OP 1

Abstract Number : 68

Elucidation of the role of NOA1 in host response to infection by South African Cassava Mosaic Virus

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1 - Wits

Background

Arabidopsis thaliana nitric oxide associated protein 1 (AtNOA1) has previously been reported to be differentially regulated in response to biotic and abiotic stress, suggesting an involvement in both pathways. AtNOA1 is a cyclic GTPase (cGTPase), and member of the YlqF/YawG family with nucleic acid and protein binding abilities. GTP/GDP are involved in various processes in a cell, however the function of cGTPase in plants and mammals has not yet been clarified. The aim of this study was to assess the impact of *South African cassava mosaic virus* (SACMV) infection on the expression of NOA1 homologues in *N. benthamiana,* and cassava cultivar T200 (susceptible) and TME3 (tolerant).

Methods and Results

A bioinformatics approach was used to identify putative NOA1 homologues in cassava. Using the cassava genome data on Phytozome, 3 putative cGTPases, namely transcripts 4.1_007735m, 4.1_002874m and 4.1_025372 were identified. Based on their protein sequences, they share a respective 68%, 27% and 27% similarity to their *A. thaliana* protein homologue and 65%, 45% and 45% sequence identity to *N. benthamiana*, respectively. GTPase specific motifs, G4, G5, G1, G2 known to be found in that order in other characterized cGTPases, is only found on transcripts 4.1_002874m and 4.1_025372, while transcript 4.1_007735m appears to be truncated, lacking motifs G2 and G3. An infectivity study was carried out to assess the expression of NOA1 in SACMV- infected *N. benthamiana*, and three cGTPases in SACMV-infected T200 and TME3. Absolute real-time PCR was used to quantify viral load in all three pathosystems. NOA1 expression in SACMV infected *N. benthamiana* was measured at 8, 14 and 28 dpi and found to be significantly down regulated at all 3 time points ($p<0.01$). In SACMV-infected cassava, transcript 4.2_007735m was significantly downregulated at 56 dpi in T200 ($p<0.01$), while it was unchanged at 28 dpi in T200 and at 28 and 56 dpi in TME3. Expression of transcript 4.1_002874m and 4.1_025372 remained unchanged at both time points.

Conclusion

These findings suggest a possible role of cGTPase/NOA1 in SACMV response in cassava and *N. benthamiana.* The nature of its involvement is currently being be elucidated.

OP 2

Abstract Number : 11

Antibacterial activity and the mode of action of Persea americana Mill. (avocado) seeds extracts on the cell morphology.

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Abstract

Persea americana Mill. (Avocado) seeds extracts were evaluated for antibacterial activity in this study. The mechanism of action of the seeds extracts on the bacterial cell morphology was investigated using Scanning Electron Microscope. The avocado seeds were extracted with acetone, chloroform and water and tested for their inhibitory potential on five Gram-negative and five Gram-positive bacterial pathogens that cause serious infections in humans. Agar dilution method was used to determine the Minimum Inhibitory Concentration (MIC) of the extracts. The MIC of the extracts ranged between 0.1 and 10 mg/mL. The acetone extract was the most active with the MIC of 0.1 mg/mL against *S. aureus*, *S. typhimurium*, *P. aeruginosa* and *P. shigelloides*. The chloroform extract showed the lowest MIC of 1.0 mg/mL while water extract exhibited lowest MIC of 0.5 mg/mL against *P. aeruginosa*. The water extract did not show any activity against *P. shigelloides*. The bacterial cells were prepared for ultrastructural observations after treatment with the extracts. A comparison was made between bacterial cells exposed to the extracts with those which were not exposed to the extracts (control strains). Positive effects of avocado seeds extracts on bacterial cells were clearly observed in this study.

Key words:

Persea americana; Antimicrobial activity; Scanning Electron Microscope
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OP 3

Abstract Number : 62

Isolation and characterization of bacterial endophytes from gnotobiotically grown Marama bean seedlings

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Marama bean [*Tylosema esculentum* (Burchell) Schreiber] is indigenous to the dry parts of Southern Africa. It is a staple food for the Khoisan and Bantu people from those areas. In Namibia it grows wild mainly in Omaheke and Otjizondjupa regions. Marama, which is currently being domesticated, has a large tuber and pods containing 1-2 oil and protein-rich seeds with a nutritional value similar to soybean. Protein content is 30-39% (similar to soy bean) and oil content is 30-43%. In addition it produces a edible tuber tht is rich in starch. Unfortunately, harvesting in the wild is very extensive and random by local people with such intensity that some genotypes of marama bean are becoming endangered with extinction or have already become extinct. One big enigma that remains is solving the question of the source of nitrogen that marama uses to synthesize the proteins in the seeds. The aim of this work was to determine the presents ob bacterial endophytes that many have a growth promoting effecty on the marama bean especially nitrogen fixing potential. Inorder to achieve this objective seed swere carefully surface sterilised and gnotobiotically grown to 2 week old seedlings. After that bacterial endophytes were isolated using three media. After obtaining pure cultures, DNA was isolated and used as template to perform 16S rDNA and *nif* H PCR amplification. Using BLAST searches, 34 bacterial species from 16 genera including *Bacillus*, *Rhizobia*, *Curtobacterium*, *Pantonea*, *Microbacterium*, *Enterobacter*, and *Burkoholderia* were isolated. Of these species, indole acetic acid production activity and nitrogen fixing activity was detected. From our data were can conclude that marama bean has a high species diversity of seed associated bacterial endophytes that have plant growth promoting activity.

OP 4

Abstract Number : 121

Vegetative Growth and Yield Response of Five Amaranthus Cruenthus to Poultry Manure (PM), Arbuscular Mycorrhizal (AM), Combination of AM-PM, and Inorganic Fertilizer

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Abstract

Background
Although poultry manure promotes and enhances the growth and yield of vegetable plants, not all macro nutrients are readily available for plant intake, and this could bring about slow growth and poor yield. *Arbuscular mycorrhiza* fungus (AMF) help plants to capture nutrients such as phosphorus, sulphur, nitrogen and micronutrients from the soil. The effect of combined *Arbuscular Mycorrhiza* and poultry manure (AM-PM) in different concentrations has been tested in recent times as a new way guaranteeing efficiency in soil productivity. This work evaluated the vegetative growth and yield response of five accessions of *Amaranthus cruenthus* to treatments of Poultry manure (PM), *Arbuscular mycorrhiza* (AM), combination of AM-PM, and NPK.

Methods

The experiment was a randomised complete block design, with five treatments and three replications; each replication consisting of 5 single row plots. The treatments were: A= Arbuscular mycorrhizal (AM) -(400kg/ha^{-1}), B= Poultry manure (PM) - (400kg/ha^{-1}PM),C= Arbuscular mycorrhizal/ Poultry manure - (200kg/ha^{-1}AM), (200kg/ha^{-1}PM), D=NPK fertilizer-(400kg/ha^{-1}NPK), E=Control

Result

Data was collected on five vegetative and yield related characters. The combined analysis of variance showed significant treatment, accession and accession X treatment interaction effect, on all the characters evaluated at 0.001 and 0.005 probabilities. PM gave significantly highest Total leaf weight per plant (24.83g), total root weight per plant(5.68g) and plant weight at six weeks (16.22g), while AM-PM gave significantly highest plant height at six weeks (29.48) and produce leaf size that had no significant difference with NPK, Suggesting that AM-PM could be an

alternative to NPK. Furthermore BUAM 004 performed best in the entire yield characters evaluated and can be considered for yield improvement in *Amaranthus,* while BUAM 005 was the poorest of the accession.

Conclusion

Treatment of *Amaranthus* with 400kg/ha^{-1}of Poultry manure (PM) gave a better leaf weight yield as well as better root weight and total plant weight. These characters constitute the bulk of economic yield in *Amaranthus.* Combination of AM-PM performed adequately better than single treatment of AM and inorganic fertilizer alone and therefore could be considered as a possible alternative to inorganic fertilizer

OP 5

Abstract Number : 263

The effect of processing techniques on the microflora of cowpea leaves

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Background

Cowpea (*Vigna unguiculata* or *Vigna sinensis*), also known as black eye beans or southern peas, are important grain legumes and vegetables in Africa and other developing countries. They serve as good sources of protein, energy and other nutrients and have been reported to possess numerous health benefits.

Method

Fresh samples of cowpea leaves were harvested from Agricultural Research Council (ARC) farm in Pretoria and were processed at the CSIR Pretoria. The processing techniques used were: tap water wash, purified water wash, blanching, drying and milling. Samples were collected at each stage of processing and were analysed according to CSIR SOP's for total aerobic mesophiles, coliforms, lactic acid bacteria and yeast and mould counts.

Results and discussion

The average level of aerobic mesophiles on fresh unprocessed leaves ranged from 7.6 x 10^8 colony forming units (cfu)/g to 1.2 x10^4 cfu/g. However, the average level of total coliforms, lactic acid bacteria, yeast and mould counts were (37, 19, 19) MPN 100mL^{-1}, 1.8 x 10^8 Cfu/g, 1.87 x 10^7 Cfu/g respectively (average of three triplicate values)

In terms of the reduction in microbial load, the first tap water wash had no effect; while the second tap water wash was more effective than the sanitiser wash. Steam tunnel blanching caused a higher reduction in microbial load than pot blanching. Best results were achieved by washing cowpea leaves twice with tap water and then blanching in a steam tunnel.

Based on the most probable number (MPN) count and other microbiological test carried out, the blanched leaves were found to have the highest population of coliforms, followed by the fresh leaves and the dried leaves had the least microbial load.

The high level of contamination in the blanched samples could possibly be because of poor quality of water used during preparation. Moreover, water is one of the major sources of sewage contamination.

Conclusion

The result of microbial counts of cowpea leaves indicated that the agricultural practices, harvesting, transporting points are poor and therefore, the higher microbial load could represent a risk for public health.
Key Words: coliforms, cowpea, lactic acid bacteria, processing, yeast and moulds

OP 6

Genome based identification and characterization of putative genes specific to Fusarium circinatum

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Background:

Fusarium circinatum is an important pathogen of pine trees and its management relies largely on early detection particularly in pine seedling nurseries. All existing detection methods for the pathogen require sophisticated laboratory facilities and highly skilled technicians. A simple robust detection method that can be used in the nurseries is urgently needed for this pathogen. The fact that the entire genome of this pathogen is available opens new avenues for the development of diagnostics for this fungus. In this study we identified open reading frames (ORFs) specific to Fusarium circinatum and evaluated their potential as diagnostic candidates.

Methods:

The ORF identification process involved bioinformatics-based screening of all the putative F. circinatum ORFs against public databases. Putative genes that showed less than 50% nucleotide identity based on a BLASTn analysis and encoded proteins with less than 30% positive identities based on a BLASTp analysis were classified as unique to F. circinatum. This was followed by functional characterization of ORFs found to be unique to F. circinatum. The predicted amino acid sequences were further analyzed using the programs CDD, Pfam, SMART, WoIF PSORT and Vaxjen. We used a PCR based approach to confirm the presence of selected unique genes in different strains of F. circinatum and their absence from other closely related Fusarium species for which a genome sequence data is not yet available.

Results:

Thirty-six ORFs were identified as potentially unique to F. circinatum. Nineteen of these encode proteins with known domains while seventeen encode proteins of unknown function. The results of our PCR analyses showed that four of the selected genes were present in all of the strains of F. circinatum tested. Furthermore, corresponding PCR products were not generated for any of the related Fusarium species. These data thus indicate that the selected genes are common and unique to F. circinatum

Conclusion:

The unique putative genes identified in this study could be good candidates for use in rapid in-the-field diagnostic assays specific to F. circinatum. These putative genes are also interesting candidates for both functional and evolutionary studies as they may be the consequence of horizontal gene transfer.

OP 7

Integrated DNA sequences in the cassava genome associated with cassava mosaic begomoviruses: A bioinformatics study

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Background Integrated exogenous genomic or viral sequences have been reported in many plant genomes. In some instances, these sequences affect disease symptom progression and sometimes may â€œjump outâ€ of the host genome to become infectious episomal agents. Â One example is plant-infecting badnaviruses.Â Two non-homologous episomal circular satellite-like ssDNA sequences (DNA-II and III) (AY826366 and AY836367) were identified in cassava field samplesÂ infected with Â African cassava mosaic virus (ACMV) or East African cassava mosaic virus (EACMV), and were found to modulate symptom phenotype and severity in the tolerant cultivar TME3 and model host Nicotiana benthamiana. The recently sequenced cassava genome has not, until recently, been scrutinized for genomic DNA sequences or elements that may be associated with cassava mosaic begomovirus pathogens, but examination of the cassava EST database surprisingly revealed different-sized DNA-II and DNA-III fragments. The relationship between these episomal and integrated DNA sequences, cassava and the associated begomoviruses is unknown. Methods and Results In this study, BLASTX searches of the cassava genome (www.phytozome.net) showed multiple truncated fragments homologous to integrated DNA-II and DNA-III sequences arranged in tandem, or inverted repeat patterns typical of transposable elements. The largest insertions were almost full-length when compared to the episomal query sequences, showing 99.2 and 84.1% homology, found on scaffolds 12498 and 12725, respectively. The gene ontology classifications of flanking host genes were mostly nuclear genes involved in nucleotide or nucleic acid processing. Â Also prevalent, were genes encoding protein synthesis, binding and processing factors. RT-PCR analysis of some of the flanking genes indicated that both the host genes and the integrated DNA sequencesÂ are expressed, possibly as polycistronic mRNAs. Conclusions We propose that the integrated DNA sequences are involved in regulatory host functions, whose cognate proteins may be responsive to invading begomoviruses.

OP 8

Preliminary Phytochemical Screening and Antibacterial Properties of Crude Stem Bark Extracts and Fractions of Parkia biglobosa (Jacq).

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Preliminary Phytochemical Screening and Antibacterial Properties of Crude Stem Bark Extracts and Fractions of Parkia biglobosa (Jacq). Olayinka A. Aiyegoro 1, Emmanuel O. Abioye 2, David A. Akinpelu 2, Anthony I. Okoh3 1GI Microbiology and Biotechnology Unit, Agricultural Research Council- Animal Production Institute, Irene, South Africa. 2Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. 3Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa.

Abstract:

Parkia biglobosa is a plant that has shown potential as a source of chemotherapeutic compounds, while many folkloric and ethnobotanical applications of this plant have been reported. This present study, therefore investigated the preliminary phytochemical composition, and antibacterial potential of the stem bark of the plant. The methanolic crude extract of Parkia biglobosa was prepared and later partitioned with different solvents ranging from n-hexane, chloroform, ethyl acetate, and butanol according to their order of polarity. Phytochemical screening of the extract revealed the presence of alkaloids, tannins, saponins, flavonoids, steroids, glycoside and sugars. The zones of inhibition exhibited by the extract against tested bacteria ranged between 14 Â± 0.00 mm and 28 Â± 0.71 mm. The smallest zone of inhibition (14 Â± 0.00 mm) was observed against Escherichia coli and the highest zone of inhibition (28 Â± 0.71 mm) was observed against Pseudomonas aeruginosa. The MIC of methanolic extract of P. biglobosa against isolates ranged between 0.63 mg/mL and 5 mg/mL, while the MIC values exhibited by the n-hexane and aqueous fractions ranged between 0.63 mg/mL and 10 mg/mL. The extract and fractions of P.biglobosa used in this work was found to possess antimicrobial properties, Â these observations make this plant a potential source of bioactive compounds that can be used in management of bacterial infections. The use of this plant as herbal medicaments in African countries and the reports on the toxicity of the plant show that the plant is non-toxic to humans. Keywords: minimum inhibitory concentration (MIC); stem bark extract; fraction; phytochemistry; resistance; infections; bioactive; antibacterial; antibiotics.

OP 9

Abstract Number : 224

Screening of Ceratotheca triloba plant extracts for antibacterial and anticancer activity

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Studies have shown that the root extract of *Ceratotheca triloba* contain an anthraquinone derivative, 5,8-dimethoxy-2,3,10,10a-tetrahydro-1H,4aH-phenanthrene-4,9-dione (Mohanlall *et al*., 2011). This anthraquinone has been shown to inhibit the human topoisomerase II enzyme. Topoisomerase inhibitors are known to exhibit antibacterial (example: fluoroquinolone) and anticancer (example: doxorubicin) activity. However due the resistant of some bacteria and cancers (Wong *et al*., 2006; Szakacs *et al*., 2006; Hooper, 2001), there is a need to research new bio-actives for treatment. Therefore the aim of this study was to determine the antibacterial and anticancer activity of *C. triloba* extracts. The root and leaf hexane extracts of *C. triloba* were tested on Gram positive and Gram negative bacteria by using the disk diffusion assay. The minimum inhibitory concentration (MIC) of the root extract was determined. The hexane root extract was fractionated by using column chromatography. Over 100 fractions were collected from the column. Similar fractions were combined and weighed for determination of the percentage yield. These fractions were screened at 25 and 12.5 µg.ml^{-1} on two cancer cell lines, A375 (melanoma) and MDA-MB-231 (breast cancer) by using the MTT cytotoxicity assay. The IC_{50} values of the fractions were also determined. The disk diffusion assay showed that the leaf and root extracts produced a zone of inhibition on *Bacillus cereus* and *Micrococcus luteus* cultures. The MIC's for the root extract were between 0.04 - 2.50 µg.ml^{-1} for five bacterial cultures; *Salmonella typhimurium*, *Staphylococcus*

aureus, *Escherichia coli*, *B. cereus* and *M. luteus*. A total of 10 combined fractions were collected from the column. Fractions; F2, F4, F5 and F8 showed cytotoxicity against the A375 (89%, 89 %, 90 and 75 %, respectively) and MDA-MB-231 (91%, 89 %, 92 % and 74 %, respectively) cell lines. The IC_{50} values of the fractions for the A375 and MDA-MB-231 cell lines were between 0.696 - 2.459 $\mu g.ml^{-1}$ and 1.124-5.290 $\mu g.ml^{-1}$, respectively. Collectively, the results showed the extracts of *C. triloba* exhibited antibacterial and anticancer activity. Therefore *C. triloba* extracts could provide a source of potential antibacterial and anticancer compounds. These compounds will be identified in future research.

OP 10

Abstract Number : 71

Effect of growth rate on the production of a pharmaceutical peptide under regulation of the hp4d promoter in Yarrowia lipolytica

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Background:

Exenatide (Byetta®) is a Type 2 diabetic drug which decreases the blood glucose level. It is expensive due to production by chemical synthesis. A potential cost effective alternate for the production of exenatide, is the use of recombinant protein production technology. *Yarrowia lipolytica*, a dimorphic yeast, was genetically engineered to produce the exenatide peptide (39 amino acids) as a fusion to Lip2 (lipase) protein under the regulation of the *hp4d* promoter. The regulation of the promoter has not been elucidated and is currently reported to be quasi-constitutive. In this study, the regulation of the *hp4d* promoter was established and a fed-batch fermentation strategy for the production of fused exenatide was developed.

Methods:

Transformed *Y. lipolytica* strains were screened for the production of extracellular chimeric exenatide. A transcript profile of a positive clone harbouring the *hp4d* carrying expression vector (pKOV410-*Lip2-Exe*) was determined using an optimised mRNA sandwich hybridisation methodology. Batch fermentation (1.2 L) was used to establish transcription and production profiles during growth of *Y. lipolytica*. Continuous fermentation (1 L) determined the effect of growth rate on the transcription levels of the *hp4d* promoter. Subsequently, a fed-batch fermentation (10 L) strategy was developed.

Results:

A maximum of 580 $mg.L^{-1}$ of extracellular protein was produced at 70 h during batch fermentation. The mRNA transcription profiles during batch fermentation showed that the *hp4d* promoter was constitutive. Continuous fermentation, used to evaluate the effect of growth rate on *hp4d* promoter driven expression, revealed a maximum mRNA level at a growth rate of 0.02 h^{-1}. This validated that the *hp4d* promoter is growth rate dependent and not growth phase dependent as reported in literature. A fed-batch process strategy for the production of fused exenatide under the regulation of the *hp4d* promoter was established and yielded a maximum of 3 $g.L^{-1}$ of extracellular protein after 45 h. This chimeric protein can then be cleaved from its fusion partner to release the intact exenatide peptide.

Conclusion:

The synthetic *hp4d* promoter is growth rate dependent. A fed-batch strategy was developed for the production of a pharmaceutical peptide under the regulation of this constitutive promoter in *Y. lipolytica*.

OP 11

Abstract Number : 268

The microbial ecology of moderately thermophilic BIOX reactors and the impact of organic carbon and solids loading on community structure.

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Background

Recent studies performed in our laboratory have shown that archaea dominate both commercial and laboratory-scale

bioleaching reactors operated between 42°C and 50°C, with *Acidiplasma cupricumulans* prominent in many cases. These archaea, with the possible exception of *Ferroplasma acidiphilum*, are considered heterotrophic or mixotrophic. The commercial reactors are not supplemented with organic carbon, so it is most likely that the autotrophs are releasing sufficient organic matter into the tanks to sustain the archaea. We have sought to characterise the moderately thermophilic community in greater detail, as recent metagenomic studies on acid mine drainage revealed novel archaeal lineages belonging to the order Thermoplasmatales, indicating far greater diversity. This has not been studied within heap and tank bioleach operations to date. The response of the community to changes in organic carbon content and solids loading have been assessed.

Methods

The research has been conducted in pH-controlled, continuously stirred reactors inoculated with a culture from a commercial operation. The bioleaching performance was monitored by analysis pH, redox potential, soluble iron and extent of sulphide oxidation. The microbial community was characterised using 16S rRNA gene sequencing and changes in community structure, in response to organic carbon content and solids loading assessed by cell counts and qPCR. . Organic carbon was measured using High Performance Liquid Chromatography.

Results

The presence of uncharacterised archaea in the inoculum was confirmed. Organic carbon supplementation, in the form of yeast extract, had a significant impact on microbial community structure, confirming published data indicating that some autotrophs (eg *Leptospirillumferriphlium*) are inhibited by low soluble organics. However, there is now evidence to suggest that organic compounds provided through cell leakage and lysis are able to sustain heterotrophic archaea within tank bioleaching systems.

Conclusions

The archaeal diversity in commercial bioleaching operations is greater than previously recognised, although their contribution to overall performance is still being quantified. Our data suggests that autotrophic bacteria, particularly *Acidithiobacillus caldus* are able to support the growth of these heterotrophic archaea, up to high cell densities, by releasing organic carbon. This research could have a profound effect on the way tank bioleaching operations are managed.

OP 12

Abstract Number : 100

Comparative secretome analysis of Trichoderma asperellum S4F8 and Trichoderma reesei Rut C30 during solid-state fermentation on sugarcane bagasse

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Background:

The lignocellulosic enzymes of *Trichoderma* species have received particular interest for biomass conversion to biofuels, but the production cost of these enzymes remains a significant hurdle for its commercial application. In this study, we quantitatively compared the lignocellulolytic enzyme profile of a newly isolated *Trichoderma asperellum* S4F8 strain with that of *Trichoderma reesei* Rut C30, cultured on sugarcane bagasse (SCB) using solid-state fermentation (SSF).

Materials and Methods:

SSF opimization included temperature adjustments, controlling realtive humidity (RH) during incubation and exposure to constant fluorescent light. Optimized conditions were evaluated with regards to its enzyme production relavent to lignocellulose breakdown using a combination of enzymology and LC-MS/MS (LTQ-Orbitrap Velos).

Results:

Comparison of the lignocellulolytic enzyme profiles of S4F8 and Rut C30 revealed significant higher hemicellulase and β-glucosidase enzyme activities in S4F8. LC-MS/MS analysis of the two fungal secretomes enabled the detection 815 proteins, with 418 and 397 proteins detected for S4F8 and Rut C30, respectively, and 174 proteins common between the two strains. In-depth analysis of the associated biological functions and glycoside hydrolase (GH) family representation within the two secretomes indicated that the S4F8 secretome was enriched with a higher diversity of hemicellulases and β-glucosidases combined with increased protein abundance from some these proteins compared to

that of Rut C30.

Conclusions:

In SCB SSF, *T. asperellum* S4F8 produced a more complex lignocellulolytic enzyme cocktail with enhanced hemicellulose and cellobiose hydrolysis potential compared to *T. reesei* Rut C30. This bodes well for the development of a more cost-effective, more efficient lignocellulolytic enzyme cocktail from *T. asperellum* for lignocellulosic feedstock hydrolysis.

OP 13

Abstract Number : 278

Optimization of UptiBlue™ assay for rapid, accurate and reliable determination of IGF-I bioactivity

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Background:

Insulin-like growth factor-I (IGF-I) is a potent mitogen which is an attractive alternative to the controversial use of serum and insulin as growth factor in cell culture. Recent biotechnological advances have led to the development of novel technologies to produce IGF-I of high purity at low cost in South Africa, thereby enabling the widespread availability of IGF-I in the commercial market. However, little is known about the bioactivity of this product as well as how it fared against commercial IGF-I and insulin. Thus, this study was aimed at determining the bioactivity of IGF-I using the UptiBlue™ assay as well as critical comparison of this novel assay with traditional cell proliferation assays for indirect measurement of IGF-I bioactivity. A comparative evaluation of recombinant IGF-I as an alternative to commercially available IGF-I and insulin in terms of bioactivity was conducted.

Methods:

The bioactivity of recombinant IGF-I, commercial IGF-I and insulin were determined using three standard cell proliferation assays namely, the crystal violet, MTS and UptiBlue™ assay. Each of the assays were optimized with regard to cell number and dye incubation time for Balb/c 3T3 fibroblast, C2C12 mouse myoblast and CHO cell lines.
Results: The data obtained demonstrated that the UptiBlue™ assay compared favourably well with the two traditional assays and supports the hypothesis that it can be used for the indirect measurement of IGF-I bioactivity. In addition the IGF-I product displays high bioactivity as expressed by EC_{50} values ranging from 4.5-8 ng/ml. The data further show that the recombinant IGF-I product is significantly more bioactive than insulin at a considerably lower concentration but in the same bioactivity range as commercially available product.

Conclusion:

It can be concluded that locally produced South African recombinant IGF-I is a potent promoter of cell growth that displays high bioactivity at low concentrations in serum free media as shown in this study, thereby representing a new and cost effective alternative to serum for culturing mammalian cells.

OP 14

Abstract Number : 104

Gas Bubbles Exposed inside Fermenting Yeasts

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Background

Fermentation is at the heart of most important biotechnological processes known to man. These processes, which include the leavening of bread and production of alcoholic beverages, exploit the capabilities of yeasts to produce increased concentrations of ethanol and CO_2. Since yeasts vigorously release CO_2 into the surrounding medium during fermentation, it is expected that cells would be filled with gas bubbles. However no sign of CO_2 bubbles has been reported inside cells even though the yeast fermentation process is well established. The lack of reports on intracellular gas bubbles is considered to be the missing link since it is not clear what happens to the CO_2 between fermentation, when it is produced, and eventual release from the cells. Therefore the main aim of this study became to find this missing

link inside fermenting yeast cells.

Methods

The brewer's yeast, *Saccharomyces pastorianus* and the baker's yeast, *Saccharomyces cerevisiae*, were grown in fermentable and non-fermentable media respectively and analyzed with various microscopic techniques to determine the presence of gas bubbles inside the cells. Imaging techniques used included light microscopy, transmission electron microscopy (TEM), and Auger-architectomics (http://en.wikipedia.org/wiki/Auger_architectomics).

Results

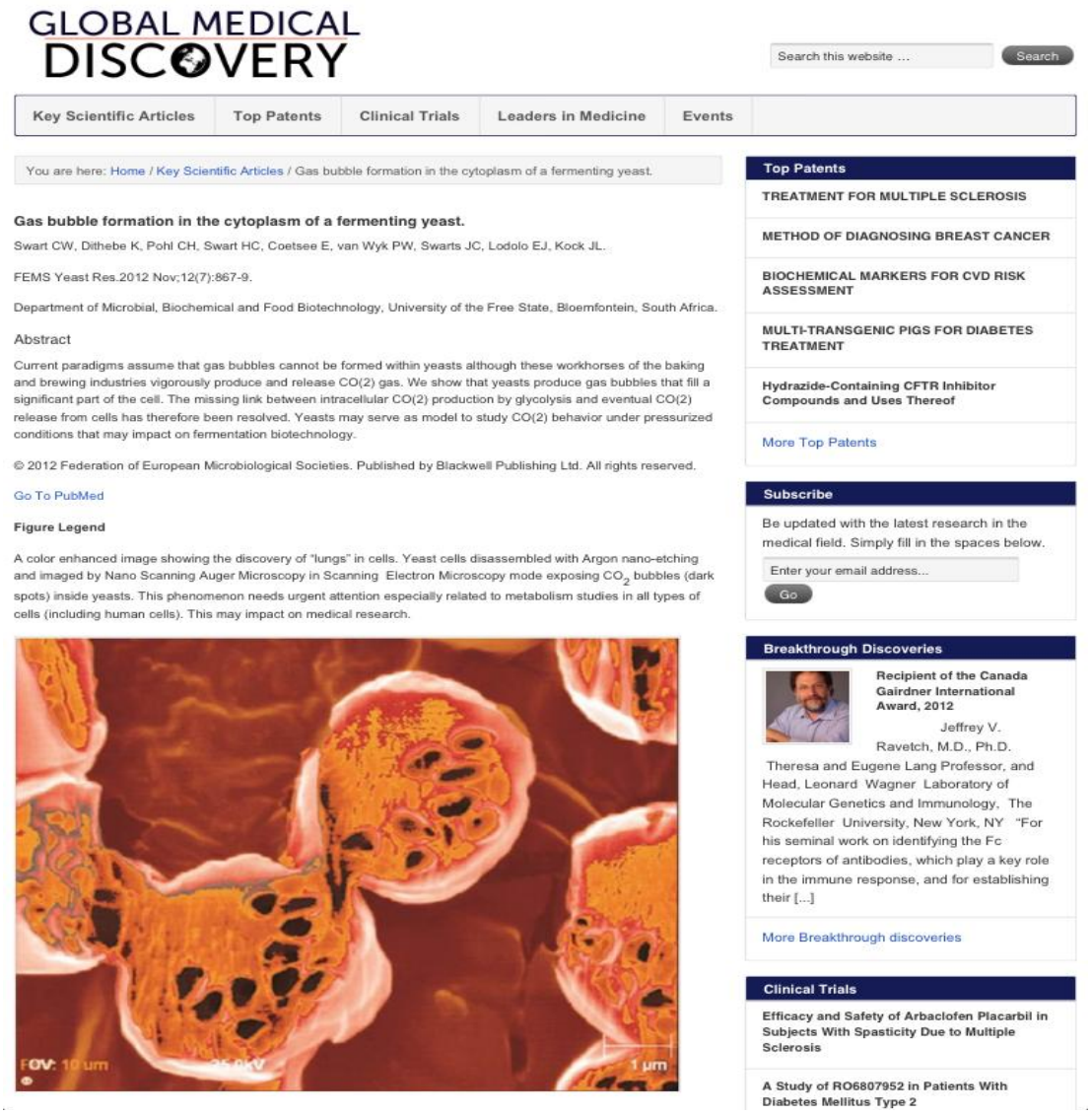
Strikingly, using light microscopy the fermenting cells had a granular appearance, similar to that observed when gas vacuoles were discovered in the Cyanobacteria. However, much less granules were observed in cells grown in non-fermentable media. Further studies using TEM confirmed the presence of gas bubbles filling a significant part of the cells when grown in fermentable media. This was further visualized with NanoSAM (Auger-architectomics) that revealed an interconnecting maze of bubbles. Transmission electron microscopy revealed that these gas bubbles compress and deform organelles inside the cells although they contained no enveloping membranes.

Conclusions

The missing link between intracellular CO_2 production by glycolysis and eventual CO_2 release from the cells has been discovered. Gas bubbles are produced in large numbers inside the cells, filling a significant part of the cells, thereby deforming and compressing cell organelles. The effects of this interaction on metabolism and the coding functions of yeasts should now be addressed.

Reference

Swart et al., 2012.*FEMS Yeast Res* 12(7): 867 - 869.



OP 15

Abstract Number : 85

The over-expression of native Saccharomyces cerevisiae SNARE-genes for increased heterologous protein production.

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Introduction

A major bottleneck for the use of *S. cerevisiae* as host organism for the single step hydrolysis of cellulolytic material to fermentable sugars and their subsequent fermentation to ethanol (Consolidated Bio-Processing), is the low heterologous cellulase secretion capacity of the host organism. SNAREs (Soluble NSF Attachment Receptor proteins), which are required at the majority of membrane fusion events during intracellular membrane transport, can be differentially classifiedbased on the localization of their action, being either v-SNAREs, located on the secretory vesicles, or t-SNAREs, located on the target membranes. The collection of SNARE genes responsible for the formation of the membrane-specific SNARE complex at the plasma membrane, facilitating SNARE-mediated exocytosis, are: *SNC1*&*SNC2* (v-SNAREs) *andSSO1*, *SSO2* and *SEC9* (t-SNAREs).

Methods

We constitutively over-expressed these SNARE genes in differential combinations. This was achieved utilizing the *S. cerevisiaePGK*1 promoter &terminator, using a range of yeast expression vectors (pBKD1, pBZD1 &pBHD1) and antibiotic selection markers (*Kan MX, Sh ble, hph*) to facilitate transformations through delta-integration. Two episomal plasmids, ySFI_*CEL3A*&pMI529_*CEL7A*, were transformed into the Y294 *S. cerevisiae* strain, creating two parental strains, each expressing a different cellulase reporter protein. These strains served as parental strains to create an ensemble of 26 strains over-expressing the respective SNARE genes and the secretory capabilities of these strains were then determined using extracellular enzymatic activity assays.

Results

We were able to increase the secretory titers of *S. cerevisiae* for two cellulase reporter proteins (*S. fibuligera* Cel3A (BGL1) &*T. emersonii* Cel7A (CBH1)) by up to 71% and the quantitative confirmation of an increased extracellular protein concentration was also established using SDS-PAGE. These increases were facilitated without significant deleterious effects on the growth capability of the yeast. Using quantitative RT-PCR we were able to show that the delta integration events averaged around 1-2 per transformation.

Conclusions

The over-expression of native *S. cerevisiae* SNARE genes provides a novel strategy for improving titers of secretory proteins that are currently attainable. As is often the case with protein secretion studies, improvements are largely protein specific, with larger increases being prevalent for the Cel7A reporter protein.

OP 16

Isolation of Polyketides Producing Actinomycete Isolates via PCR-Based Genome Screening for PKS Gene

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Objective: To assess the antibiotic biosynthetic potential of actinomycetes isolated from rhizosphere soils. Methods: The actinomycete isolates genomic DNA was screened by PCR for the polyketide biosynthetic gene cluster. Phylogenetic analysis of the PKS gene nucleotide sequences was conducted using bioinformatics softwares. Results: In this study 341 bacterial isolates were screened by PKS-specific PCR. Twenty-four were characterized based on the presence of the expected size of the PCR-amplified DNA fragment in the genome. The nucleotide sequencing of the PCR-amplified DNA fragments showed that each of the 24 bacterial isolates contained the polyketide gene. Culture extracts from 4 of these isolates showed a typical polyketide-like high-pressure liquid chromatography (HPLC) chromatogram profile, and also strong antibacterial activity against the test organisms. Conclusion: PCR screening of the genomes of

actinomycetes for specific antibiotic biosynthetic gene clusters allows for the rapid determination of the antibiotic biosynthetic potential of these actinomycete isolates. This also gives advanced knowledge of the type of antibiotic(s) to expect and the appropriate methods of antibiotic purification to apply. Keywords: actinomycetes; polyketides, PKS gene; antibacterial activity.

OP 17

Abstract Number : 181

Blooming Algae Offshore

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Introduction

A new production method for low-cost cultivation of phytoplankton was field tested in St. Helena Bay, Western Cape, South Africa, to evaluate the potential to produce high-quality animal feed.

Methods

Eight locally isolated phytoplankton strains were grown under ambient conditions in a series of high rate ponds to determine their growth rate, nutrient, CO_2, sunlight and mixing regime requirements. These included three chlorophytes and five diatoms. The algae were grown with seawater enriched to 40-80 mM nitrate to replicate or double nutrient conditions found during seasonal upwelling events. The primary objective of the field trials was to determine whether high-rate growth could be sustained over an extended period of time to increase the algal biomass to a sufficient density and volume to support an animal feed production system. The unconventional flow-through production system operated without CO_2 addition, very low mixing energy and 10% of nutrient used in comparable biofuel productions systems to reduce input and operational cost.

Results

During the June and July winter months, at low rates of insolation, with significant rain episodes and low night-time temperatures, the five of the tested algal strains maintained exponential growth, more than doubling per day, for 9-12 day stretches attaining harvestable cell densities in excess of 300,000 cells/ml.

Conclusions

Given these preliminary results, at this rate of production and low operational costs, this alternative production system warrants further evaluation for the low-cost production of phytoplankton biomass for animal feed.

OP 18

Abstract Number :29

Turning waste into value: crude glycerol and Streptomyces albulus a genomics perspective

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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 is 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 microorganism optimally use glycerol as a carbon source, glycerol from biodiesel production contains

contaminants such as methanol, salts and water and in particular the high salt concentration hinders most microorganisms growth with the exception of *Streptomyces albulus*. Metabolic engineering therefore needs to take place not only to increase glycerol uptake but also to optimise the production of value products. In order to successfully engineer a microorganism the genetics, biosynthetic pathway and flux of the metabolite of interest needs to be known. Little is known about the genetics of the gram positive bacteria called *Streptomyces albulus* which produces the value product -poly-L-lysine (PL). PL is an antimicrobial agent and is used as a food preservative in countries such as USA and Japan. In order to use the biodiesel production by-product glycerol as a main carbon source to make PL more is needed to be known about the genome and transcriptome of this species. *S. albulus* sequencing was performed on a MiSeq (Illumina) at the Agricultural Research Council (ARC) Biotechnology Platform. The genome was assembled using the *de*

novo assembly tool in the *CLC Genomics Workbench* version 6 (CLC Bio). This assembly produced 243 contigs withan average length of 39,836 bp. The *S. albulus* genome was 9.4 Mb in size with a high GC content (72.2%) similar to other *Streptomyces* species. Genome annotation was performed using the NCBI's Prokaryote Automated Annotation Pipeline (PGAAP), resulting in 9,177 protein coding sequences (CDS) being identified. This genome will provide valuable insight into the functioning of *S. albulus* and will provide the reference onto which transcriptomics will take place. This information will be used in order to optimise glycerol uptake and the production of PL through metabolic engineering.

OP 19

Abstract Number : 113

Detection of Blaoxa-24/40 carrying acinetobacter baumannii isolates obtained from wastewater treatment plants

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Background

The first known Acinetobacter baumannii strain resistant to carbapenemswere reported as early as 1985. Since then, incidences of carbapenemase resistant *A.baumannii have increased dramatically. The* emergence of carbapenemases, such as class D β-lactamases poses a significant problem to healthcare practitioners, particularly the treatment of Gram-negative pathogens, such as *A.baumannii.* Currently, 200 variants of *oxacilinases* have been described in literature of which 108 have been detected in *Acinetobacter* species. The*bla*$_{\text{OXA-24/40}}$ gene was first reported by 2002 and has been reported in Europe, South America and Asia. Acquisition of these resistance genes can be mediated by environments, such as wastewater treatment plants. Wastewater treatment plants have been recognized as a major reservoir for antibiotic resistance bacteria and genes. The aim of this study was to determine the resistance profiles of *A. baumannii* isolates obtained from wastewater treatment plants.

Methods

A grab sampling approach was done on eight wastewater treatment plants.Samples collected were incubated in Baumann enrichment broth and plated out on CHROMagar*Acinetobacter* selective media. Colonies were identified using the Vitek2 or MALDI-TOF. Antimicrobial susceptibility was performed using Vitek®2 AST cards.Total DNA extraction was performed using the ZR Fungal/Bacterial DNA MiniPrep™kit. A multiplex PCR assay was used to determine the circulating *bla*$_{\text{OXA}}$ genes.

Results

A total of 35 *A.baumannii* isolates were identified from eight wastewater treatment plants.Two distinctgene profiles were observed.The majority of isolates showed a resistance profile containing *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-23}}$ genes. While the second resistance profile showed two isolates containing *bla*$_{\text{OXA-51}}$and *bla*$_{\text{OXA-24/40.}}$ No antimicrobial susceptibility variation was seen amongst these two gene profiles.

Conclusion

The*bla*$_{\text{OXA-24/-40}}$gene is established amongst the *A.baumannii*. Furthermore, the hospital setting could be a source of extremely resistant *A.baumannii* and may have a greater environment impact than previously thought. To the best of our knowledge, this is the first reported case of *bla*$_{\text{OXA-24/40}}$ carrying *A.baumannii* in South Africa.This poses serious public health concerns as these opportunistic pathogens may become established in the community.

OP 20

Abstract Number : 125

A molecular analysis of oxalate-degrading intestinal bacteria in black and white South Africans

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Background

The incidence of kidney stone disease in the South African black population is extremely rare, while that in the white population is comparable to moderate/high risk populations in other countries. It has been suggested that

oxalate-degrading intestinal bacteria play a role in the metabolism of dietary oxalate, which is a significant component of kidney stones. Therefore, we aimed to examine whether an enhanced diversity or abundance of oxalate-degrading bacteria in the gastrointestinal tracts of black South Africans plays a role in the low risk of kidney stones in this group.

Methods

Stool samples were obtained from healthy black and white South African male volunteers and an analysis of *Oxalobacter formigenes*, *Lactobacillus* spp. and *Bifidobacterium* spp. present carried out using DGGE- and qPCR-based approaches. Bacterial pools were also prepared from each of the samples and the ability of these to degrade oxalate was assessed *in vitro*. Oxalate-degrading strains were selectively isolated, one of which was characterised further in terms of its probiotic potential and the ability to degrade oxalate *in vivo* in a reconstituted *Lactobacillus*-free (RLF) mouse system.

Results

Samples from the black population showed a greater diversity of *Lactobacillus* spp. and a higher relative abundance of *Bifidobacterium* spp. than those from the white group, while *O. formigenes* was present only at very low levels in either group. Bacterial pools prepared from samples provided by black volunteers degraded oxalate more efficiently *in vitro* than the corresponding pools from white volunteers. A potential probiotic strain of *Lactobacillus gasseri* (strain B72) isolated from the stool of a black volunteer was able to degrade oxalate and showed good colonisation potential *in vitro*. However, it was not able to colonise a RLF mouse host or to degrade oxalate *in vivo*.

Conclusions

The South African black population harbours a diverse and abundant pool of potential oxalate-degrading bacteria that may help to protect this group from developing kidney stones. *L. gasseri* B72 shows good probiotic potential *in vitro*, however the RLF mouse *in vivo* system used in the current study does not appear to be a good tool for evaluating strains isolated from humans.

OP 21

Abstract Number : 12

The Identification of Novel Picornaviruses Associated with Acute Infantile Gastroenteritis in South Africa.
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Background:

Acute gastroenteritis is a diarrhoeal disease affecting both adults and children worldwide. It is a leading cause of infantile mortality, killing approximately 2.5 million children per annum, and is caused by many pathogens including bacteria and viruses. Rotavirus and norovirus (NoV) are prime causative agents, but several picornaviruses such as Aichi virus (AiV), Cosavirus and Saffold virus (SAFV) are also found in association with diarrhoea (Knox *et al*., 2012). However, there are a large number of cases, up to 40%, which are of unknown etiology. Rotavirus is monitored by the MRC Diarrhoeal Pathogen Research Unit, MEDUNSA, South Africa, but many cases test negative for the virus. It is therefore important that these novel or unrecognised viral pathogens are identified and characterised as they may be clinically significant. This study aims to identify picornaviruses (such as Aichi virus) that are associated with gastroenteritis in other countries but have not yet been found in South Africa.

Methods:

Thirty rotavirus-negative stool samples from patients with diarrhoea, virus-infected oysters which caused viral gastroenteritis, and waste water samples from the Belmont Valley treatment plant, Grahamstown, South Africa, were obtained and screened by transmission electron microscopy (TEM) for viral particles. To identify specific viral sequences in the samples, RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen) and oligonucelotides targeting regions of the AiV, NoV and SAFV genomes have been designed for RT-PCR which was performed using the Verso 1-Step RT-PCR Hot-Start kit (ThermoScientific).

Results:

Picornavirus-like particles and other viral particles resembling adenovirus were present in all the samples screened by TEM. RT-PCR assays using primers for the capsid region detected NoV GII sequences in the tested samples.

Conclusion:

TEM and RT-PCR have been used to identify the presence of viral particles and specific regions of the NoV GII genome in waste water, infected-oysters and stool samples. Future work will involve synthesis of double stranded cDNA for use in 454-pyrosequencing.

Reference:
Knox, C., Luke, G., Dewar, J., de Felipe, P. &Williams B. (2012).Rotavirus and emerging picornaviruses as aetiological agents of acute gastroenteritis.South African Journal of Epidemiology and Infection. 27(4), 141-148.

OP 22

Cytokine production by epithelial cells infected with dominant clinical strains of *Mycobacterium tuberculosis* in KwaZulu-Natal (South Africa)

Authors: Nontobeko E. Mvubu, Balakrishna Pillay and Manormoney Pillay

Background:

Mycobacterium tuberculosis, (MTB), the causative agent of tuberculosis (TB) remains one of the major causes of mortality worldwide, leading to 1.4 million deaths each year. Specific strains of MTB dominate in different geographic regions globally. In KwaZulu-Natal, the KZN strain has been associated with drug resistance since 1994 and was responsible for XDR TB, and high mortality rates at Tugela Ferry in 2005. MTB is able to infect and replicate in pulmonary epithelial cells that are present in greater abundance in the alveolar space. In this study, we determined differential host response to infection by measuring cytokine/chemokine response of pulmonary epithelial cells infected by dominant strains in KZN.

Methods:

The *in vitro* host response to infection of A549 pulmonary epithelial cells infected with the KZN, F28, F11, Beijing, Unique and H37Rv strains was investigated for cytokines/chemokines (CCs) production. CC production was measured at 0, 24, 48 and 72 hr post-infection using the Bio-Plex assay.

Results:

Eighteen CC (IL-1rα, -2, -4, -6, -8, -9, -17, , Rantes, Eotaxin, GM-CSF, TNF-α, IFN-γ, MIP-1α, MIP-1β, MCP-1, IP-10 and PDGF-bb) were detected using the BioPlex Pro Human Cytokine 27-plex assay. The BioPlex Pro Human Cytokine 16-plex kit confirmed the presence of 11 CC, (IL-6, IL-8, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, Rantes and TNF-α,) in the supernatants of the infected A549 cells at all-time intervals for the different strains. The concentrations of these CCs were found to be significantly higher ($P<0.05$) in the infected cells compared to the uninfected cells. In comparison to other strains, F28 and KZN induced a higher production of IL-8, IFN-γ, TNF-α, G-CSF, GM-CSF and Rantes at both 48 and 72 hr. The laboratory strain H37Rv induced the highest production of IL-6, TNF-α, G-CSF, GM-CSF, Rantes, MIP-1α and MIP-1β at 24 hr after infection. There was no trend in the response of pulmonary epithelial cells and induction of different CCs, suggesting the presence of different virulence factors in these clinical strains.

Conclusion:

Our results indicate that cytokine production in pulmonary epithelial cells is strain dependent and may explain differential host response to various strains of *M. tuberculosis*.

OP 23

Abstract Number : 323

Demographic and clinical profile of HIV infected patients accessing care at different health care Centers in the Limpopo Province, South Africa

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HIV and AIDS patients due to their weakened immune system regularly experience a wide range of opportunistic infections. However, a comprehensive study has not been conducted in Africa and in Limpopo in particular to understand the specific patterns of these infections. Therefore the present study was carried out to identify the most common pathogens infecting HIV and AIDS patients.

Patients attending different treatment centres in the Vhembe district particularly from Tshilidzini and Donald Fraser hospitals as well as patients in the community in the Tshakuma region were recruited in the study. Once the patient had agreed to participate in the study, they were requested to sign a consent form and fill a survey questionnaire. Samples were also collected from the patients including sputum, urine and mouth wash. The samples were analyzed using standard microbiological analysis. Molecular methods were also used for the identification and characterization of specific pathogens. Statistical analyses were conducted in order to determine the correlations between the different infections and syndromes experienced by the patients.

A total of 149 patients were recruited in the study. However, only 122 agreed to fill in the questionnaires while the other 27 refused to fill in the questionnaires but agreed to provide the samples. Most of the patients 89 (73%) were females. The age of the patients varied between 3 and 78 years old. The mean age of the patients was 39.41 ±12.103 years. The sexual transmission was the most prevalent mode of transmission. However, blood transfusion; care for HIV and AIDS patients and rape were reported as secondary mode of transmission of the virus. The most common opportunistic diseases reported by the patients included sores on the lips (24.6%), sores in the genitals (18%), genital warts (19.7%), mouth thrush (30.3%), vision problems (27.9%), breathing difficulties (23%), tuberculosis (25.4%) and diarrhea (28.9%). Symptoms of sexually transmitted diseases reported by the patients included burning sensation during urination, cream white discharge, itching, syphilis and others.

OP 25

Abstract Number : 220

Heavy metal-resistant, hydrocarbon-degrading bacteria from the lagos and ologe lagoon in nigeria

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Background

This work aimed to isolate bacteria from polluted lagoon sediments that are able to utilize selected polycyclic aromatic hydrocarbons (PAHs) in the presence of heavy metals**.**

Methods

Using a selective, aerobic enrichment procedure with naphthalene, anthracene, phenanthrene, fluorene, fluoranthene, pyrene, chrysene and benz[a]anthracene supplied as sole carbon and energy sources in the presence of a heavy metal mixture (Hg, Cr, Cd, Ni, Pb), 21 hydrocarbon-utilizing heavy metal-resistant bacterial isolates were obtained from sediment samples.

Results

Three of the isolates were selected for further studies owing to their ability to productively catabolize selected hydrocarbons in the presence of the heavy metal mix. They were identified based on morphological and physiological properties as *Pseudomonas* sp. strain K6, *Arthrobacter* sp. strain K14 and *Alcaligenes* sp. strain K10; confirmed by 16S rRNA gene sequence analysis for strains K6 (JQ277727) and K14 (JQ277728). DGGE analysis of sediment samples demonstrated that members of proteobacterial genera such as *Burkholderia* dominated in these sediments. Using naphthalene, anthracene, phenanthrene, fluorene, fluoranthene, pyrene, chrysene and benz[a]anthracene as sole sources of carbon, strains K6, K10 and K14 exhibited in batch cultures (25°C, 200 rpm) specific growth rates and doubling times in a range of about 0.02-1.58h^{-1} and 0.44-36h. The EC_{50} value (growth inhibition) for the 5 heavy metals in the presence of naphthalene, chrysene and benz[a]anthracene was in a range of about 0.8-15mM for the 3 isolates. *Arthrobacter* sp. strain K14 cells were able to bind chromium as was established by scanning and transmission electron microscopy. This is a possible mechanism explaining the removal of the heavy metals by the test organisms from microcosms. The level of heavy metal removal was within a range of 20-80% of the nominal concentration. The presence of Cd, Cr and Pb induced the formation of additional proteins (size range from 28-205 KDa) in cell extracts of all 3 isolates - probably involved in metal binding or detoxification - as was demonstrated by SDS-PAGE.

Conclusions

The 3 strains utilized several PAHs as carbon and energy source in the presence of toxic heavy metals thereby indicating their potential for bioremediation processes in co-contaminated environments.

OP 26

Abstract Number : 286

Characterisation of bradyrhizobia associated with southern African Crotalarieae and Genisteae

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Background

Rhizobia are Gram-negative soil bacteria capable of nodulating and establishing nitrogen-fixing symbioses with legumes. In South Africa, little is known regarding the rhizobial symbionts of indigenous legumes, especially those in the tribes Crotalarieae and Genisteae. Previous work elsewhere has shown that these tribes may be nodulated by rhizobia in the genus *Bradyrhizobium*. Here we investigated the novelty of the bradyrhizobia associated with South African Crotalarieae and Genisteae by making use of a multilocus sequence analysis approach. Our ultimate goal was to determine the placement of the South African isolates within the larger biogeographic framework for this genus of root-nodulating bacteria.

Methods

Twenty two isolates, obtained from *Argyrolobium* (Genisteae), *Pearsonia* and *Leobordea* (Crotalarieae) species collected on the Great Escarpment, were used in this study. The sequences for seven housekeeping genes (i.e., *recA*, *gyrB*, *rpoB*, *dnaK*, *atpD*, *glnII* and 16S rRNA), as well as nodulation gene *nodA* and nitrogen-fixation gene *nifD* were determined. After constructing multiple alignments, the various datasets were subjected to phylogenetic analyses using Maximum Likelihood and Bayesian approaches.

Results

Phylogenetic analyses of the housekeeping genes separated our set of 22 isolates into three clusters. One of these clusters consistently grouped with indigenous Australian isolates that have not yet been described. The second cluster appeared to be related to *Bradyrhizobiumelkanii* and *B. pachyrhizi*, while the third cluster grouped with *B. yuanmingense*. Based on the *nodA* data, the majority of South African isolates examined here represent a novel clade designated Clade XI, with only three of the isolates forming part of the cosmopolitan Clade III. However, the *nifD* isolate clusters did not correspond to those in the *nodA* phylogeny.

Conclusions

The housekeeping gene trees revealed that our set of isolates potentially include one novel species as well as members of *B. elkanii*, *B. pachyrhizi* and *B.yuanmingense*. However, the *nodA* and *nifD* genes support discordant evolutionary patterns, suggesting that the *nod* and *nif* loci are unlinked. Furthermore, most isolates form part of a novel *nodA* clade, indicating that South African bradyrhizobia holds important clues for understanding the evolution of nodulation capabilities in this group of bacteria.

OP 27

Abstract Number : 174

Cloning, expression, purification and characterization of dienelactone hydrolases (TfdEI and TfdEII) from Cupriavidus necator JMP134

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Background

Dienelactone hydrolases (DLH) catalyse the hydrolysis of dienelactone to maleylacetate during the microbial degradation of chloroaromatics via chlorocatechols in many bacteria and fungi. In this study, DLH (TfdEI and TfdEII) distantly located on plasmid pJP4 of *Cupriavidus necator* JMP134 were cloned, purified, characterized and three dimensional structures predicted.

Methods

The TfdE1 (NCBI GeneID:2847423,705bp) and TfdE2 (NCBI GeneID:2847410,708bp) genes were amplified from plasmid pJP4, cloned into pET21b vector and expressed in *E. coli* BL21(DE3) by inducing with 1mM IPTG. Recombinant TfdEI and TfdEII enzymes were purified to about 130 fold by sequentially passing through Ultra-membrane filter centrifuge tubes (MW cut-off 55kDa and 10kDa), anion-exchange QFF column, followed by gel-filtration column

(Sephacryl HR100). The enzyme activity was determined using *cis*-dienelactone as a substrate (ε280=17,000$M^{-1}cm^{-1}$). The three dimensional structures of enzymes was predicted using SWISS-MODEL workspace and the biophysical properties were determined on ExPASy server.

Results:

Both TfdEI and TfdEII (M_r 25kDa) exhibited optimum activity at 37°C and pH 7.0. The enzymes retained approximately 50% of their activity after 1h of incubation at 50°C, and showed high stability against Tween80 and TritonX-100. The TfdEI and TfdEII converted *cis*-dienelactone to maleylacetate at a rate of 0.26 and 0.18 μMs^{-1}, with a K_m value of 87µM and 305µM, respectively. The TfdEI and TfdEII k_{cat}/K_m ratios for *cis*-dienelactone were 0.36 and 0.39 $s^{-1}\mu M^{-1}$, respectively. The presence of metal ions like Ca^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, and Zn^{2+} in the reaction did not affect enzyme activity even though DLH's activity is reported to be metal dependent.

Conclusions:

The k_{cat}/K_m ratios for *cis*-dienelactone show that both enzymes catalyse the reaction with same efficiency even though K_m value differs significantly. The enzymes belong to α/β hydrolase family containing a catalytic triad composed of Cys-His-Asp in the active site as determined from the reported catalytic sites of very closely related DLH's from *Pseudomonus* sp. B13 (PDB:1DIN). Both enzymes share a 41.5% homology at gene level and 19.68% homology at protein level. The reason why *Cupriavidus necator* JMP134 harbours two structurally different and distantly located DLHs still remains unclear at this stage and requires further investigation.

OP 28

Abstract Number :99

Metaviromics of extreme terrestrial habitats: fundamentals and tools

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Bacteriophages play a crucial role in shaping microbial populations in extreme environments, driving carbon turnover and possiblycontributing beneficial genes or functions during lysogenic conversion. In addition to an ecological role, bacteriophages have several potential applications in the modern biotechnology industry and in advancing molecular biology-based research. Due to their highgenetic diversity, enormous abundance, and richness in genetic novelty, phages represent the largest reservoir of unexploredgenetic information in the biosphere. Moreover, phages are rich and powerful tools for the development of genetic systems ingenetically naive bacterial species. In order to tap into this diversity, phage metagenomic analysis, or metaviromics, of the Namibian desert has been conducted. The Namib desert, located along the Atlantic coast of southern Africa, is considered the oldest in the world, and has been hyperarid for the last five million years, with a recorded mean annual rainfall of 25 mm in the last 50 years. Through an annual cycle, surface soil and rock temperatures range from 0°C to over 50°C. The microbial populations in these environments are specifically adapted to these extreme conditions with specialized stress response and tolerance mechanisms.

Samples from several Namib Desert habitats, including gravel plains, hypolithic biomass, dune sand and hypersaline springs have been collected and processed. Samples were subjected to transmission electron microscopy and next-generation dsDNA metagenomic sequencing of the hypolithic phage community is presented. Typically, approximately half of the sequences showed sequence identity to known bacteriophages. Within this category, roughly 85% were identified as *Caudovirales*, with siphoviruses being most abundant, while the remaining 15% was mostly made up of unclassified phages or unclassified viruses. The sequencedata obtained additionally serves as a resource for the identification of biotechnological relevant genes, and for the development of a high throughput culture-independent method for the identification of phage-host pairs.

OP 30

Abstract Number : 124

Cytotoxity of the metabolites produced by Stenocarpella maydis

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Background:

Stenocarpella maydis (Berk.)Sutton, formerly known as *Diplodia maydis* (Berk.)Sacc., is encountered throughout the world as an economically important pathogen of maize. Diplodiosis, a nervous disorder of cattle and sheep, results from the ingestion of maize cobs infected by *S. maydis*. It is characterized by reluctance of the animals to move, standing with a wide-based stance, incoordination, muscle tremors, paralysis and death. The aim of this study was to investigate the cytotoxicity of the three *S. maydis* metabolites, namely the diplodiatoxin, diplonine and dipmatol toxins, on the Neuro-2a, CHO-K1 and MDBK cells.

Methods:

Cytotoxicity of the *S. maydis* metabolites was determined using the real-time cell analyzer (RTCA) xCELLigence and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays following exposure for up to 72 h of the cell lines.

Results:

The xCELLigence and MTT assays demonstrated a concentration-dependent cytotoxic response on the Neuro-2a, CHO-K1 and MDBK cells after they were exposed to the diplodiatoxin and dipmatol toxins. In general, the diplonine toxin did not induce any cytotoxicity to the three cell lines. The diplodiatoxin was the most cytotoxic *S. maydis* metabolite followed by the dipmatol toxin. The diplonine toxin was not cytotoxic.

Conclusion:

Our results have demonstrated that the three cell lines have the potential to be used as suitable in vitro toxicity testing models for the *S. maydis* metabolites.

OP 31

Abstract Number : 239

Are heterotrophic bacteria contributing to the oxidative removal of iron and manganese from borehole water in a biofiltration system?

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Background

The objective of this study was to verify the presence of iron and manganese oxidizing bacteria and metabolically active biofilms in a biofiltration system employed to treat borehole water and to verify the potential contribution of individual microorganisms to the removal of these metals.

Methods

Viable counts for biofilter water and matrix samples were established by employing R2A medium for heterotrophic bacteria and MSVP medium plus either added iron or manganese to target Fe(II) and Mn(II) oxidizing bacteria. Presumptive Fe(II) and Mn(II) oxidizing isolates were selected and characterized by light and electron microscopy, physiological tests, MALDI-TOF MS and 16S rRNA gene sequence analysis. Colorimetric assays using ferrozine and leucoberbelin blue as well as XRD analysis were employed to confirm Fe(II) and Mn(II) oxidation for selected isolates. The presence of metabolically active biofilms was verified by CLSM analysis of biofiltration matrix samples.

Results

Biofilter water and matrix counts were $10^6/10^5$cfu.ml and $10^7/10^7$cfu.g for Mn(II)/Fe(II) oxidizers. A Gram and oxidase-negative, non-motile, plump rod-shaped isolate able to form biofilms was identified as a member of the genus *Acinetobacter* as confirmed by MALDI-TOF MS and 16S rRNA gene sequence analysis. XRD analysis of crystals formed in batch cultures containing MSVP plus added iron or manganese confirmed the ability of the strain *Acinetobacter* sp. LB1 to oxidize both Fe(II) and Mn(II). In the presence of active *Acinetobacter* sp. LB1 cells, the rate of Mn(II) oxidation was significantly higher than that observed for abiotic controls. However, in the presence of active cells of *Acinetobacter* sp. LB1 at neutral pH, the rate of Fe (II) oxidation was in the same range as that of abiotic controls. CLSM analysis of matrix samples stained with CTC confirmed the presence of metabolically active biofilms.

Conclusion

Heterotrophic bacteria present within an established biofiltration system in which metabolically active biofilms are formed can contribute to the oxidative removal of Mn(II) at neutral pH thereby demonstrating that species within the

proteobacterial genus *Acinetobacter* are potentially involved in the geochemical cycling of manganese. However, at neutral pH the removal of iron by microbial activity appears to be indistinguishable from abiotic iron oxidation.

OP 32

Abstract Number : 149

Characterisation of β-lactamase encoding Klebsiella pneumoniae subsp. Pneumonia

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1 - University of Pretoria 2 - University of Pretoria/NHLS 3 - Medical Research Council

Background:

Klebsiella pneumoniae is a common commensal of the human body as well as an important contributor to both community-acquired and nosocomial infections in immune-compromised hosts, often responsible for urinary, blood and respiratory infections. Branded as one of six bloodstream pathogens of significance in South African public hospitals and an important contributor to in-hospital mortality, the pathogen has acquired resistance towards antibiotics, such as β-lactams, with up to 75% of *K. pneumoniae* isolates tested being extended-spectrum β-lactamase (ESBL) producers in 2009. Treatment has been further complicated by the sporadic emergence of carbapenem resistant *K. pneumoniae*, which has rapidly gained global distribution, since the introduction of carbapenems. Presence of resistance genes in antibiotic resistant isolates could exist through natural selection of resistant clonal lineages or acquisition through mobile genetic elements. The aim of the study encompassed the molecular detection of Ambler molecular classes A, B and D β-lactamase genes in ESBL producing *K. pneumoniae* subsp. *pneumoniae* as well as genetic discrimination of strains circulating within the clinical setting.

Methods:

One hundred unrepeated ESBL-producing *K. pneumoniae* subsp *pneumoniae* isolates were collected from the National Health Laboratory Service after routine identification and antibiotic susceptibility testing using the Vitek®2 Automated system (bioMérieux, France). Beta-lactamase genes were detected utilising conventional multiplex-polymerase chain reaction assays and genetic discrimination determined by pulsed-field gel electrophoresis (PFGE) utilising the Rotaphor VI system (Biometra, Germany) as well as by multilocus sequence typing.

Results

All three ESBL associated genes, namely SHV (100%), CTX-M (94%) and TEM (81%), were detected as well as additional β-lactamase encoding genes, particularly OXA-1-like (95%) and OXA-48-like (94%) genes. All the strains were typeable by PFGE using *Xba*I, which discerned multiple pulsotypes.

Discussion/Conclusion

The β-lactamase encoding genes CTX-M, SHV, TEM, OXA-1-like and OXA-48-like were all highly prevalent within the *K. pneumoniae* clinical isolates, the majority encoding four or all five genes. Genetic typing identified several pulsotypes with a few highly related clusters. Genetic typing of *K. pneumoniae* is useful in outbreak investigations, understanding transmission, managing hospital infections and for epidemiological referencing, whereas the detection of β-lactamase encoding genes serves as an indicator of current genetic resistance profiles.

OP 33

Abstract Number : 153

Molecular detection of Mycobacterium tuberculosis Complex using Step-One plus real-time assay and Genotype MTBDR plus assay on formalin fixed paraffin embedded pleural biopsy specimens

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Background

Tuberculosis (TB) remains a leading cause of morbidity and mortality in HIV-infected patients. This disease often presents a diagnostic challenge because of its diverse clinical manifestation and low yield of acid-fast bacilli especially in tissue specimens. Although, culture offers better sensitivity, the incubation time may take up to 6 weeks due to the slow growth of mycobacterium, hence the need for rapid and sensitive detection of *Mycobacterium tuberculosis* especially in

tissue specimens. Histopathology analysis is considered the gold standard for tissue samples. The aim of this study was to compare the performance of StepOne™*plus* real-time PCR assay against Genotype MTBDR *plus* V.2 assay for the detection of *M. tuberculosis* complex in pleural biopsy samples.

Methods

100 pleural biopsy specimens were obtained from 56-suspected cases of tuberculosis and 44 non-tuberculosis cases. Pleural specimens were collected over a period of six months, and analysed by StepOne™*plus* real-time PCR (PrimeMix MTB universal assay) and Genotype MTBDR plusV.2 assay.

Results

Out of 100 specimens, 56 were TB positive on histopathology and ZN staining. The remaining 44 specimens were negative. The overall status reaction of StepOne™*plus* real-time PCR assay on suspected tuberculosis cases were found to be TB positive in 33 cases while Genotype MTBDR *plus* assay were positive in 15 cases. Of the remaining 44 non-TB specimens, three cases were positive by StepOne™*plus* real-time PCR assay whereas Genotype MTBDR *plus* assay did not detect *M. tuberculosis* complex. StepOne™*plus* real-time PCR assay showed a better sensitivity as compared to Genotype MTBDR *plus* assay. Our results demonstrated that real-time PCR assay is able to detect *M. tuberculosis* complex in FFPE tissue specimens, and this could be used to increase diagnostic accuracy in patients who have perplexing diagnostic problems associated with a granulomatous tissue response

Conclusion

Real-time PCR assay is simple, rapid and useful for early detection of tuberculosis in tissue specimens where morphologic features are suggestive but not confirmatory. This assay can be used as a stand alone diagnostic tool especially in samples where culture is deemed unsuitable, and this might assist physician with early medical intervention and prevention of spread of the infection

OP 34

Abstract Number : 157

Impact of the GenotypeMTBDRplus assay on case detection and MDR identification in the Free State, South Africa, 2012.

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Introduction

GenoType MTBDRplus is a molecular line probe assay (LPA) used for detection of *Mycobacterium tuberculosis* (MTB) and resistance to the drugs rifampicin (RIF) and isoniazid (INH). Resistance to both these drugs is classified as multidrug resistance (MDR) and indicates dramatic changes in treatment for which early detection is vital. This assay has been used since 2010 in the Free State NHLS laboratories to increase the case finding rates and early detection of MDR.

Aim

To retrospectively determine the impact of the LPA test on case finding and early identification of drug resistance in the Free State province in 2012 before the use of the GeneXpert .

Methods

Data was collected using s*tructured query language* for sputum specimens received from suspected cases of pulmonary tuberculosis (PMTB) or those currently on treatment for PMTB. These specimens were tested with one or more of the followingmethods: smear microscopy using auramine staining (AUR), direct LPA, Mycobacterial Growth Indicator Tube (MGIT) culture and MGIT drug susceptibility testing (DST) at the NHLS laboratory in Bloemfontein. Data was analysed using Excel software.

Results

Preliminary data analyses are presented for 9896 sputum specimens that did not contain any visible blood and were screened with at least AUR. Of the specimens 732/9896 tested AUR+ and 9164 AUR-. A further 641 AUR- specimens were positive using MGIT and LPA testing. All of the AUR-/MGIT+ specimens tested with LPA gave congruent results (sensitivity, 100%). A total of 8420 AUR- specimens were negative with all three methods (100% correlation).

Direct LPA testing provided rapid RIF and INH susceptibility results for 575 (sensitivity, 100%) AUR+ specimens. Twelve AUR+ specimens that were initially LPA+ and MGIT- were later negative for both methods. This can be attributed to the ability of LPA to detect DNA from non-viable bacteria.

Based on LPA testing the prevalence of MDRTB was determined as 8.4% of the specimens analysed.

Conclusion

For sputum testing, the sensitivity of MTBDRplus correlates to the bacillary load of the specimen. Our results suggest that the MTBDRplus test provides rapid results for cases with MDRTB and contributes to timeous treatment.

OP 35

Abstract Number : 158

Laboratory based surveillance of shigella, its serotype and resistance patterns in johannesburg south africa

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Infectious diarrhoea, like shigellosis, causes considerable morbidity and mortality especially in young children, immune compromised and HIV/AIDS individuals. It is endemic in developing countries and in Sub-Saharan Africa, including South Africa, a region where unique geographic, economic, political, sociocultural, and personal factors interact to create distinctive continuing challenges to prevention and control, diarrheal disease remains a leading cause of mortality and morbidity. Our study was undertaken to establish incidences of *Shigella,* its serotype and resistant pattern of isolates from human faeces from residence of Johannesburg, South Africa.

All stools received between January to April 2013 from private health care system were cultured on standard media and *Shigella* was confirmed by standard biochemical reactions and serological method. Antibiotic sensitivity test was determined by agar diffusion method.

A total of 11009 samples from age range of 22days to 94 years yielded 110 *Shigella* species, of which 47 (43%) were *S. flexneri,* 61 (55%) were *S. sonnei* and 1 (1%) was *S.dysenteriae* and 1 (1 %) was *S. boydii*. Majority of patients were children between <1 to 5 years old 76 (69%) followed by those between 6 to 10 years 13(12%). From the four species *S. sonnei* was confirmed in 52 cases (59%) and *S. flexneri* in 36 cases (41%) in children up to 10years. An overall of 53 (48%) males and 57 (52%) females were infected.

One hundred percent susceptibility to ciprofloxacin, and ceftriaxone was noted. There were high levels of resistance to other antimicrobials: Co-trimoxazole (83%), tetracycline (72%), and ampicillin (26%). From 110 strains, 96 (87%) were resistant to one or more drugs while 14 (13%) were fully susceptible.

Our results show the presence of shigellosis and *S. sonnei* followed by *S. flexneri* more prevalent as aetiology. Ceftriaxone and ciprofloxacin are effective drugs against all four *Shigella* species.

OP 36

Abstract Number : 279

Amplified growth during co-culture with Mycobacterium tuberculosis strains suggests in vitro trans-complementation

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Introduction:

Mixed infections are a significant contributor to disease in KwaZulu-Natal (Andrews *et al*., 2008) and occur when two strains with different phenotypes (i.e. growth kinetics and drug resistance profiles) co-exist in the same individual (van Rie *et al*., 2005). Drug-resistant strains often demonstrate decreased competitive ability against susceptible strains in the absence of the drug (Gagneux *et al*., 2006). Microbes may display 'fitness costs' in various forms, including: decreased transmission rates (Randall *et al*., 2008) and growth *in vivo* (Majcherczyk *et al*., 2008) or *in vitro* (Andersson, 2006), enhanced clearance rates (Gustafsson *et al*., 2003) or reduced invasiveness (Fernebro *et al*., 2008). The aim of this study was to determine the relative fitness of resistant *Mycobacterium tuberculosis* strains during competition with susceptible strains in broth culture.

Methods:

A total of 15 clinical strains representing the F15/LAM4/KZN, Beijing, F11 and F28 genotype families were included. The competitiveness of multidrug- (MDR) or extensively drug-resistant (XDR) and susceptible (S) pairs of isolates of the same strain family was assessed by competition assays using isoniazid, rifampicin, kanamycin and ofloxacin. Relative fitness indices (*W*) were calculated as follows: $W = \ln (R_f/R_i) \div \ln (S_f/S_i)$. The fitness of the susceptible strain was taken as 1.

Results:

An "overgrowth" of susceptible strains was observed in co-culture, with the exception of one pair (V9124 + X162). This was statistically significant for several pairs. All the F15/LAM4/KZN resistant strains had higher fitness indices paired with V9124 (S) than V4207 (S), with significant differences for the KZN605 (XDR) pairs ($p = 0.017$). Similarly, the Beijing MDR strains had higher fitness indices with B910 (S) than B1528 (S). Co-culture growth rates were consistently higher than independent growth rates in 13 of 14 competition pairs.

Discussion/Conclusions:

Pairwise competition revealed heterogeneous fitness profiles for resistant strains, suggesting a manner of strain dependence. The persistence of resistant strains at low CFUs could consequently support the acquisition of additional drug resistance-conferring mutations and/or the evolution of compensatory mechanisms. Moreover, amplified growth in co-culture may be suggestive of *in vivo trans*-complementation, whereby either strain may be promoting the growth of the other.

OP 37

Abstract Number : 213

Is the antimicrobial susceptibility pattern of neisseria gonorrhoeae isolated from pretoria, south africa changing?

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Background

Diseases caused by *Neisseria gonorrhoeae* have been successfully treated with antibiotics for the past 70 years. However, a disturbing trend worldwide is the increasing prevalence of strains with resistance to inexpensive and widely available antibiotics. The antimicrobial resistance of *N. gonorrhoeae* is constantly changing. As a consequence, there is a need for area-specific research to strengthen control programmes. Periodic monitoring of the prevalence of *N. gonorrhoeae* and its susceptibility profiles can provide essential information for updating local syndromic management guidelines and early detection of the emergence of drug resistance. The aim of this study was to determine the antimicrobial susceptibility and minimum inhibitory concentration (MIC) of *N. gonorrhoeae* clinical isolates to eight antibiotics: penicillin, cefoxitin, ceftriaxone, cefpodoxime, tetracycline, ciprofloxacin, ofloxacin and spectinomycin.

Methods

Endourethral and urine specimens (100) were collected from adult men with symptoms of urethritis. Vaginal swabs (100) were collected from women with symptoms of vaginal discharge attending two healthcare facilities, namely the Antiretroviral and Sexually Transmitted Infections clinics. These specimens were plated onto enriched media for isolation of *N. gonorrhoeae*. Antimicrobial susceptibility of the bacteria was performed using the disc diffusion and E-test methods.

Results

A prevalence of 5% (10/200) was obtained by culture method. Over 50% of the culture positive isolate had a MIC (0.016 µg/ml to 0.023 µg/ml) within the susceptible range for the recommended first line treatment, cefotaxime. Two isolates (20%) had slightly elevated MIC values (0.125 µg/ml and 0.75 µg/ml) for cefotaxime. Three isolates (30%) was resistant to ciprofloxacin and ofloxacin.

Conclusion

This study suggests that cefotaxime is still effective as a first line drug for the treatment of *N. gonorrhoeae* in the public healthcare setting in Pretoria. However, there have been reports of *N. gonorrhoeae* isolates that is resistant to

ceftriaxone and cefixime, third-generation cephalosporins. Hence, there is a need to constantly monitor the antimicrobial susceptibility pattern of *N. gonorrhoeae* in order to detect the emergence of resistance to this drug. Furthermore, clinicians are advised to be vigilant about cases of cefotaxime treatment failure.

OP 38

Abstract Number : 242

The influence of L-carnitine on oxidative stress resistance in the yeast Saccharomyces cerevisiae requires the transcription factor Yap1p and phosphatidylcholine homeostasis

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Background

L-carnitine acts as a shuttling molecule for activated acyl residues in eukaryotic cells. In yeast, specifically, these residues are generated in either the peroxisomes or the cytoplasm and needs to be transferred to the mitochondria to access the tricarboxylic acid cycle. The transfer of activated acetyl-residues from CoA to carnitine is catalysed by carnitine acetyl transferases (CATs). The generated acetyl-carnitine is subsequently transported to the mitochondria. This system is referred to as the carnitine shuttle and is conserved in its function from yeast to humans. In higher eukaryotes, however, evidence suggests that carnitine has additional biological functions that are not directly linked to the shuttle activity. In agreement, we have recently shown that carnitine protects yeast from oxidative and weak acid stress while increasing the damaging impact of DTT on yeast cells. These outcomes were, furthermore, shown to be independent of shuttle activity and to require genetic mediation.

Methods

To investigate the effect of carnitine on a genetic level and identify genetic mediators, two separate screens where used. Firstly, microarray analysis was performed to determine the effect of carnitine on global transcription. The second approach used a high-throughput chemigenetic screen of the yeast deletion collection. Genes identified as potential targets where investigated using standard genetic, molecular and phenotypic analysis.

Results

The outcome of high-throughput screens identified specific genetic targets involved in carnitine's influence on oxidative stress resistance. Mitochondrial homeostasis is specifically shown to be important for the establishment of the carnitine related phenotypes. Furthermore, the activities of Cho2p and Opi3p, which catalyse successive reactions in the synthesis of phosphatidylcholine from phosphatidylethanolamine, are required for both protective and detrimental carnitine related phenotypes. In addition, the data indicates that the transcription factor, Yap1p, is central to the impact of carnitine.

Conclusions

The conserved interplay between eukaryotic metabolism and cellular stress responses is a topic of considerable research importance. By using yeast as a genetic model system and focusing on the central metabolite, L-carnitine, the outcome of this study contributes uniquely to the understanding this dynamic interface.

OP 39

Abstract Number : 244

An investigation into the synergistic action of exo-type and endo-type cellulases on the hydrolysis of complex substrates

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Background

With the depletion of fossil fuels and the environmental issues that are associated with crude oil usage, extensive research has been initiated into the bioconversion of renewable cellulosic biomass to bio-ethanol. The biphasic crystalline structure of cellulose makes enzymatic hydrolysis very challenging. Enhancing cellulose hydrolysis through enzyme synergy has become important in achieving complete degradation of complex lignocellulosic substrates.

Methods

This study investigated the synergistic interaction between three classes of cellulolytic enzymes, namely cellobiohydrolases (CBH1 from *Talaromyces emersonii* and CBHII from *Chrysosporium lucknowense*), endoglucanases (EGI from *Aspergillus fumigates* and EGII from *Trichoderma reesei.*) and β-glucosidase (BGL from *Saccharomycopsis fibuligera*) on the degradation of Avicel. The protein was kept at a constant loading of 50 ug/ml.

Results

All enzyme combinations displayed synergy on Avicel after 24, 48 and 72 hours. Smaller protein fractions of EG in combination with CBH resulted in higher yields of sugar as well as synergy. Maximum reducing sugar yield (1.45g/ml) was found with the enzyme combination CBHII: EGI in a protein ratio of 75:25 % and highest degree of synergy (1.91) was found with the enzyme combination CBHI: EGII in a protein ratio of 75:25 % on Avicel. These results were obtained after 72 hours.

Conclusions

The results from this study showed that in order to achieve efficient cellulose degradation, the enzymes needed to act in a co-operative manner. Enzyme mixtures giving rise to the highest yield of sugar did not always correspond to enzyme mixtures generating the highest synergy. Synergy increased with time, however at longer digestion times, hydrolysis slowed down which may have resulted from a decrease in substrate accessibility. The results also demonstrated the importance of cellobiohydrolases in synergy studies as they are the key enzymes required for effective and optimal crystalline cellulose degradation.

Further studies will investigate the synergistic action on the degradation of steam-exploded bagasse and paper waste.

OP 40

Abstract Number : 248

An overview comparison of the influences on the Crocodile River water by tributary rivers that flow through areas polluted by mining-related pollution

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Background:

The Crocodile River flows from GP near Krugersdorp and Johannesburg, through part of the NW and finally joins the Limpopo River in the Limpopo province. It has several tributaries which flow through areas known to be polluted by mining-related wastes. The extent and impact of these tributaries on the quality of the Crocodile River water is unknown. The commercial farmers are the major water users in the Crocodile River catchment. The first aim of the study was to measure the levels of mining-related pollutants in selected Crocodile River tributaries. The second aim was to establish the correlation between the bacterial diversity in the water and the prevailing biochemical characteristics.

Methods:

Water samples were collected from eight tributary rivers monthly to obtain a representation of the final water quality that will enter the Crocodile River. A sample upstream and downstream the Crocodile River was also obtained monthly. Levels of heavy metals in the samples were determined on the inductively coupled plasma-mass spectrometry (ICP-MS) except iron and aluminium, whose levels were determined using a spectrophotometer. Biochemical oxygen demand (BOD), dissolved oxygen (DO) and chemical oxygen demand (COD) were measured following the Hach™ instruments manufacture guidelines. Total heterotrophic counts of the samples were estimated using plate count technique. Bacterial diversity in samples was measured from the total metagenomic DNA using PCR-DGGE, based on 16S rRNA gene sequences.

Results:

Prelimary results show that all rivers have high BOD measurements (ranging between 203.25 - 340.50 BOD). The Hennops River showed the highest COD measurement (259.57 mg/L COD) of all tributaries while Magalies (10.44 mg/L) and Muldersdrift (9.93 mg/L) Rivers had the highest DO content. The Bloubankspruit River had the highest aluminium content (0.88 mg/L Al^{3+}).

Conclusion:

It can be concluded that the tributaries have high levels of organic pollution. This pollution will serve as carbon and energy source for utilization by aerobic microorganisms. This will mean poor microbiological quality of the Crocodile River due to polluted water flowing in from the tributaries. However, data from molecular analysis will be used to support this claim and increase confidence in conclusions drawn from the study.

OP 41

Abstract Number : 82

Specific wine yeast interactions revealed by metabolomic and proteomic approaches

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1 - Stellenbosch University

During wine fermentation, *Saccharomyces cerevisiae* interacts with other yeast species. This interaction impacts on wine composition, especially when a non-*Saccharomyces* yeast is co-inoculated at a high cell density. The results of this interaction are fairly well documented, but the molecular mechanisms thereof are mostly unknown. The progress of systems biology techniques and the recent availability of genetic data for some non-*Saccharomyces* allow such investigation to be considered. In this study, *Metschnikowia pulcherrima* and *Lachancea thermotolerans*, two commonly occurring wine yeasts species were co-inoculated with *S. cerevisiae* in synthetic grape juice. Fermentation progress and population dynamics were monitored throughout fermentation and the concentrations of primary and secondary metabolites were determined using enzymatic assays, GC-FID and GC-MS. The experiment was repeated and the extracellular proteins were harvested and identified using mass fingerprinting. In all experiments, pure cultures were inoculated as controls. *M. pulcherrima* only delayed the growth of *S. cerevisiae* by a few days and the population declined as soon as that of *S. cerevisiae* increased. Interestingly, when *M. pulcherrima* was present with *S. cerevisiae*, high concentrations of medium chain fatty acids and a killer toxin secreted by *S. cerevisiae* were found in the medium. A correlation with the early decline of *M. pulcherrima*'s population could be hypothesized. *L. thermotolerans* impacted much more severely on the overall fermentation kinetics and both yeast populations remained similar in density. When the pure *S. cerevisiae*'s fermentation was compared to the co-fermentation with *L. thermotolerans*, the absence of glycolysis-related proteins of *S. cerevisiae* was noticed in the supernatant of the co-fermentation. This could correlate with a weaker general metabolism and/or with the delayed autolysis, both as a result of the slow fermentation rate. Moreover, *L. thermotolerans* impacted on the medium composition by producing specific secondary metabolites and by lowering the amount of acetic acid produced. The results confirm that interactions occur between the yeasts and that the nature of these interactions depends on the yeast species present. The antagonistic activity of *S. cerevisiae* against *M. pulcherrima* is currently being further investigated as well as the overall impact of *L. thermotolerans* on *S. cerevisiae*'s metabolic activity.

OP 42

Abstract Number : 108

Assessment of synteny in the genomes of Fusarium circinatum and Fusarium verticillioides

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Background:

Co-localization of genetic loci, referred to as synteny, represents an important feature at the genomic level, in understanding the evolution of a species. For example, breaks in synteny can reflect important evolutionary events within and between species. In this study, we assessed the level of synteny between the genomes of two phylogenetically closely related fungi, *Fusarium circinatum* and *Fusarium verticillioides*.

Methods:

Previously generated contigs in the *F. circinatum* genome were assembled into scaffolds using SOLID mate-pair sequencing data and the SSPACE genome scaffolding computer software. These scaffolds were then orientated and ordered into chromosomes based on BLAST searches against a local database of the *F. verticillioides* genome. The online Genome Synteny Viewer was then used to generate synteny maps between the chromosomes of the two species.

Results:

A total of 583 scaffolds were assembled for the *F. circinatum* genome, with an average scaffold size of 75 000 base pairs (bp). The smallest scaffold was 500 bp and the largest was 1 213 886 bp in size. According to the N50 assembly statistic, more than 50% of these contigs were 363 633bp in length or longer. Although a high level of synteny was detected between the eleven chromosomes of the two fungi, numerous breakpoints were also detected. These arose from small chromosomal rearrangements, inversions mainly at the terminal ends of chromosomes and indels that ranged in size from a few base pairs up to 125 000bp.

Conclusions:

The use of mate-pair sequencing data has greatly improved the published *F. circinatum* genome assembly. Comparisons have revealed extensive synteny between the two species of *Fusarium* considered here. The syntenic breakpoints observed also suggest that the evolution of these two taxa, involved numerous changes at the chromosomal level. Further analyses of these breakpoints, especially where they are caused by large and apparently unique insertions, will allow for a more detailed understanding of the forces that drive speciation in these and other *Fusarium* species.

OP 43

Abstract Number : 289

Metabolomic and proteomic characterisation of hybrid yeasts used for varietal Sauvignon blanc wine production

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Background.

The locally produced commercial yeast i.e. VIN 7 is a known producer of aromatic Sauvignon blanc wine, due to its ability to release bound aroma-inactive thiols and esters (metabolites) during fermentation. However, VIN 7 has other undesirable characteristics eg. volatile acidity (VA) formation which could have a commercial impact. Previous research has shown that yeast enzymes (proteins) are involved in the release of these metabolites during fermentation. This necessitated an in-depth study into wine yeast protein expression and metabolite release and its effect on the organoleptic quality of Sauvignon blanc wine if the South African wine industry is to remain globally competitive. Therefore, a trial utilising hybrids conserved in the ARC Infruitec-Nietvoorbij culture collection was initiated.

Methods.

Hybrid yeast strains was trialled against the top local and imported commercial 'thiol-releasing' wine yeast (TRWY) i.e. VIN 7 and VIN 13 (Anchor Bio-Technologies), Zymaflore X 5 and VL 3 (Laffort Oenologie) and Fermicru 4F9 (DSM Oenology) in small-scale Sauvignon blanc wine production during the 2013 harvest. Final wines were subjected to chemical, sensory and gas chromatography coupled to a flame ionisation detector (GC-FID) analyses. Fermenting yeasts were also subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE).

Results.

Most hybrid yeast strains produced wine with chemical parameters equal to, or better than that produced with the references, especially concerning VA production. Sensorial, VIN 7 and VL 3 was the only commercial TRWY that produced wine with more vegetative than tropical notes, whilst the hybrid yeast strain i.e. NT 07/1 overall produced wine with the most tropical notes. However, the VIN 7 produced wines had the highest levels of esters (metabolites which imparts fruitiness). Various fermenting yeasts showed similar protein expression patterns following SDS PAGE.

Conclusions.

The choice of the most promising hybrid yeast/s will be based on the organoleptic quality of Sauvignon blanc wine.

OP 44

Abstract Number : 34

The isolation and genetic characterisation of a novel South African Phthorimaea operculella granulovirus

(PhopGV)

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South Africa is the 31st largest producer of potatoes in the world with a harvest of R5.3 billion per annum. The potato tuber moth (PTM), *Phthorimaeaoperculella* (Zeller) is a destructive pest causing extensive damage in South Africa and internationally. This industry typically relies upon the use of chemical insecticides to control the pest but with increased global pressure to restrict the use of these insecticides, alternative control strategies are required. A baculovirus biopesticide specific to this pest may contribute to crop protection and the safety of food production in South Africa.

A laboratory culture of PTM was maintained at 27°C with a 12:12 light:dark cycle. Diseased larvae were collected, and the virus isolated and purified using a glycerol gradient protocol. Samples were examined by transmission electron microscopy (TEM) for baculovirus occlusion bodies (OBs).

Genomic DNA was extracted from purified OBs using a CTAB extraction protocol and used for PCR amplification of *granulin* and *ecdysteroid UDP-glucosyltransferase* (*egt*) genes, and single restriction endonuclease (REN) digestion. Amplified products were sequenced to identify the virus and to allow phylogenetic analysis to be performed. DNA profiles were compared to those of known *Phthorimaeaoperculella* granulovirus (*Phop*GV) isolates.

TEM showed the presence of granulovirus like particles with an average size of 355 nm by 188 nm. Alignment of *granulin* and *egt* gene sequences identified the virus as *Phop*GV, and showed similarities of 100% and 93% respectively to the reference isolate 1346 (NC_004062.1). The DNA profile obtained by REN analysis contained most major bands observed in those of other *Phop*GV isolates, but also submolar bands present in profiles of strains isolated in Chile and Ecuador.

A phylogenetic analysis using the *egt* sequence data from a range of *Phop*GV strains grouped the South African isolate with *Phop*GV isolates from Ecuador, Chile, Indonesia and Kenya.

The discovery of this virus may lead to the development of a new biopesticide for control of PTM in South Africa.

OP 45

Abstract Number : 51

RNA packaging by Helicoverpa armigera stunt virus: understanding the molecular mechanisms by which RNA viruses packaged their genomes

Mr. N None ,Mr. N None ,Mr. N None ,Ms. R Dorrington

The tetraviruses are small, positive sense single-stranded RNA viruses with particles exhibiting a unique *T*=4 icosahedral symmetry. They are the only RNA viruses that exclusively infect insects and their host range is confined to the Order Lepidoptera. *Helicoverpa armigera* stunt virus (HaSV) is an omegatetravirus belonging to the *Alphatetraviridae* family. These viruses have a bipartite genome with RNA1 encoding the viral replicase. RNA2 encodes the capsid protein precursor (CP) as well as a smaller, overlapping open reading frame encoding a predicted protein (p17), which is expressed at low levels in the midgut cells of HaSV- larvae and is packaged by wild-type virus particles. The virus capsid comprises 240 copies of the CP, which undergoes autoproteolytic cleavage during maturation and each particle encapsidates both genomic RNAs. The structure and assembly of tetravirus particles has been well studied, but the mechanism that results in the packaging of both genomic RNAs is yet to be elucidated. The aim of this study is to investigate the packaging of vRNAs in the absence of viral replication using a recombinant baculovirus expression system expressing HaSV RNA2, to produce virus-like particles (VLPs) in *Spodoptera frugiperda* cells. RNA extracted from these VLPs was analysed using RT-PCR and northern analysis to characterise the packaged RNAs. The results show that sequences present in the 5' and 3' UTRs of RNA2 are not required for packaging and that the VLPs potentially exclude non-viral, baculoviral mRNAs. The function of p17 in directing the encapsidation is still unclear.

OP 46

Abstract Number : 67

Comparison of stacked mismatched ACMV AC1/4:AC2?3 hairpin RNA silencing constructs for resistance against *African cassava mosaic virus*

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1 - Wits

Background

Cassava (*Manihot esculenta* Crantz) accounts for up to 60% of the daily calorie intake in sub-Saharan Africa. However, a major constraint to cassava cultivation is the 30-50% yield loss due to cassava mosaic disease (CMD). CMD is caused by several circular ssDNA cassava begomoviruses (CBVs), including *African cassava mosaic virus* (ACMV); *East African cassava mosaic virus* (EACMV) and *South African cassava mosaic virus* (SACMV), found to be endemic to South Africa. Current strategies for obtaining resistance to CMD are through genetic engineering based on RNA silencing induction via transgenic expression of virus-derived hairpins or inverted repeats (IR) to generate siRNAs that target homologous viral sequences for degradation. The IR fragments are separated by a spliceble intron required for correct folding of hairpin. The aim of the study was to develop an improved method for generation of SACMV resistant transgenic cassava targeting the movement protein (BC1 ORF) on DNA-B.

Methods and Results

A mismatched BC1 IR was constructed, where the sense arm was designed to contain C-T mutations to inhibit the formation of cruciform structures associated with IR sequences and therefore aid in correct folding of the hairpin. Cassava friable embryogenic callus (FEC) from model cassava cultivar, cv.60444, and South African landrace, T200, were transformed with a RNA silencing construct derived from a selected sequence within the BC1 ORF of SACMV. FEC was co-cultivated with *Agrobacterium* LBA4404 transformed with pCambia 1305.1 harboring the mismatched construct. Here we report selection of regenerated cv.60444 lines transformed with a SACMV BC1 mismatched construct and agro-infected with SACMV infectious clones for virus challenge. Leaf material was collected and symptom severity scored at 12, 32, 55 and 67 days post infection (dpi). Of 17 lines screened, eight had significantly lower severity phenotypes compared to infected non-transgenic controls.

Conclusion

Molecular characterization (viral load using Real-Time PCR; siRNAs and transgene expression) of transgenic and untransformed controls will be completed in order to confirm virus resistance status in these lines.

OP 47

Abstract Number : 127

Investigating the role of non-structural protein, p130 in the viral life cycle of Providence virus (Tetraviridae:Carmotetraviridae)

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Tetraviruses are a group of small, single-stranded, positive-sense RNA viruses that exclusively infect lepidopteran insects. Tetraviruses have non-enveloped capsids that display T=4 icosahedral architecture. The capsid, derived from 240 copies of a single capsid protein precursor, undergoes autoproteolytic cleavage to form large and small subunits during maturation. Tetraviruses exist as single-stranded (ss) and positive-sense (+ve) viral RNA particles. Providence virus (PrV) is the only member of Carmotetraviridae family and is unique among the tetraviruses in that it is able to replicate in cell culture and thus offers opportunities to study the replication biology of tetraviruses. PrV is also unique among the tetraviruses due to the relatedness of its replicase with those of positive-sense RNA plant viruses belonging to the Tombusviridae and Umbraviridae families. In addition to the viral replicase and capsid protein precursor, which are present in all tetraviruses, the PrV genome encodes a unique ORF (designated p130), with an unknown function. Sequence analysis of p130 revealed a putative 2A-like processing sequence which mediates the production of two translation products from one ORF. This involves a ribosome "skipping" effect with the hydrolysis of the ester bond between the nascent peptide and glycyl-tRNA within the ribosome. In addition, we have identified a putative RNA binding domain in the p130 coding sequence that may be functionally important for PrV replication. This study aimed to determine the function of p130 in the viral life cycle. We show that the protein is expressed in PrV-infected cells and also report the results of subcellular localisation and co-immunoprecipitation studies. These results lead us to hypothesise that p130 may function as an RNA chaperone protein that facilitates the initiation of virus replication in infected cells.

OP 48

Abstract Number : 198

Identification of antigen-specific serological cross-reactivity among survivors of Crimean-Congo Haemorrhagic Fever

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Background

Crimean-Congo haemorrhagic fever virus (CCHFV) is a member of the *Nairovirus* genus belonging to the family *Bunyaviridae*, which consists of diverse RNA viruses. CCHFV has the propensity to cause nosocomial infections with a high fatality rate and is endemic in South Africa. Handling of the virus requires biosafety level 4 (BSL-4) conditions, which limits diagnostic capacity. Advances in molecular techniques have allowed preparation of safe recombinant antigens that are useful in diagnosis and serosurveillance of CCHFV. The purpose of this study was to examine the global nucleic acid and amino acid diversity between isolates worldwide; clone and express a recombinant CCHFV nucleoprotein (NP) from a southern African CCHFV and distantly related Greek CCHFV strain and determine the antigenic cross-reactivity between the two isolates.

Methods

Phylogenetic analyses based on NP gene of 45 isolates was performed. Nucleotide sequence diversity and amino acid diversity between groups, within groups and pairwise distances were calculated. A previously expressed codon optimized NP from a South African isolate, SPU 415/85 was subcloned into pColdTF vector and was expressed in a bacterial system. Similarly, the gene encoding the NP of a Greek isolate AP92 was codon optimized and expressed in *Escherichia coli* host cells. Recombinant NP were used to develop in house ELISA to detect IgG antibody against CCHFV in South African patients who survived infection.

Results

Phylogenetic analyses using nucleotide and amino acid sequences of the NP revealed six different groups. The most diverse strain, AP92, displayed the greatest amino acid difference with SPU415/85 (8.7%). Both proteins were expressed with the aid of a chaperone and were purified from the soluble phase. A total of 14/14 sera reacted with the South African recombinant NP and 13/14 reacted with the Greek recombinant NP. The cross reactivity suggests the presence of highly conserved epitopes.

Conclusion

Phylogenetic analyses reveal a high genetic diversity and lower amino acid diversity which suggested synonymous changes in nucleotides, resulting in fewer differences at protein level. The serological cross-reactivity of the two NP antigens suggests that recombinant antigens prepared from geographically specific CCHFV strains will have diagnostic and epidemiological applications worldwide.

OP 49

Abstract Number : 252

Identification of linear b-cell epitopes in the capsid, ns4a and domain iii region in the e glycoprotein of yellow fever virus

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1 - UFS 2 - None Yet

Background:

Yellow fever virus (YFV) is a mosquito-borne virus that causes viral hemorrhagic fever in tropical parts of both Africa and South America. The virus has re-emerged and has become a public health concern across the world, despite the availability of a highly efficacious vaccine. This vaccine cannot be administered to immune-compromised individuals, thus the identification and mapping of viral epitopes is important for development of subunit vaccines and improved diagnostics. Our aim was to identify immuno-dominant antigenic regions and screen for linear B-cell epitopes on the capsid, NS4a and domain III (EDIII) region of the E glycoprotein of YFV.

Methods:

Putative hydrophobic and hydrophilic regions along the length of each protein were predicted using BepiPred and ABCpred. Surface accessibility and antigenicity were done using the Immune Epitope Database (IEDB) version 2.0 software (www.ImmuneEpitope.org). Bioinformatics was used to identify 28 overlapping peptides (8 mer length offset by 3) representing predicted epitopes on the capsid protein, NS4a protein and EDIII protein. Using an in-house ELISA, the peptides were screened for reactivity against YFV antibody using immune sera from 12 patients with a history of vaccination against YFV.

Results:

Overlapping peptides 1-5 covering the region (IPSSASPWSWPDLDLKPGAA) of NS4a reacted with 6/12 patient sera. Overlapping peptides 6-9 covering the region (KTKQIGNRPGPSRGVQG) of capsid reacted with 4/12 patient sera. Overlapping peptides 10-28 of EDIII reacted with 4/12 patient sera. Peptides 14, 15, 22, 23 and 28 of EDIII showed consistent reactivity against all the patient sera. Overlapping peptides 22 and 23 showed higher reactivity compared to the other peptides.

Conclusion:

Bioinformatics was used to identify epitopic regions to reduce the cost of synthesizing peptide libraries that would span the entire length of the three proteins that were selected for the study. The use of whole virus antigen requires biocontainment for preparation and the antigen cannot differentiate between closely related flaviviruses. In summary, a potential epitope, TGHGTVVMQ, from amino acid 21 to 29 on the EDIII protein was identified using bioinformatics and was shown to have some reactivity against immune sera.

OP 50

Abstract Number : 274

Expression of rotavirus VP2 and VP6 structural proteins in yeast

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1 – UFS

Background:

Rotavirus is the leading cause of severe gastroenteritis worldwide in children less than five years of age. Although there are two licensed vaccines (RotaTeq® (MERCK) and Rotarix® (GlaxoSmithKline)) against rotavirus that have been proven to be effective and safe, a need exists for an efficacious, low cost vaccine for use in Africa. Production of virus-like particles is an alternative approach to vaccine development. The current study focusses on the production of rotavirus double-layered particles in yeast using a rotavirus strain prevelant in Africa. This will be achieved through the cloning of the VP2 and VP6 coding regions using the wide-range yeast expression system.

Methodology:

The wide-range yeast expression system, which contains the *Yarrowia lipolytica* TEF promoter, *K. marxianus* inulinase region and the hygromycin B resistance gene (hph) was previously developed. This system allows for the simultaneous expression of more than one gene in any transformable yeast strain. Open reading frames (ORFs) encoding rotavirus (strain G9P[6]) VP2 and VP6 were codon optimized for expression in *Kluyveromyces lactis and Arxula adeninivorans* purchased from GenScript. Codon optimized VP2 and VP6 ORFs for the G9P[6] rotavirus strain for expression in *Pichia pastoris* was obtained from the University of Stellenbosch. The optimized VP2 and VP6 ORFs were cloned into pKM173 and pKM177, respectively using suitable restriction enzyme sites to facilitate cloning. The dual expression vector was constructed by subcloning the expression casette containing the VP6 coding region into pKM173 containing the VP2 coding region.

Results:

Three constructs (pKM173_VP2, pKM177_VP6 and pKM173_VP2/6) for each optimized set of genes were successfully cloned and verified by sequencing across the restriction/cloning sites. Seven yeast strains, namely *Kluyveromyces marxianus, Debaryomyces hansenii, Yarrowia lipolytica, Hansenula polymorpha, Candida deformans,Pichia pastoris and Arxula adeninivorans* were selected for transformation and screening. Following successful transformation of the recombinant plasmids, integration of the ORFs were evaluated.

Conclusion:

Dual expression vectors were successfully constructed and will be tested in a wide range of yeasts to evaluate expression. *Saccharomyces cerevisiae* will be included as reference since VP2 and VP6 was previously expressed in this yeast.

OP 51

Abstract Number : 277

Identification of hpv-11 variants associated with recurrent laryngeal papillomatosis based on lcr-e6 sequence data, southern Africa

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1 - UFS 2 - None Yet

Background

Recurrent laryngeal papillomatosis (RLP) is a chronic disease that affects adults and children and is caused by human papillomavirus (HPV)-11 and HPV-6 which are classified as low risk genotypes. Patients present with varying degrees of severity of disease requiring multiple surgical procedures. Previous studies suggested that HPV-11 was associated with more severe disease within our cohort of patients. The aim of this study was to determine if there are variants of HPV-11 circulating in patients in the Free State and if there is any correlation between HPV variants and disease severity.

Methodology

In this study we identified HPV-11 variants by amplifying the long control region (LCR) and E6 region of the HPV genome. A total of 15 HPV-11 isolates from patients in the Free State were included. DNA was isolated from tissue biopsy and amplified using a primer pair that flanked the LCR and E6 region. Amplification resulted in 1287bp-1450bp amplicons that were sequenced using Sanger method. Nucleotide sequences were edited using ChromasPro, aligned with 111 sequences of the LCR region and 106 sequences of E6 region retrieved from GenBank for HPV-11 isolates from geographically distinct regions worldwide Three isolates were selected for complete genome sequencing. The clinical presentation of the disease in patients infected with novel variants was considered.

Results

For identification of variants, the sequence data was compared with the HPV-11 prototype retrieved from GenBank. Five novel variants were identified. Three variants previously described in Slovenia were present in the cohort. One potentially novel subtype with 91.3% identity in the LCR region was selected for complete sequence analysis.

Conclusion

Novel variants of HPV -11 were identified within the cohort of patients from the Free State province. The presence of isolates genetically related to variants from Slovenia suggests that the virus is not geographically restricted. There was no correlation between variants and disease severity.

OP 52

Abstract Number : 285

Preparationof a dna-launched replicon encoding the ed-III protein of yellow fever virus

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1 - UFS 2 - None Yet

Background:

Yellow fever virus (YFV), a re-emerging positive sense single-stranded RNA virus endemic in the tropical regions of Africa and South America is transmitted by the bite of an infected mosquito. The World Health Organization has estimated the annual prevalence of yellow fever to be 200 000 cases, of which more than 90% is attributed to Africa. Since 2001, vaccine-associated adverse events have been reported for the available live attenuated vaccines against YFV, therefore a safer alternative is required. The aim of this study is to prepare a DNA-launched Sindbis replicon encoding the envelope domain III (ED-III) protein of YFV and to characterize the expression of the ED-III protein in a mammalian expression system.

Methods:

The gene encoding the wild-type Asibi strain YFV ED-III protein was synthesized by GenScript and procured from pUC57 by a double restriction digestion with *Not*I and *Xho*I restriction endonucleases. The gene was cloned into a replication-deficient backbone using the *Not*I and *Xho*I restriction sites. The replicon was purified using the Qiagen Plasmid Mini kit and BHK21 cells at 80% confluency were transfected at a ratio of 1 µg DNA : 1µl lipofectamine. A control replicon containing a gene encoding the green fluorescing protein (GFP) was used as a transfection control. After incubating the cells for 24 hours, the cells were fixed using methanol/acetone and permeabilized with 0,5% Triton X-100. The expression of the recombinant antigen was detected by an indirect immunofluorescence assay (IFA) with mouse monoclonal antibodies directed against the hexahistidine tag at the 5' end of the ED-III gene and anti-mouse anti-IgG

monoclonal antibody labeled with fluorescein isothiocyanate (FITC).

Results:

The DNA sequence of the construct was confirmed, as well as the DNA sequence encoding the YFV ED-lll insertion. Expression of the YFV ED-lll was confirmed by IFA when a ratio of 1 µg DNA:1µl lipofectamine was used for the transfection of cells. Approximately 50% of the cells were transfected.

Conclusion:

A DNA-launched Sindbis replicon expressing the codon optimized gene of YFV ED-lll has been constructed and the immunogenicity of the construct will be evaluated in an animal model.

OP 53

Abstract Number : 221

Bioflocculant Production by Brachybacterium sp. and Cellulomonas sp. Consortium and Media Optimization through Surface Response Model

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Background:

Flocculants aggregates suspended particles in solutions thus, reducing turbidity. The industrial application of this process includes water/wastewater treatment and downstream processing. Flocculants could be inorganic or organic which may be synthetic or natural. These inorganic, organic and synthetic are non biodegradable, and have been implicated in deleterious health effects. Conversely, bioflocculants are non-toxic, biodegradable and environmentally safe thus, a valuable alternative to the conventional flocculants.

Methods:

In this study, two species of actinobacteria previously isolated from Tyume River in the Eastern Cape Province of South Africa and identified by 16S rDNA sequence as *Cellulomonas* and *Brachybacterium* species were evaluated as a consortium for enhanced production of bioflocculant.

Results:

The carbon, nitrogen and cation sources most suitable for the production of bioflocculants were glucose (92% flocculation activity; 4.06 ± 0.62 g/l yield), peptone (89% flocculation activity; 3.88 ± 0.432 g/l yield) and magnesium chloride (83% flocculation activity; 3.99 ± 0.79 g/l yield) respectively. Media components screening, following Plackett-Burman experimental design, revealed glucose, peptone and magnesium chloride as critical in the production of bioflocculant. Hence, optimal concentrations of glucose (16 g/l), peptone (1.5 g/l) and magnesium chloride (1.6 g/l) achieved through central composite design showed flocculation activity of about 98% and bioflocculant yield of 4.11 g/l. The central composite design adequacy was shown by the high regression coefficient obtained (R^2 = 0.865) and at the same time, the predicted values were quite close to the observed response (flocculation activities). FTIR spectrometry of the bioflocculant indicated the presence of carboxyl, hydroxyl and amino groups, typical for heteropolysaccharide, while SEM imaging revealed amorphous membranous pleated sheets with interstice between horizontal pleated sheets of less than 1 µm.

Conclusion:

Cellulomonas and *Brachybacterium* species consortium produced heteropolysaccharide bioflocculant in high yield and with high flocculation activity. Response surface model was critical in yield optimization and the high flocculation activity shown by the bioflocculant suggests suitability for water/wastewater treatment and downstream process.

OP 54

Abstract Number : 204

Antimicrobial and anti-biofilm properties of commercially-available buchu, olive leaf, pepperbark and sutherlandia extracts

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Background:

Members of the family *Flavobacteriaceae* demonstrate intrinsic multi-drug resistance and have a high affinity to form biofilms, resulting in increased persistence and making eradication of these organisms difficult. Phytochemicals which are bioactive, non-nutrient plant compounds inhibit microbial growth as defence/stress mechanisms as well as displaying anti-adhesion effects.

Methods:

The present study investigated the bactericidal effect of commercially available 10% ethanol-based Buchu (*Agathosma betulina*), Olive leaf (*Olea europaea*), Pepperbark (*Warburgia salutaris*) and Sutherlandia (*Sutherlandia frutescens*) extracts on members of the family *Flavobacteriaceae* using agar well diffusion assays. Each extract (120 µl, 150 µl, and 200 µl) was tested against fish-associated *Chryseobacterium, Flavobacterium* and *Myroides* spp isolates, as well as selected Flavobacteriaceae type strains. The anti-biofilm potential of these extracts against selected isolates was determined using microtitre plate assays.

Results:

Flavobacteriaceae type strains and *Myroides* spp. isolates were most susceptible to 200 µl of Olive leaf extract with 57.14% of isolates displaying resistance whilst for Pepperbark 85.29% and 71.43% of isolates, respectively, were resistant. Increased susceptibility of isolates to Pepperbark and Olive leaf extracts was observed with exposure to increased extract volumes. Exposure to the Sutherlandia extract resulted in limited antimicrobial activity against isolates belonging to the family *Flavobacteriaceae*, except for *F. johnsoniae*-like isolates. The anti-adhesion activity of Pepperbark extract decreased with increased volume. Olive leaf extract displayed high anti-adhesion activity although a reduction in efficacy was observed with higher volumes. Buchu and Sutherlandia extracts displayed the best reducing activity. Sutherlandia extract was the most effective as an anti-adhesion compound, reducing biofilm formation by ≥ 90% for 90.91% of isolates. Sutherlandia exposure reduced adherence at all volumes whilst biofilm inhibition was observed with Buchu in a dose-dependent manner.

Conclusion:

Although they have limited antimicrobial activity, these extracts display potential as sustainable, alternative anti-pathogenic agents, especially in biofilm prevention of aquaculture pathogens.

OP 55

Abstract Number : 5

1-Aminocyclopropane-1-carboxylate deaminase activity as a marker for identifying plant-growth promoting rhizobacteria in cultivated soil

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1-Aminocyclopropane-1-carboxylate (ACC) is the immediate precursor of the stress hormone ethylene in plants. Some rhizobacteria have the ability to hydrolyse ACC into ammonia and α-ketobutyrate due to the presence of ACC deaminase (ACCd) activity. This study screened soil samples isolated from Ngaka Modiri Molema district SA, for ACCd activity. A total of 169 isolates were obtained from the different rhizospheric soils collected. Results indicated that 11% (18) of these isolated strains had the ability to utilize ACC as the sole source of nitrogen but with different degrees of efficacy. The cultural, morphological, biochemical, physiological and molecular characterization of these isolates revealed that eleven strains belonged to the genus *Bacillus.* Pot experiments showed that selected isolates containing ACCd activity increased plant growth and development over uninoculated control. The use of plant growth promoting rhizobacteria (PGPR) containing ACCd activity along with other innovations could prove to be an environmentally friendly strategy to ensure sustainable agriculture. These isolates are likely to be potential candidates for biofertilizers, and biotechnological application of commercial value.

Keywords: 1-Aminocyclopropane-1-carboxylate, ACC deaminase, ethylene, mechanism, PGPR, rhizobacteria

OP 56

Abstract Number : 128

Application of conventional multiplex PCR and real-time multiplex PCR to detect virulent Escherichia coli genes in surface water sources.

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Background:

Escherichia coli found in surface waters originates mainly from municipal wastewater discharges, septic leachate, agricultural or storm water run-off, wildlife populations, or non-point sources of human and animal waste. Depending on the virulence factors present, certain strains such as Enteroaggregative *Escherichia coli (EAEC*), Enterohaermorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC), Enterotoxigenic *Escherichia coli* (ETEC) and Enteropathogenic *Escherichia coli* (EPEC), are classified as pathogenic and can cause various health-related illnesses such as urinary tract infections, diarrhoea and respiratory illnesses. The aim of this study was to optimise and compare conventional and real-time multiplex PCRs for the detection of pathogenic *Escherichia coli* genes in surface water sources.

Methods:

Conventional multiplex and real-time multiplex PCR assays targeting genes associated with EAEC, EHEC, EIEC and EPEC strains, respectively, were first optimised using control *E. coli* strains. Thereafter river water samples collected at sites close to informal settlements along the Berg and Plankenburg Rivers were concentrated by centrifugation, DNA was extracted and the optimised conventional multiplex and real-time multiplex procedures were applied.

Results:

Results from the conventional multiplex indicated that the dominant pathogenic *E. coli* strain present at the Berg River site was EAEC (69%), with EHEC and EIEC detected in 15% and 31% of the samples analysed. At the Plankenburg River site, only the EAEC strain (23%) was detected in the water samples analysed. Correspondingly, the optimised real-time multiplex detected EAEC in 69% of the water samples collected in the Berg River system, with EHEC, EIEC and EPEC present less often throughout the sampling period. In addition EAEC, EIEC and EPEC were detected sporadically in samples collected at the Plankenburg River site.

Conclusions:

The results obtained in the study demonstrated that real-time multiplex PCR was more rapid and sensitive compared to the conventional multiplex PCR in the detection of virulent *E. coli* genes in river water samples. A single multiplex PCR assay is thus time- and cost-effective and allows for the simultaneous detection of numerous target genes in a single sample. It can also be used for the routine detection of pathogenic *E. coli* in surface water samples without initial pre-culturing.

OP 57

Abstract Number : 106

Annotation and comparative analysis of three Ceratocystis mitochondrial genomes

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Background

Mitochondrial genomes are generally thought to evolve at an accelerated rate independently of their nuclear genome counterparts. Accordingly, mitochondrial sequences are potentially valuable in the phylogenetic differentiation of cryptic species complexes such as those in the genus *Ceratocystis*, or their relatives within the order Microascales. However, very little is known regarding the mitochondrial genomes of these fungi. The aim of this study was, therefore, to determine and compare the mitochondrial genome sequences for two plant pathogenic (a root and tree pathogen) and one non-pathogenic (a saprophyte) species of *Ceratocystis*.

Methods

The genomes of *C. fimbriata sensu stricto*, *C. albifundus* and *C. moniliformis* were sequenced using a combination of next generation platforms. The assembly, annotation and comparative analysis of the different mitochondrial genomes

was achieved using the CLC Genomics Workbench software program.

Results

The mitochondrial genomes of *C. fimbriata s.s* and *C. albifundus* was respectively 135 104 base pairs (bp) and 126 447 bp in size. The non-pathogenic species, *C. moniliformis,* had a smaller (110 987 bp) mitochondrial genome. All three genomes contained the 15 archetypal mitochondrial genes and various clusters of tRNA genes. A variety of group I type introns were present across all the genomes, with cytochrome oxidase containing the highest number of introns. For *C. fimbriata s.s* we found a complement of 31 putative tRNA genes that recognized all the amino acids coded for by its mitochondrial genome. In *C. albifundus* and *C*. *moniliformis*, a total of 33 and 24 putative tRNA genes respectively were observed.

Conclusions

The major differences between the three mitochondrial genomes could be ascribed to the intron presence when compared computationally. The genomes of *C*. *fimbriata* and *C*. *albifundus* were most similar to each other and very different to that of *C*. *moniliformis*, most noticeably the number of tRNAs, intron presence and overall size of the genome. The variation observed in the tRNAs can be attributed to the same anticodon recognizing different tRNA sequences. This is consistent with their phylogenetic relationships. Overall, the results of this study provide a framework for further genomic comparisons in *Ceratocystis* and other fungi in the Microascales.

OP 59

Abstract Number : 52

Assessment of the microbial quality of the Kat River and Fort Beaufort abstraction water and the prevalence of Escherichia coli pathotypes in the surface waters

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1 - University of Fort Hare

Exposure to contaminated water constitutes an important mechanism for the transmission of gastrointestinal pathogens. Pathogenic *Escherichia coli* strains cause enteric infections in humans and include eight different categories according to virulence factors. This study aims at evaluating the microbial quality of Kat River and Fort Beaufort abstraction water in the Eastern Cape, using *E.* coli as an indicator organism, as well as elucidating the prevalence of E. coli pathotypes in the surface waters. Surface water samples were collected over a three month period (August 2012 to October 2012) at six locations of the Kat River and five around the Fort Beaufort abstraction dam. *E. coli* counts were done by standard filtration method and cultivated using specific Chromogenic agar. Presumptive E. coli isolates were identified and differentiated into the pathotypes by polymerase chain reaction techniques. *E. coli* counts in the Kat River and abstraction water were in the range of 1.1×10^1 to 9.3×10^2 and 2.4×10^1 to 9.1×10^3 respectively for the entire sampling period, suggesting that the microbial quality of the Kat River and abstraction water exceeded the acceptable maximum limit according South African Standards of 0 cfu/100ml for *E. coli.* Since these surface waters are also used for recreation, irrigation and domestic purposes in its raw state, our findings point to a possible threat to the health of the consumers. This indicates the potential risk of infection for residents around the Nkonkobe district. A total of 400 presumptive E. coli isolates are currently undergoing identity confirmation and characterization into the different pathotypes.

Keywords: Kat River, *E. coli*, microbial quality, pathotypes.

OP 60

Abstract Number : 55

Characterization of a bioflocculant produced by Micrococcus species isolated from sediment of Algoa Bay in Eastern Cape Province

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1 - University of Fort Hare

Background information:

Compared with chemically synthesised flocculants, bioflocculants are safe, biodegradable, lack of secondary pollution and harmless to humans and the environment.

Method:

In this study, the physicochemical and flocculating properties of the bioflocculant produced by a *Micrococcus* species were investigated. The bioflocculant was extracted from culture broth by ethanol precipitation and kaolin suspension was used to measure the flocculating activity.

Results:

The purified bioflocculant flocculated kaolin suspension optimally at a dosage of 0.2 mg/ml under acidic conditions of pH 4 in the presence of Al^{3+} as the cation resulted in flocculating activity of about 88%. The bioflocculant retained more than 70% of its flocculating activity at 100^{o}C. Chemical analysis of the purified bioflocculant showed that it was composed of polysaccharide 28.4% (w/w), protein 2.63% (w/w) and uronic acid 9.7% (w/w). Fourier transform infrared spectra revealed the presence of hydroxyl, carboxyl and amino group as the main functional groups. **Conclusion:** These properties showed that the bioflocculant had a good flocculating activity and could be used as alternatives to chemical flocculants commonly used in waste/drinking water treatment and also in other industrial processes.

Key words: *Micrococcus* specie, Bioflocculant, Flocculating activity, Thermostable

OP 61

Abstract Number : 61

The effect of carbon input on the soil microbial community in the central hyper-arid Namib Desert

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The Namib Desert is hyper-arid with low and variable rainfall (> 20 mm p.a) and subjected to wide daily temperature fluctuations (0°C to 50°C). These factors impose extreme environmental constraints on edaphic microbial communities. In such arid regions, high temperatures in association with limited and sporadic rainfall results in restricted plant growth and microbial activity. However, with the increase of global levels of CO2 in the atmosphere, increases in droughts and episodic floods are expected. These pulse-precipitation events may result in increasing periods of temporal vegetation cover, increasing the bioavailable carbon input into otherwise oligotrophic soils.

In order to investigate the effect of carbon input provided by seasonal vegetation on the soil microbial communities in the Namib Desert, surface soil samples were randomly collected from a 8100 m2 plot, colonized by seasonal Stipagrostis grass, near the Gobabeb Training and Research Centre over a 1 year period. Microbial community fingerprinting by Terminal-Restriction Fragment Length Polymorphism, functional microbial potential as determined by fluorescein diacetate hydrolysis and chlorophyll a measurements, as well as abiotic analyses (soil chemistry, temperature and humidity) were performed.

These studies demonstrate that bacterial communities in the Namib Desert are shaped by temporal scales, rather than spatial heterogeneity. We also show rapid bacterial community responses (both structural and functional) to variations in water bioavailabilty and temperature, never previously shown in desert ecosystems (3 days). Identifying indicator species and alternative (a)biotic factors mediating the soil microbial community structure in the Namib Desert, will help us resolve the microbial contribution to the terrestrial carbon cycle in this extreme environment.

OP 62

Abstract Number : 169

Development of a reporter system for the analysis of Xylophilus ampelinus Type III Secreted effectors

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Background

Xylophilus ampelinus, the causal agent of bacterial blight of grapevine has long been a threat to the table grape industry in the Western Cape, leading to severe economic losses due to reduced productivity and shortened lifespan of infected grapevines. Very little is known about the genetic make-up of the organism, especially with regard to the genetic factors that contribute to pathogenicity. Therefore, the aim of this study was to generate a reporter plasmid system that will allow

the identification and classification of potential pathogenicity factors as members of the Type III Secretion class of effectors.

Methods

The first part of this study was to identify *avr* genes from the wild type *X. ampelinus*. Since expression of *avr* genes in non-host plants result in a hypersensitive response (HR), two identified coding sequences (CDs) were cloned and transiently expressed in *Nicotiana tabacum* using *Agrobacterium*-mediated gene transfer to determine functionality. Deletions of the *avrBs1*CD were tested for HR-inducing ability, and then the minimal fragment required for HR was used to construct the reporter plasmid by cloning it upstream of the 3xFLAG tag in the broad host range vector pBBR1.MCS5.

Results

Two coding sequences (CDs) with similarity to avirulence genes of the *avrBs1*-family were identified. The cloned *avr* genes were constitutively expressed under control of the CaMV promoter and they were able to elicit HR on tobacco plants when transiently expressed through *Agrobacterium*-mediated expression. The reporter plasmid was constructed using the C-terminal domain of *avrBs1,* the 3xFLAG tag and the broad host range vector, pBBR-MCS5.

Conclusion

Two *avr* genes isolated from *X. ampelinus* induced HR in tobacco via *Agrobacterium* transient expression. A reporter plasmid for identification of potential Type III secretion effectors in *X. ampelinus* has been constructed using the HR-inducing domain of avrBs1 and will be validated through translocation and secretion assays in the future.

OP 63

Abstract Number : 9

Emergence of new root nodule bacteria from members of the beta-Proteobacteria group

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Inoculation of rhizobia on legume seeds has been the biggest 'success story' in the field of applied soil microbiology. These symbiotic nitrogen fixing rhizobia have been described for long to fall into four 16S rDNA rhizobial branches of the α-proteobacteria. Studies conducted in recent years to explore the phylogenetic diversities of N_2 fixing legume symbionts has led to the discovery of new members of bacteria phylogenetically different from the α-sub group of proteobacteria. The first such report of nodulation of legumes by the β-sub class of proteobacteria particularly members of the Genus *Burkholderia* has been published in *Letters to Nature* which triggered more exploration of the existence of symbiotic bacteria outside of the 'rhizobia' group capable of nodulating legumes.

A study was conducted to characterize the rhizobia which nodulate and fix atmospheric nitrogen in Rooibos (*Aspalathus linearis* burm f.), an indigenous South African legume commonly used to make the popular beverage 'rooibos tea'. This indigenous legume is adapted to grow in soils characterized by high acidity and poor nutrient conditions. However, like many other legumes, it forms a symbiotic relationship with certain group of rhizobia and has the ability to fix well over 100kg N per hectare per year. For several years, the symbiotic rhizobia associated with the nodulation and nitrogen fixation in rooibos were believed to belong to members of the slow growing Bradyrhizobium strains.However, our study revealed that rooibos is nodulated by a diverse group of rhizobia including both the α- and β-Proteobacteria. The alpha Proteobacteria which colonize and nodulate rooibos roots mainly belong to the Genera *Mesorhizobium* and Rhizobium while the beta sub group of Proteobacteria were represented by members of the Genera *Burkholderia* and *Herbasprillum.* For a better understanding of the occurence of symbiotic interaction between rooibos and these non-rhizobia group of the beta-Proteobacteria, this study warrants further meolecular studies to investigate the occurence of the common nodulation (nodABC) genes in these bacteria.

OP 64

Abstract Number : 276

Antibiotic producing Streptomyces spp. inhabit the infructescences of Protea spp. in South Africa

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Background

The fungal diversity associated with the infructescences of *Protea* spp. includes a specialized group known as the ophiostomatoid fungi. These fungi have spore-bearing structures adapted for insect dispersal and reside in two different orders; the Microascales and the Ophiostomatales. Because of their dominant occurrence in the *Protea* infructescences, we have hypothesized that antibiotic producing actinomycetes could be responsible for restricting the growth of common non-ophiostomatoid saprophytic fungi in the infructescences of *Protea* spp.

Methods

Using a culture-based survey, we isolated actinomycetes from the infructescences of *P. repens* and *P. neriifolia*, from two locations in the Western Cape province. Isolates were identified using 16S rRNA sequence analysis and antifungal activity was determined using *in vitro* bioassays. Chemical analysis was performed on isolates of the actinomycetes as well as directly on fresh infructescences, for the presence of antifungal compounds using liquid chromatography-mass spectrometry.

Results

A total of 86 different isolates were obtained and could be separated into five different phylogenetic clades, each representing a distinct species. Members of three of these clades had antifungal activity against the common saprophytes against which they were tested. Interestingly, one of these species had distinctly lower inhibitory activity against the ophiostomatoid fungi than against the test saprophytes. One of the compounds produced by the actinomycetes was identified as the antibiotic Pentamycin and this was also detected in the tissue of various parts of *P. repens* infructescences.

Conclusions

The results of this study suggest that the presence of antibiotic producing actinomycetes is at least in part, responsible for the restricted fungal diversity and the dominance of the ophiostomatoid fungi in the *Protea* infructescences. In this regard, it also appears that antibiotic producing actinomycetes associated with fungal niches such as these infructescences could be a source of novel antifungal antibiotics and this line of research is being actively pursued.

OP 65

Abstract Number : 94

Phylogeny of fungal root endophytes associated with Erica chamissonis and Erica caffra and in-vitro assessment of fungal activity against plant pathogenic fungi.

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Ericaceous plants are associated with a diversity of fungi, which are primarily mycobionts in the ericoid mycorrhizal association (ERM). This enables the host plants to assimilate mineral nutrients from a wide range of organic sources in their nutrient depleted habitats. In this genetic diversity not all fungi are involved in the ERM therefore they may have other ecophysiological functions beneficial to the host. The use of organic modified media to optimize the commercial production of *Vaccinium corymbosium* plants in the Ericaceae family has resulted in the persistence of root diseases. The common pathogens include *Pythium, Phytophthora* and *Alternaria* species. The aim of this study was to isolate and identify fungal endophytes associated with *Erica chamissonis* and *Erica caffra* and evaluate their potential in the control of these pathogens.

Endophytes were isolated in pure culture from the hair roots of the respective *Erica* host plants. Isolates were identified and analysed phylogenetically after DNA extraction, PCR, sequencing and BLAST analysis. Assessment of anti-fungal activity was conducted in dual agar plate experiments on Malt Extract agar. Twenty five fungal endophyte isolates were selected and tested against the fungal pathogens *Alternaria*, *Pythium*, *Phytophthora* and *Fusarium* sp.

A total of 47 ascomycetous fungal species were identified from both *Erica* species. *Leohumicola* spp. of the Leotiomycetes constituted 19% of the total isolates from both host plants. Other species of interest included Helotiales species with affinities to unidentified Epacrid root endophytes from the Southern hemisphere and other fungal taxa such as *Lachnum* sp., *Humicola* sp., dark septate endophytes such as *Leptodontium* sp. and the typical ericoid fungus *Oidiodendron maius*. Of all the isolates tested for anti fungal activity, *Leohumicola* sp. produced an olive brown soluble pigment in the media which suppressed the growth of *Alternaria* and *Phytophthora sp.* Inhibition was not significant for *Fusarium* sp. and no effect was recorded against *Pythium* sp. No effects were recorded for the remaining isolates tested. These results highlight the importance of fungal endophytes in plant production.

OP 66

Abstract Number : 41

Transcriptional alterations in model host, Nicotiana benthamiana, in response to infection by South African cassava mosaic virus

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1 – Wits

Background

Successful systemic infection by plant viruses is the outcome of complex molecular interactions between host and viral pathogen, leading to spatial and temporal alterations in plant gene expression. *Nicotiana benthamiana* has been used extensively as a model host to study plant virus-host interactions, and in this study, transcriptional alterations in *N. benthamiana*, in response to infection by *South African cassava mosaic virus*, was undertaken.

Methods and Results

Results from a microarray study using the commercially available custom-made 60-mer oligo NimbleGen Platform (representing 13 014 ESTs) (Roche) revealed that expression levels of many transcripts were altered in response to SACMV at 21 dpi (representing full systemic infection), including encoded proteins involved in transcription networks, defence responses and plant hormone signalling. Approximately 4.7% (611 of 13 014) of the transcripts were significantly altered in response to SACMV infection. Of these, 483 genes were found to be induced and 128 genes were suppressed. GOslim functional group analysis illustrated that differentially expressed genes in infected leaf tissue, compared to mock inoculated, were primarily overrepresented in the cellular component category for nuclear (19.92%) and other cellular components (14%), while categories corresponding to transferase activity (14.42%) and other binding (13.43%) were overrepresented for Molecular Function. Cellular processes (24.93%) and other metabolic processes (18.05%) were overrepresented for Biological Process. Notably from our data, we were able to detect transcript changes in several defence-related and sucrose and starch metabolic pathways. Alterations in genes associated with the cytoskeleton, cell wall and plasmodesmata, namely myosin heavy chain, beta-tubulin, Ras-GTPase (Rab6A), ß-1,3-glucanase, pectinesterase and pectate lyase , collectively suggest possible roles in intracellular vesicle-assisted movement to the plasmamembrane and release into the adjacent cell via the plasmodesmata (Pd).

Conclusions

Understanding host responses to virus pathogen attack can contribute to building of metabolic pathway models or interactomes that can inform more effective control strategies.

OP 67

Abstract Number : 56

Comparison of microRNA populations in South African cassava mosaic virus-infected tolerant and susceptible cassava cultivars

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1 - None Yet 2 – Wits

Background

Cassava serves as a staple food crop for over 500 million people and is particularly important for the sustainable livelihoods for resource-poor farmers in sub-Saharan Africa. It is also used as a biofuel crop, animal feed and industrial raw material. *South African cassava mosaic virus*-[South Africa:99] (SACMV) belongs to the family *Geminiviridae* and is one of several distinct species causing cassava mosaic disease (CMD). CMD is the most economically important disease of cassava and is endemic in all cassava-growing countries in Sub-Saharan Africa. MicroRNAs (miRNAs) are short RNA molecules that control gene expression by silencing complementary mRNA. They play a crucial role in stress response in plants, including biotic stress. Some miRNAs are known to respond to begomovirus infection in *Nicotiana benthamiana* , but it is currently unknown whether these responses are conserved in cassava and whether novel species-specific miRNAs could have a role in infection or defence.

Methods and Results

This study aimed to understand the changes in miRNA populations in SACMV-infected cassava, and to compare these with a tolerant (TME3) and susceptible (T200) cultivar. Next-generation sequencing (Illumina Platform) was used for

analysing small RNA libraries from healthy non-infected cassava with leaf tissue infected at 12 (pre-symptomatic), 32 (full systemic infection) and 67 dpi (recovery in TME3; full susceptibility in T200) with SACMV. A full repertoire of cassava miRNAs was characterized, which included conserved and novel cassava-specific families. Differences between TME3 and T200 were noted, as well as differences in miRNA levels and patterns between different time points during the infection period, and these will be discussed further. Endogenous targets were predicted in the cassava genome for many miRNA families.

Conclusion

The identification of miRNAs that are altered apon SACMV infection may provide novel targets for control strategies aimed at developing a CMD-resistance cassava cultivar.

OP 68

Abstract Number : 136

Design and Delivery of TALE-Nucleases in the treatment of chronic Hepatitis B

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Hepatitis B Virus (HBV) infection is hyper-endemic to sub-Saharan Africa, where up to 8% of the population is chronically infected. Close to 600 000 deaths are directly linked to chronic HBV annually. HBV has a small genome which can be maintained as an episomally stable "mini-chromosome", cccDNA, and can remain dormant in the nucleus of infected hepatocytes for decades. Current therapies only target post-transcriptional events in the HBV life cycle; thus the cccDNA remains unaffected. This may result in the re-initiation of HBV replication. The properties of cccDNA make it an attractive but challenging target for novel drug therapies.

Transcription activator-like effector (TALE) nucleases are an emerging class of sequence-specific nucleases. Work completed in our laboratory, showed excellent *in vitro* and *in vivo* activity of TALENs targeting the Surface ORF and the Core ORF of the HBV genome. This work is the first to use TALENs against a viral target and has illustrated the potential of TALENs as a novel therapy in the treatment of HBV. TALENs are encoded by ~3.5kB of DNA, must be delivered in pairs and efficacy is dependent on high liver transduction. This project focuses on the use of 2 families of viral vectors to deliver TALENs both *in vitro* and *in vivo*, Helper-Dependent Adenoviral (HDAd) vectors and Adeno-Associate Viral Vectors (AAVs). HDAds, are able to accommodate up to 32 Kb of transgene DNA, making them attractive for the delivery of both TALEN constructs simultaneously. AAVs are small viral vectors and although they have a limited capacity for trans-genes (4.8Kb), their higher transduction efficiency makes them attractive for the introduction of TALEN constructs.

With the continued global health burden of HBV the design and delivery of novel therapeutics remains a research priority. This project focuses on the pre-clinical proof of concept for the use of AAV/HDAd vectors for the delivery of anti-HBV TALENs, as a precursor to clinical evaluation.

OP 69

Genetic identification of two sweet potato-infecting begomoviruses in South Africa

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Abstract:

The complete genome sequence of two monopartite begomovirus isolates (genus *Begomovirus*, family *Geminiviridae*) that occurred either alone or in mixed infection in sweet potato (*Ipomoea batatas*) plants collected in Waterpoort, South Africa is presented. One of the isolates corresponds to that of S*weet potato mosaic associated virus* (SPMaV; SPMaV-[ZA:WP:2011]) with which it shared 98.5% nucleotide identity, whereas the second isolate corresponds to a new variant of*Sweet potato leaf curl Sao Paulo virus* (SPLCSPV; SPLCSPV-[ZA:WP:2011]) with which it shared 91.4% nucleotide identity. The phylogenetic and recombination relationships of these isolates to other monopartite *Ipomoea*-infecting begomoviruses were also investigated. SPLCSPV-[ZA:WP:2011] was found to be a natural recombinant of swepoviruses consisting of two distinct parental genomic sequences from SPLCSPV and sweet *potato*

leaf curl Georgia virus (SPLCGV). Results of continued surveillance of swepoviruses in all sweetpotato growing provinces and their characterization by 'circomics' (Rolling circle amplification and next generation sequencing) will also be discussed.

Acknowledgement: This work was financially supported by the National Research Foundation (NRF), South Africa.

OP 71

Abstract Number : 19

Molecular identification of fungi associated with leaf lesions on Marama bean seedlings in Namibia

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Background:

In this study and during a greenhouse experiment at the University of Namibia, signs of necrosis were observed on marama bean (*Tylosema esculentum*) leaf tissue of seedlings. We describe the findings of an investigation to determine the causative agent(s) of the leaf spotting.

Methods:

Experimental samples were collected from leaves showing necrosis and brown spotting. These were subjected to molecular analysis and species identification of various fungal isolates associated with detected symptoms. Leaves were surface-sterilized and used as leaf discs (2 mm X 2 mm), to inoculate PDA (potato dextrose agar). The inoculated media was incubated at room temperature (25°C) under white fluorescent light for 5 days, and an array of fungal isolates grew. The control leaves (showing no symptoms) were treated similarly and no fungi were observed on them. For each isolate, a pure culture was obtained by repeatedly sub-culturing the fungal hyphal edges onto new PDA at 25°C until a pure culture was obtained. A single spore culture was aseptically isolated for each pure culture and developed into fully grown mycelium by incubation into PDB (potato dextrose broth) at room temperature for 14 days. DNA isolation together with the internal transcribed spacer (ITS) region amplification and sequencing followed with a comparison of the DNA sequences with GenBank.

Results:

A total of 8 single spore cultures were obtained and molecular analysis revealed the presence of a complex of fungal strains on the affected leaves with 8 known species and associated similarity: *Penicillium brevicompactum* (94%)*, Epicoccum sorghi* (99%), *Rhizopus stolonifer* (100%)*, Alternari solani* (96%), *Fusarium equiseti* (98%)*, Penicillium olsonii* (98%)*, Fusarium chlamydosporum* (98%) and *Fusariumincarnatum* (99%).

Conclusions:

These fungi are commonly known to be involved in plant diseases of some common legumes and other agronomically important crop plants. The involvement of these species in marama leaf decay should be noted with great concern and interest as marama bean has been identified and earmarked as a potential leguminous crop for domestication. The fungal species identified could be potential pathogens that could affect yields in marama bean in the case of its adoption for both domestication and farming efforts.

OP 72

Abstract Number : 50

In silico predicted Babesia bovis antigens are indicative of potential candidate diagnostic antigens

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Babesia bovis is an obligate intracellular protozoan parasite, imposing important constraints on livestock health and economic development in tropical and subtropical regions throughout the world. This organism is responsible for the most prevalent and costly tick borne disease, namely bovine babesiosis. The current control approaches have many limitations enabling babesiosis to remain prevalent worldwide. In this study, we identified two novel genes of B. bovis encoding two proteins from B. bovis, named Bbov 16 and B.bov 22. The proteins are potentially novel and secreted proteins that may be used as diagnostic antigens. Bioinformatics analysis indicated that they have signal sequences,

are hydrophobic, have multiple epitopes and are immunogenic suggesting that they may also be used as candidate vaccine antigens. Both genes were obtained by Polymerase Chain Reaction and ligated into the pJET 2.1 cloning vector. The recombinant plasmid constructs were identified by colony PCR and sequencing. The positive clones were double digested and inserted into the expression vector pGEX3x and recombinant constructs were expressed as GST-fusion proteins in the E. coli BL21 (DE3) cells. Analysis with SDS-PAGE revealed the correct sizes of the proteins as 42KDa and 48kDa for Bbov 16 and Bbov 22 respectively. Further studies will be undertaken to develop an ELISA diagnostic assay that will use these recombinant proteins as antigens and evaluate it with field samples from infected cattle.

OP 73

Abstract Number : 111

Mating type markers reveal unexpected patterns of sexual compatibility in Leptographium sensu lato

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Background

Species of *Leptographiumsensu lato* (Ophiostomatales, Ascomycetes) are sap-stain fungi vectored by bark beetles (Coleoptera, Scolytinae) and some species cause or are associated with root diseases in conifers. Sexual states have been reported for more than 30 species in this group and following the dual nomenclature system, these have been treated in the sexual genus *Grosmannia*. For at least 59 additional species, no sexual state is known and these reside in the asexual genus *Leptographium*. The discovery of sexual states for species of *Leptographium* relies mainly on the presence of fruiting bodies on host tissue at the time of isolation and/or intensive laboratory mating studies, which commonly have a low success rate. The aim of this study was thus to develop markers to diagnose mating-type and to study sexual compatibility of species in *Leptographium sensu lato* using these markers.

Method

Available mating type sequences for species of *Leptographium sensu lato* and *Ophiostoma* were obtained, aligned and used to design primers. These primers were then used to amplify portions of *MAT1-2-1* and *MAT1-1-3* genes from a number of *Grosmannia* and *Leptographium* species. Phylogenetic analyses were also conducted using the ITS2-LSU as well as the *MAT* gene sequences from to study pattern of sexual compatibility in these species.

Results

Portions of *MAT* genes in 40 *Grosmannia* and *Leptographium* species were amplified and sequenced. This made it possible to recognise the thallism of these species, in many cases for the first time. The results showed that heterothallic species are abundant in this group fungi (37 of 40 species investigated) when compared to the limited number of homothallic species (3 of 40 species investigated).

Conclusion

MAT markers for *Grosmannia* and *Leptographium* species were successfully developed in this study. These markers have made it possible to amplify and identify the mating types of 40 species residing in *Leptographium sensu lato*. Many species previously considered to be asexual were shown to be heterothallic. These markers present an opportunity for the discovery of sexual structures of these fungi. They will also facilitate various genetic studies on these species that would otherwise not have been possible.

OP 74

Abstract Number : 150

Optimizing recombinant expression and purification of an aspartic protease from Metschnikowia pulcherrima

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1 - Stellenbosch University

Wine proteins mostly originate from grapes and constitute an unutilised source of nitrogen for most microorganisms. Indeed, most *Saccharomyces cerevisiae* strains do not secrete proteases able to degrade proteins under winemaking conditions. These proteins also tend to form haze in white wine leading to the depreciation of the bottled wine. In this context, the extracellular acid proteases of non-*Saccharomyces* yeasts could fulfil a number of roles in winemaking,

which include increasing the available nitrogen sources for the growth of fermentative microbes, affecting the aroma profile of the wine (via production of higher alcohols and esters), and potentially reducing protein haze formation. One such protease-encoding gene named *MpAPr1* originating from *Metschnikowia pulcherrima* IWBT Y1123 has previously been isolated and its corresponding enzyme shown to be active against grape proteins. In this study, the gene was cloned into the expression vector pET14b and transformed into *E. coli* Rosetta-gami 2™ for recombinant expression in order to further characterise the properties of MpAPr1. After extensive optimisation, the following conditions were validated for induction of MpAPr1 overexpression: cells grown to an OD_{600nm} of between 0.8-1.0, addition of 0.4mM IPTG and incubation of the cells for 48h at 14°C on a rotary shaker. The nature of the enzyme caused it to be expressed in inclusion bodies, which made native extraction techniques (freeze-thaw method) unsuccessful. Extraction of the over-expressed enzyme could thus only be performed under denaturing conditions, which included the use of urea. MpAPr1 was then purified by making use of Ni-NTA spin columns, but yielded an inactive enzyme. Refolding the protein into its native conformation from denatured samples is currently being optimized in parallel with extraction of the enzymes under native conditions.

OP 75

Abstract Number : 202

The characterization of three α-L-Arabinofuranosidases identified from a compost-derived metagenomic library

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Background:

Second generation biofuels production requires a suite of lignocellulolytic enzymes, acting synergistically to liberate the fermentable monosaccharides contained within agricultural waste materials. The use of thermostable lignocellulosic enzymes in a high-temperature process represents a number of advantages over their respective mesophilic counterparts, including increased solubility of the polymeric lignocellulosic substrates, enhanced enzyme processivity and reduced risk of bacterial and/or phage contamination during fermentation (Turner *et al.,* 2007; Viikari *et al.,* 2007). Alpha-L-arabinofuranosidases (AFases) participate in the deconstruction of lignocellulosic materials by hydrolysing the arabinofuranosyl bonds contained within the hemicellulosic portion of lignocellulose. In this study, three AFases isolated from compost-derived metagenomic DNA were characterised.

Methods:

Three genes derived from fosmid metagenomic library constructed from 70°C compost were cloned into the pET21a(+) expression vector and expressed in *E. coli* BL21. The heterologously expressed proteins, AFase_H4, AFase_E3 and AFase_D3, were subsequently purified and their biochemical characteristics determined.

Results:

All three AFases were shown to be active between pH 4.0 and 6.0. AFase_H4 and AFase_E3 displayed the highest activity at 60°C, while AFase_D3 had an optimum temperature optimum at 25°C. Furthermore, the three AFases had differing thermostability profiles. In particular, AFase_E3 maintains 100% residual activity following 60 min incubation at 80°C and 24 hour incubation at 60°C. All three AFases have activity upon p-Nitrophenyl-arabinofuranoside and none against a range of alternative p-Nitrophenyl-glycosidic substrates. Phylogenetic analysis of the catalytic domain, identified within the amino acid sequences of the AFases, suggests that these AFases are belong to glycoside hydrolase (GH) family 51.

Conclusion:

All three AFases indicated similar biochemical and biophysical characteristics. AFase_E3 was deteremined to be the most thermostable amongst all three AFases. In the assessment of the three AFases respective suitability for inclusion in thermogenic bioethanol production processes, AFase_E3 was concluded as a suitable candidate for hydrolysis and synergistic testing on natural substrates.

OP 76

Abstract Number : 266

Characterization of C. albicans deletion mutants of genes expressed during PGE2 production

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Background

The pathogenic yeast, *Candida albicans*, can form prostaglandin E_2 (PGE_2) from host derived arachidonic acid (AA). A study that was conducted to evaluate the effect of AA on the genome wide expression of genes by *C. albicans* revealed several open reading frames (orf's) to be significantly upregulated in the presence of AA. Further analysis revealed that seven of these orf's may be involved in yeast to hyphal transition as well as biofilm and germ tube development in *C. albicans*. According to Candida Genome Database two of these orfs were found to represent genes coding for uncharacterized proteins. Furthermore, we hypothesize that signaling networks [e.g. Regulation of *Ace2*Morphogenesis (RAM) and SPS (Ssy1, Ptr3, and Ssy5)], in which some of these orf's play roles (e.g. Hym1 and Csy1), are regulated by AA/PGE_2.

Methods

We obtained sequences of seven open reading frames including their flanking sequences from Candida Genome Database (http://www.candidagenome.org/). These were cloned into pGEM-T easy and pSMART vectors and target regions were then replaced by the *SAT1* construct (Reuß *et al*., 2004).

Results

Seven knockout plasmids, with the *SAT1* flipper as our main deletion marker, were designed. So far, five *Candida albicans* deletion mutants, each represented by two or more independed isolates, were constructed and characterized. We have already observed distinctive morphologies, in the budded to hyphal and white to opaque switch, on different carbon sources by some of the mutant strains. Deletion mutants will be analyzed in presence of exogenous AA and PGE_2.

Conclusions

Few studies have attempted to generate and analyze deletion mutants in the presence of AA or PGE_2 (Alem &Douglas, 2005; Erb-Downward &Noverr, 2007; Erb-Downward *et al*, 2008) although they can inform us on how to characterize PGE_2 production in the context of regulatory circuitries influenced. In this research we will analyze deletion mutants expressed during AA metabolism in order to understand the genetic mechanisms orchestrated by or orchestrating the AA pathway and PGE_2 production. This work will further allow us to tackle important questions pertaining to the genetics of AA metabolism into different prostanoids.

References

Available from the authors

OP 77

Abstract Number : 163

Mitochondrial recombination suggests hybrid speciation within the Gibberella fujikuroi species complex.

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Background:

The *Gibberella fujikuroi* species complex (GFC) is a monophyletic assemblage of fungi that includes many economically important plant pathogens. Previous studies have shown that multi-gene phylogenies based on nuclear and mitochondrion-encoded gene regions are incongruent. Although the source of this incongruence could be ascribed to various factors, recombination is the most reasonable explanation especially as it matches the biology of mitochondria and the history of the GFC. The aim of this study was to determine whether recombination is responsible for the observed phylogenetic incongruence among individual mitochondrion-encoded genes.

Methods:

In order to detect recombination, minimal ancestral recombination graphs (ARGs) were constructed using the BEAGLE branch and bound algorithm in SNAP Workbench 2.0. For this purpose, segregating sites and indels were excluded in order to assume the infinite-sites model of mutation. We utilized five mitochondrion-encoded genes (i.e., *nad*3, *nad*5, *nad*6, *atp*6 and *cox*2) in the 10 biological species of the GFC.

Results:

The ARG analysis revealed extensive recombination in the sequences of some of the mitochondrial genes, especially *nad*5 and *nad*6. Analysis of the concatenated gene dataset further showed that recombination also occurred both within and between the clades of the GFC. To confirm these findings, future analyses should focus on how selection influences mitochondrial genes and to eliminate the possible effects of analytical artefacts arising from factors such as substitution saturation, which could also cause gene tree incongruence.

Conclusions:

The evidence for recombination between the mitochondrial genomes of GFC species could be linked to the life history of this species complex. Historical recombination between the clades supports the hypothesis that the origins of the GFC could be associated with an ancient hybridization event, since recombination among mitochondrial genomes is common when parental leakage occurs during inter-specific hybridization. Although the mechanism by which recombination might have occurred between mitochondrial genomes in GFC requires further investigation, the fact that recombination was not detected in all genes examined suggests intron homing as opposed to recombination via dispersed repeats. Recombination via intron homing is also consistent with the overall gene order conservation observed in these intron-rich genomes.

OP 78

Abstract Number : 271

Heterologous expression and substrate specificity of Aspergillus terreus CYP505E3

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Background

In 2008, Vatsyayan *et al*. reported the presence of a self-sufficient cytochrome P450 with terminal alkane hydroxylase activity in cell free extracts of *Aspergillus terreus*. Two open reading frames (ORFs) encoding possible self-sufficient CYP450s were identified in the sequenced genome of *A. terreus*. One of the two ORFs had a crucial domain missing; hence it is not likely to be an active CYP450. The second CYP450 ORF, which encodes a CYP450 classified as CYP505E3, was investigated in this study.

Methods

An N-terminal variant of CYP505E3, similar to the N-terminal variant described for CYP505A1, another fungal self-sufficient CYP450, was constructed for heterologous expression in *Escherichia coli*BL21(DE3) cells. Whole cell biotransformation was carried out with the expressed CYP505E3 variant using hexadecane and hexylbenzoic acid as substrates.

Results

Soluble CYP450 recovery after cell disruption was improved from 5 nmol per 1 L flask to 13 nmol per 1 L flask (159%) using a Plackett-Burman experimental design. No hydroxylated products of hexadecane were detected. Hydroxylated products of hexylbenzoic acid were identified as omega-4 hexylbenzoic acid and omega-2 hexylbenzoic acid using a GC-MS.

Conclusion

Hexylbenzoic acid, which is a substrate of self-sufficient sub-terminal fatty acid hydroxylases such as CYP102A1 and CYP505A1, is also hydroxylated by CYP505E3. We therefore concluded that CYP505E3 is probably also a sub-terminal fatty acid hydroxylase, rather than a terminal alkane hydroxylase.

References

Vatsyayan, P, Kumar, A K, Goswami, Papori, &Goswami, Pranab (2008) Broad substrate Cytochrome P450 monooxygenase activity in the cells of *Aspergillus terreus* MTCC 6324.*Bioresource Technology* 99: 68-75.

Kitazume, T, Tanaka, A, Takaya, N, Nakamura, A, Matsuyama, S, Suzuki, T, &Shoun, H (2002) Kinetic analysis of hydroxylation of saturated fatty acids by recombinant P450foxy produced by an Escherichia coli expression system. *European Journal of Biochemistry* 269: 2075-2082.

OP 79

Abstract Number : 21

Evaluation of esx sequence &protein profile variations within mtb clinical &laboratory isolates

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Background:

The *Mycobacterium tuberculosis* (*Mtb*) genome harbours the *esx* family of genes (*esxA-W)* containing effector molecules influencing immunogenicity and pathogenicity. The aim of this study is to evaluate potential genetic variations, by identifying variations in sequence and protein expression profiles in clinical isolates.

Method:

25 *Mtb* genomes downloaded from TBDB, TubercuList, BCGList and BoviList databases, aligned using DNAStar MegAlign software (LaserGene 7.2, USA) for *esx* sequence variation analysis. 58 clinical isolates (20 Beijing; 25 KZN and 13 Other isolates) were sequenced and analysed relative to H37Rv *esx* genes. Whole cell lysates and culture supernatants were extracted from clinical and laboratory isolates. 10mg samples were subjected to to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoblot analysis was carried out with ESAT-6, CFP-10 and GroEL mouse monoclonal antibodies.

Result:

The clinical isolates harboured a higher number of more than one non-synonymous mutations in *esxK, L* and *V,* relative to H37Rv. Greatest diversity of esx sequences for *esxI, O, P, V* and *W* in Beijing isolates. Interestingy, the Beijing isolates showed a decreased expression of ESAT-6, CFP-10 and GroEL proteins relative to the other clinical isolates and the control laboratory strains.

Conclusion:

Publically available strains lacked all completed *esx* sequences, thus the analysis for potential sequence variations are not a true reflection of overall diversity. The *esxI, O, P, V* and *W* sequences from the Beijing isolates are potential candidates for further antigenic diversity analyses. The change in protein expression levels in the Bejing isolates compared to the other isolates is indicative of a change in the gene regulation for ESAT-6 and cfp-10. Further reasearch is needed into the specific expression of other *esx* members. Overal, the high number of nsSNPS and low protein expression levels may confer an advantageous selective phenotype on the Beijing genotype, and may contribute as potential genetic markers and play a vital role in the emergence of the Bejing family in the worldwide tuberculosis epidemic.

OP 80

Abstract Number : 44

A New Nanotechnology for Microbiology

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Background

Nano Scanning Auger Microscopy (NanoSAM), in combination with Argon-etching, is a technique usually used to analyse conductors and semi-conductors. The use of NanoSAM on biological material was discovered in 2010, where it was used to track the antifungal drug, fluconazole, in the yeast *Nadsonia fulvescens*. This novel nanotechnology consists of the Anti‑mitochondrial Antifungal Assay (^{3}A) linked to NanoSAM and is aimed at exposing novel anti-microbial, anti-mitochondrial, anti-malarial and anticancer drugs. Here, yeast sexual cells (ascospores) serve as biosensors to screen drugs with anti‑mitochondrial activity (^{3}A system) while NanoSAM is used to determine the metabolic fate of the drug. Additionally, the effect of anti-mitochondrial drugs on cell 3-D ultrastructure and chemical composition can be determined as etching with Argon proceeds in nanometre thin "slices" through the cell.

Methods

Cells of *Nadsonia fulvescens* were treated with the antifungal fluconazole by overlaying a test-strip, containing various concentrations of the drug, on a homogenous lawn of cells spread onto an agar plate. *Nadsonia fulvescens* produces an amber colour on the plate when mature ascospores are produced, yet when no mature ascospores are produced, growth remains white. After incubation three zones are therefore expected (i) an inhibition zone where limited growth occurs, (ii) a white zone (at high concentrations of the drug) where only asexual reproduction occurs and (iii) a brown zone (at low concentrations of the drug) where asexual and sexual growth occurs. The white and brown zones were then subjected to analysis with Light Microscopy, NanoSAM and Transmission Electron Microscopy (TEM).

Results

Light Microscopy revealed mature ascospores in the brown zone compared to immature ascospores in the white zone - indicating the effect that fluconazole has on the development of ascospores in this yeast. This was confirmed with TEM studies. With NanoSAM the 3-D architecture as well as the differences in chemical composition in cells from both zones could be determined. The metabolic fate of fluconazole was also demonstrated.

Conclusions

NanoSAM, as a part of the exciting new research field of Auger-architectomics (http://en.wikipedia.org/wiki/Auger_architectomics), exposes a new and interesting world in atom and structural imaging and further applications should now be explored.

OP 81

Abstract Number : 102

Bacterial vaginosis and genital mycoplasmas in pregnant antenatal clinic attendees

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Background

Bacterial vaginosis (BV) and genital mycoplasmas are reproductive tract infections (RTIs) that are associated with several infections and adverse pregnancy outcomes, such as pelvic inflammatory disease, preterm birth and pregnancy loss. Bacterial vaginosis is a dysbiosis, which is prevalent in 10% to 40% of pregnant women. Genital mycoplasmas are reported to colonise up to about 70% of sexually active women. The purpose of the study was to determine the prevalence of BV and genital mycoplasmas.

Methods

Self-collected vaginal swabs were obtained from pregnant women. Bacterial vaginosis was diagnosed using the Nugent scoring system with subsequent real-time PCR quantification of *Atopobium vaginae* and *Gardnerella vaginalis*. A multiplex PCR assay was used to detect genital mycoplasmas. The 140-kDa adhesion protein, 16S rRNA gene and the multiple-banded antigen genes were used as targets for the detection of *M. genitalium, Mycoplasma hominis, Ureaplasma parvum* and *U. urealyticum*, respectively.

Results

A total of 220 women were included in the study. The mean gestational age of current pregnancies were 26 weeks and most women [45.9% (101/220)] were in the second trimester of pregnancy. The prevalence of BV was 17.7% (39/220), whereas the prevalence of genital mycoplasmas ranged from 2.3% to 71.4%. Bacterial vaginosis was significantly (95% CI: 2.2256 to 13.7542; p=0.0002) detected in women with 1st trimester pregnancies (11/23), whereas only *M. hominis* was significantly (95% CI: 1.5916 to 8.0142; p=0.0020) isolated from HIV positive women (27/36). The concentrations of *A. vaginae* and *G. vaginalis* that were significantly associated with high Nugent scores were 10^6 to 10^7 copies/reaction. *Mycoplasma hominis* (95% CI: 1.4108 to 6.4025; p=0.0043) and *U. parvum* (95% CI: 1.0028 to 6.3739; p=0.0493) were significantly isolated from women with BV.

Conclusions

This study found that *M. hominis* and *U. parvum* are associated with BV. Infections with these bacteria may be misdiagnosed because no routine screening is done in South Africa. The low effectiveness of syndromic treatment to reduce the prevalence of asymptomatic RTIs necessitates improved diagnostic and treatment strategies. Early detection and intervention of these infections would minimise complications, such as undesirable pregnancy outcomes and decrease neonatal morbidity and mortality.

OP 82

Abstract Number : 115

Genetic diversity of clinical methicillin resistant staphylococcus aureus strains in the pretoria region in south africa usingSPA, AGR and MLST typing

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Background

Methicillin resistant *Staphylococcus aureus* (MRSA) causes severe infections in humans. The ability of MRSA to become multiresistant poses challenges to the healthcare system and has emerged as a major concern in community settings worldwide. The aim of this study was to identify and determine the genetic diversity of healthcare-associated and community-associated MRSA isolates that are circulating in the Steve Biko Academic hospital using staphylococcal protein A typing (*spa*), accessory gene regulatory typing (*agr*) and multilocus sequence typing (MLST).

Methods

Three polymerase chain reaction (PCR) assays for *spa*, *agr* and MLST typing was performed. The sequences for the seven housekeeping genes were analysed using the CLC main workbench. The MLST database where used to assign allelic profiles and sequence types. The *spa* types were determined using the Ridom StaphType software and a dendrogramme was constructed from the *spa* data.

Results

The *spa* typing results showed 12 distinct clusters (A to L). The *agr* typing indicated the majority of the isolates belonged to *agr* group I. Ten representative isolates were selected for *spa* sequencing and MLST typing, selection was based on the results from pulsed-field gel electrophoresis (PFGE) from another departmental study on the same clinical isolates. The sequence types (ST) obtained corresponded to the pandemic clonal complexes [(*spa* type t891-ST22/CC22), (*spa* type t1257-ST612/CC8), (*spa* type t037-ST239/CC8), (*spa* type t037-ST239/CC8), (*spa* type t1257-ST612/CC8) and (*spa* type t012-ST36/CC30)]. The only PVL positive isolate in this study was identified as *spa* type t891-ST22/CC22.

Conclusions

Combining different molecular techniques showed that typing assays, such as: *spa*, *agr* and MLST can be accurately used to determine the genetic diversity of MRSA. Compared to PFGE, the three PCR based techniques were rapid and

less labour intensive. The *spa* types and sequence types identified in this study are in agreement with previously characterized genotypes in South Africa and worldwide. The ST239 is referred to as the oldest pandemic clone reported worldwide while ST612 has been described as the infrequent clone isolated in South Africa and Australia. It is therefore, important to implement continuous monitoring and surveillance to obtain epidemiological data for a specific clinical setting.

OP 83

Abstract Number : 118

Capsular types and sub-types of group b streptococcus in colonised pregnant women and neonatal invasive diseases at dr george mukhari academic hospital, pretoria.

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Background:

Group B *streptococcus* (GBS) is a gram positive, catalase negative, beta hemolytic encapsulated bacteria. It is widely recognized as a leading cause of perinatal morbidity and mortality worldwide. It forms part of normal flora of the gut and genital tract and is found in up to 40 % of healthy adults especially pregnant women. In most cases, neonatal GBS infection is acquired during pregnancy or delivery. Based on the capsular polysaccharide antigen, GBS is classified into ten distinctive capsular-types. Serotypes (Ia, Ib, II - IX) correlates with pathogen virulence and clinical prognosis. Epidemiological studies that determine the proportion of capsular-types in a given population for the development of capsular base vaccine are important. Although antibiotics are effective in treating group B streptococcal infection, the best long term intervention for prevention and control of GBS infection is an effective vaccine.

Objectives:

To evaluate the prevalence of individual GBS serotypes at the Dr George Mukhari academic hospital, Ga-rankuwa Pretoria

Methods:

The samples (n=413) which constitutes of rectal, vaginal swabs from pregnant women; ear and umbilical swabs from newborns were collected over a period of 11 months. Samples were cultured and GBS was identified using morphological and biochemical tests. Capsular typing was done using Strep-B-latex (SSI) Denmark.

Results:

GBS colonization was detected in 49.6% (205/413) of pregnant women. Sixty two (62) isolates were typed and capsular types III, Ia, IV (21%, 19% and 12.9) respectively, were found to be prevalent. The study is still ongoing.

Conclusion:

This study shows that there is a high prevalence of GBS among pregnant women attending the antenatal clinic at the Dr George Mukhari academic hospital and serotypes detected include capsular types III,Ia and IV .

OP 84

Abstract Number : 120

A clinical analysis of C. difficile infection at Groote Schuur Hospital

Miss. T Brock[1]
1 - None Yet

Background

Clostridium difficile infection (CDI) is a potentially fatal gastrointestinal disease of increasing medical significance worldwide. However the incidence in South Africa at present is unknown. *C. difficile* is a spore-forming, multidrug-resistant, toxin-producing anaerobic bacterium and is the leading cause of hospital-acquired, antibiotic-associated diseases. The two main virulence factors are toxins A and B (cytotoxins), which are released in the intestine causing symptoms ranging from mild diarrhoea to pseudomembranous colitis. Various strains, such as the

recent PCR ribotype 017 and the hyper-virulent ribotype 027, are responsible for outbreaks worldwide. This is a clinical study analysing the validity of the methods currently used to diagnose CDI at Groote Schuur Hospital in Cape Town and determine the prevalence of *C. difficile* within the Gastrointestinal Unit of the hospital.

Methods

Stool samples from symptomatic patients were screened for CDI using two types of immunoassays: the ImmunoCard and the MiniVidas, which are the current methods used at the hospital. They were compared to recent PCR-based methods: the GeneXpert and the Hain test. Culture methods using three types of selective media, followed by PCR screening for toxigenic *C. difficile* were conducted in parallel with the diagnostic methods. The strain-type of *C. difficile* was determined using PCR ribotyping. Further sub-genotyping was done using multi-locus variable-number tandem-repeat analysis (MLVA).

Results

The diagnostic sensitivities of the ImmunoCard, MiniVidas, GeneXpert and the Hain test were 37.5%, 50%, 90.6% and 87.5%, respectively. *C. difficile* was isolated from 34 of the stool samples (23%). Toxigenic-types A-B+ and A+B+ were identified, as well as the non-pathogenic A-B- type. PCR ribotype 017 was predominant (47%) among the isolates, while ribotype 027 was absent. Remaining isolates were of various ribotypes. MLVA is currently underway to study further the epidemiology of *C. difficile* isolates.

Conclusion

The study showed that the currently used ImmunoCard and MiniVidas methods are not reliable for screening *C. difficile*. The GeneXpert method is recommended for CDI diagnosis. *C. difficile* ribotype 017 predominated. This pilot study is a first step in establishing the prevalence of *C. difficile* strains responsible for disease across South Africa.

OP 85

Abstract Number : 142

Mucin degradation by clinical isolates of Bacteroides fragilis from Groote Schuur Hospital, Cape Town

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1 - University of Cape Town 2 - None Yet

Background

Bacteroides fragilis is an anaerobic bacterium that is present in the human gastrointestinal tract (GIT). Through microbial-host interactions, the bacteria may function as either a commensal or pathogen. The interaction of the bacterium with the mucosal layer of the GIT is crucially important for colonisation, nutrition and virulence. Pathogenicity is initiated when the organism escapes though discontinuities of the mucosal layer which leads, ultimately, to bacteraemia and abscess formation.

This study aimed to determine whether *B. fragilis* can degrade mucin, the major component of the GIT mucosal layer, and to identify the enzymes involved in this.

Methods

Clinical isolates of *B. fragilis* (n = 23) were screened for mucinolytic capabilities using a plate degradation assay. Growth curve analyses were performed using glucose or mucin containing media to determine whether *B.* fragilis could utilise mucin as a sole carbon source. Cell associated and secreted proteins extracted from bacteria grown in a rich medium were analysed for mucinase activity using mucin zymography. The ability of mucin to induce mucinase activity was determined using bacteria grown in minimal media supplemented with mucin and subsequent mucin zymography. The mucinases were subjected to inhibition assays using a serine protease inhibitor (PMSF) or a metalloprotease inhibitor (EDTA).

Results

All the *B. fragilis* isolates degraded mucin as measured by the plate assay, and mucin could function as a sole carbon source for cell growth. Individual proteins of ~90 kDa and ~115 kDa were shown by zymography to possess mucinase activity. The mucin degradative capacity of these proteins was not enhanced by the presence of mucin in the culture medium, and additional novel mucinases were not induced under these conditions. Significant inhibition of activity of the ~115kDa protease was evident when the protein was treated with a metalloprotease inhibitor.

Conclusions

B. fragilis can degrade mucin through the activity of at least two distinct proteins which are produced constitutively under the conditions tested. The ~115kDa mucinase is possibly a metalloprotease due to its response to inhibitors. Its identity will be further investigated using MALDI. Targeting these mucinases may prove to be an option in combatting pathogenicity.

OP 86

Abstract Number : 147

Clostridium perfringens as a possible cause of enteritis in ostrich (Struthio camelus) chicks in the Western Cape Province, South Africa

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Background

Ostrich (*Struthio camelus*) chicks less than three months of age are observed to experience a high mortality rate of 30-40% that is often associated with enteritis. This study was undertaken to investigate different bacterial agents implicated in enteritis of ostrich chicks.

Methods

Samples were collected during August-March 2011 and 2012 from farms surrounding Oudtshoorn, in the Western Cape Province, South Africa. Post mortems were done on 122 ostrich chicks from one day to three months of age. Bacterial culture was performed and PCR toxinotyping of *C. perfringens* isolated from the samples was carried out. Another PCR was performed on the *C. perfringens* isolates to detect the *netB* toxin gene. Three *netB* toxin gene PCR amplicons were sequenced and analysed to determine the similarity between the sequences obtained in this study and those published in Genbank.

Results

Escherichia coli (49%) was the most frequently isolated from the samples followed by *C. perfringens* (20%), *Enterococcus* spp. (16%) and *Salmonella* spp. (7%). Other less significant bacteria and samples where no bacteria were isolated constituted 8% of samples. The majority (93%) of *C. perfringens* were type A and only 7% were type E. *Clostridium perfringens* type B, C and D were not present. The *netB* toxin gene was identified from 16% of *C. perfringens*. All *C. perfringens* type E harboured the *netB* toxin gene and 10% of the *C. perfringens* type A had this gene. The BLAST results indicated that the sequence for the *netB* toxin gene had 94% similarity to the sequences available on the National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). The sequences of the three PCR products, that were sequenced, were identical to each other.

Conclusions

The results suggest *C. perfringens* plays a role in the occurrence of enteritis in ostrich chicks, involving *C. perfringens* type A and E (with the possible influence of the *netB* toxin gene). This is the first report of *C. perfringens* type E and of the *netB* toxin gene in the enteritis of ostrich chicks. *Escherichia coli,* and *Salmonella* spp. are other bacterial agents identified in this study that may be involved in causing enteritis.

OP 87

Abstract Number : 148

Characterisation of zoonotic Gram-positive bacteria prevalent in slaughtered pigs and abattoir workers in Gauteng, South Africa

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Background

Bacterial species such as *Enterococcus* (*faecium* and *faecalis*) and *Staphylococcus aureus* (particularly methicillin-resistant *Staphylococcus aureus*) cause diseases well recognized in humans, livestock, companion and zoo

animals. The emergence of antibiotic resistant Gram-positive bacteria has caused complications in the health care settings. Most of the research to determine the prevalence of these species in abattoir settings has been conducted in Europe and the USA. However, the prevalence of these *Staphylococcus* and *Enterococcus* spp in pigs and human contacts in South Africa is unknown. The purpose of this study was to determine the characterisation of zoonotic Gram-positive bacteria prevalent in slaughtered pigs and abattoir workers in Gauteng, South Africa.

Methods

Nasal and rectal swabs were taken from 40 pigs from five farms in the Gauteng region (Total 200 pigs). Self collected nasal swabs from all volunteers (abattoir workers) were collected. The boiling method was performed for a DNA extraction followed by multiplex PCR assays for the detection of the species specific and resistance genes.

Results

Preliminary results showed that the 16S rRNA and *nuc* genes were detected in 13 isolates indicating *Staphylococcus aureus.* No MRSA (*mec*A) gene was detected and none of the *S. aureus* isolates were positive for the *luk*S/F (PVL) gene. One *E. faecium* isolate tested positive for the *van*A gene indicating vancomycin resistance.

Conclusion

The pathogens present in these agricultural settings could possibly transfer resistant genes to those present in the community. Precautionary measures should be taken when working with animal products to prevent the transmission to humans and to prevent the transfer of resistant genes between these bacteria, which may lead to an increase in resistance genes in clinical pathogens.

OP 88

Abstract Number : 216

Metagenomic and culture dependent discovery of bioactive compounds from South African endemic marine sponges.

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1 - University of the Western Cape

Background.

Bioactive natural products, such as alkaloids, terpenoids polyketides &small peptides, are the basis for almost all pharmaceuticals brought to market. These commercially and medically important chemicals are often derived from marine invertebrates or their symbiotic bacteria.

Methods.

In this project we recovered over 600 isolates from 4 endemic sponge species (*Waltherarndtia caliculatum*, *Spongia sp*.001RSASPN, *Guancha sp*. 001RSASPN &*Hamacantha esperiodied*) using a suite of 21 isolation media. As culturing techniques can only access approximately 1% of the bacterial species present, a metagenomic approach is also being taken. Metagenomic DNA has been isolated from the 4 endemic sponge species for the construction of cosmid and fosmid libraries. In the first instance libraries will be screened using degenerate PCR to identify clones with conserved domains in known biosynthetic pathways (PKS and NRPS). After conjugation into other host strains (*Streptomyces coelicolor*&*Pseudomonas putida*) the libraries will also be functionally screened against *E. coli* 1699 for the production of potentially novel bioactive natural products.

Results.

Our isolates have been screened for antimicrobial activity against 5 test strains, resulting in 26 positive hits, representing a hit rate of 4.6%. Most interestingly is the activity produced against the *E. coli* 1699 test strain. This strain is resistant to over 50 commercially available antibiotics, suggesting that 3 of these hit strains could be producing highly novel bioactive natural products.

Conclusion.

South African marine sponges harbour a wide range of natural product producing bacteria. Here we have sustainably accessed several of these biosynthetic pathways through culture-dependent and culture-independent methods. Several of these natural products may be novel and of medical use.

OP 89

Abstract Number : 179

A Meta-phylogenomic Approach to Investigate Functional Diversity of the Surface-Associated Microbiome of Splachnidium rugosum.

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Background

The microbiome associated with marine macroalgae has a significant impact on the lifecycle, health and physiological development of its host. Intertidal macroalgae and their bacterial consorts are often subjected to "extreme" abiotic fluctuations. While the microbiome is characterised by a niche genetic repertoire, community structure and composition as well as function and specific roles of bacteria are largely unknown. In order to gain better understanding of surface-associated microbial communities and (bio) chemical interactions with algae, microbial community composition was investigated using a metagenomic approach combined with next generation sequencing. Metabolic (functional) potential of the microbiome was studied through the construction and screening of metagenomic libraries as well as analysis of shotgun sequencing of the metagenome. Sequence data may link functional genes to biological networks and may assist in the elucidation of community structure.

Methods

A metagenomic DNA extraction method was developed to allow for the isolation of inhibitor-free, high molecular weight DNA the microbiome associated with *Splachnidium rugosum*. DNA was size fractionated and fragments above 23 kb were excised. The gel slice was GELase treated and precipitated DNA was resuspended in TE. The size fractionated DNA was end repaired, ligated overnight and packaged using the EPICENTRE Fosmid library construction kit. The 16S rRNA gene marker was amplified using barcoded FLX adapters with the primer set 27F-534R covering the V1 to V3 hypervariable regions and sequenced with Macrogen.

Results

Surface associated HMW bacterial DNA was successfully extracted from *S. rugosum* and used to construct a fosmid library of 120 000 colonies with an average insert size of 23 kb. The metagenomic library covers approximately 890 bacterial genomes. End sequencing revealed gene products potentially involved in core energy metabolism, sulphur and nitrogen assimilation, transporters/permeases, non-ribosomal peptide synthatases and various membrane associated proteins.

Conclusions

The *S. rugosum*'s microbiome community is distinct from that of the surrounding sea water yet displays variability on individual hosts. Furthermore, the associated microbiome is a rich genetic resource that can be exploited for commercial ventures.

OP 90

Abstract Number : 4

Quantitative Detection and Characterization of Human Adenoviruses in the Buffalo River in the Eastern Cape Province of South Africa

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Buffalo River is an important water resource in the Eastern Cape Province of South Africa. Over a 1-year period (August 2010-July 2011), we assessed the prevalence of human adenoviruses (HAdVs) at a total of 6 sites on the river and three dams along its course. HAdVs were detected by real-time quantitative PCR in about 35 % of the samples with concentrations ranging from 1.2×10^1 genome copies (GC)/l to 4.71×10^3 GC/l. HAdVs were detected at 5 of the 6 sampling sites with the detection rate ranging from 8.3 % at Rooikrantz Dam to 92 % at Parkside. The HAdV concentrations across the sampling sites were as follows: Parkside (3.25×10^2-4.71×10^3 GC/); King William's Town (1.02×10^2-4.56×10^3 GC/l); and Eluxolzweni (1.17×10^2-3.97×10^2 GC/l). Significantly ($P < 0.05$) higher concentrations were detected at the non-dam sites compared to the dam sites. A very low mean concentration of 1.86×10^1 HAdV GC/l

was observed at Bridle Drift Dam. While HAdVs were detected only once at Rooikrantz Dam (1.74×10^1 GC/l), no HAdV was detected at Maden Dam. Epidemiologically important serotypes, Ad40/41, constituted 83.3 %, while Ad21 made up 16.7 % of the all HAdVs detected and were characterized by qualitative PCR. The Buffalo River presents a public health risk heightened by the presence of Ad 40/41 and Ad21. Our results make imperative the need for assessing water sources for viral contamination in the interest of public health. This work is a significant contribution to the molecular epidemiology of adenoviruses and to the best of our knowledge this is the first report on detection of enteric virus from surface waters in the Eastern Cape.

OP 91

Abstract Number : 155

Status and molecular identification of arbuscular mycorrhizal fungi (AM fungi) on rehabilitated gold and uranium mine tailings

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Background

Phytoremeditation of mine tailings provides the most cost-effective means of alleviating their pollutant effects, including that of heavy metal contamination. However, research has shown that successful revegetation of mine tailings can be optimized by providing appropriate microbial symbionts for the plants. Legumes of the genus *Acacia* may grow as woody trees or shrubs and are suitable plants for tailings rehabilitation as they form a tripartite association with both nitrogen-fixing bacteria and mycorrhizal fungi. Mycorrhizal fungi form symbiotic interactions in or on host plant roots whereby the fungus is provided with soluble carbon sources from the host plant and in turn provides the host plant with an increased capacity to absorb water and nutrients. The aim of this investigation is to compare the arbuscular mycorrhiza fungal (AM fungal) status of indigenous *Acacia* species from two different phytoremediation trials on gold and uranium tailings in the Welkom area of the Free State gold fields and to establish the diversity of the AM fungi.

Methods

The two trials represent naturally colonised acacias versus those inoculated with a crude inoculum of the fungi. Propagation of all AM fungal species occurring in the soil was done by trap cultures. The analysis involved measuring root colonisation levels, fungal spore numbers and molecular techniques for identification of the AM fungi from root and spore samples collected in early spring.

Results

Total AM fungal colonisation of initial samples was found to be 19% for inoculated acacias and 66% for naturally colonised acacias. The total AM fungal colonisation of propagated samples for inoculated acacias increased to 32% and for naturally colonised acacias it increased to 78%.

Spore counts of initial samples determined that on average 402 spores were present per 100 g soil for inoculated acacias and 455 spores were present per 100 g soil for naturally colonised acacias. For propagated samples spore counts decreased by approximately 50%.

Molecular data is still being analysed.

Conclusion

Preliminary results show higher fungal sporulation and colonisation levels from the naturally colonised site and successful PCR amplification of spore and fungal tissue DNA has been achieved using a nested PCR approach.

OP 92

Abstract Number : 16

Expression of a recombinant PbrR metalloregulatory protein for immobilization on multi-walled carbon nanotube screen printed electrodes

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Background:

Acid mine drainage (AMD) from the mining industry poses a serious environmental problem. AMD leads to ecological destruction and contamination of human water sources by sulfuric acid and heavy metals, including arsenic, copper, and lead. Current treatment and detection technologies of heavy metals in AMD are either inefficient or too costly to be employed at most abandoned mine land sites which are sources of untreated AMD. There is a need for a cheap, sensitive technique to detect and quantify heavy metal pollutants in AMD. This study aims to investigate the modification of multi-walled carbon nanotube screen printed electrode (MWCNT-SPE) with recombinantly expressed PbrR metalloregulatory protein for detection of lead in water.

Method:

The full length gene PbrR was synthesized and inserted into the vector pGEX-3X. GST tagged PbrR protein was expressed in BL21 Escherichia coli cells and purified using Promega's MagneGST™ protein purification system. Recombinant protein was confirmed by SDS-PAGE and western blot using an anti-GST HRP-conjugated antibody. Binding assays were performed to confirm the ability of recombinant PbrR to bind lead ions in solution, pre and post immobilization on MWCNT-SPE.

Results:

The PbrR protein has been successfully expressed in BL21 E. coli cells and confirmed by SDS-PAGE and western blot using anti-GST antibodies. Binding assays with purified PbrR need further optimization to determine appropriate concentrations of protein to be used in downstream applications.

Conclusions:

Modification of disposable MWCNT-SPEs with PbrR could find potential for use as a biosensor to detect bioavailable lead in water samples.

OP 93

Abstract Number : 144

Synergistic actions of various GH family mannan-degrading enzymes on galactomannan substrates

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Background

Liquid fuels generated from plant biomass feedstock have been regarded as a promising field in terms of scientific and economic feasibility to replace the depleting petroleum-based fuels. Elucidation of the enzymatic degradation of lignocellulosic biomass is very important for the development of a sustainable bioethanol industry. Limited research has been carried out on mannan-degrading enzymes in this respect.

Methods

This study investigated the synergistic behavior of mannan-degrading enzymes, specifically focusing on differences between enzymes from different glycoside hydrolase (GH) families. The GH family classification of enzymes will affect the catalytic activity of the enzymes, the substrate specificities for different mannan substrates, the synergistic interactions with other glycoside hydrolases and end product patterns. Commercial α-galactosidases from guar seed (Aga27A, GH 27) and *A. niger* (AglC, GH 36), and β-mannosidases from *B. thetaiotaomicron* (*Bt*Man2A, GH 2) and *C. mixtus* (*Cm*Man5A, GH 5) were evaluated for their ability to synergistically interact with a β-mannanase from *A. niger* (*An*Man26A, GH 26) to hydrolyse galactomannan substrates.

Results

Both heterosynergy combinations at the protein ratios *An*Man26A 75: AglC 25 and *An*Man26A 75: Aga27A seemed to be efficient at locust bean gum hydrolysis when applied either simultaneously or sequentially. The homeosynergy combinations between *An*Man26A and *Bt*Man2A, and *An*Man26A and *Cm*Man5A, at the ratio of 75 to 25%, respectively, only cooperated synergistically when applied sequentially on locust bean gum. None of the combinations exhibited synergy on guar gum hydrolysis when applied simultaneously. Synergistic enhancement of guar gum hydrolysis was observed when 75% dosage of *An*Man26A was supplemented with Aga27A at 25% sequentially.

Conclusions

The results showed that α-galactosidases display better synergistic interactions with the *A. niger* mannanase in the hydrolysis of galactomannans (guar and locust bean gum). Guar gum hydrolysis by the enzyme combinations considered proved to be more recalcitrant compared to locust bean gum, this being due to the differences in the extent and pattern of galactose substitution on the two substrates. Sequential application of the enzyme combinations rendered higher reducing sugar yield compared to when the enzymes were applied simultaneously.

OP 94

Abstract Number : 262

Acclimatization of saccharomyces cerevisiae to elevated temperature and ethanol

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Background

Saccharomyces cerevisiae is the microorganism of choice for bioethanol production. During fermentation the yeast cells face various stress factors which may have a negative impact on growth and ethanol yields. The aim of this study, was to develop a more robust strain by acclimatizing yeast cells to elevated temperature and ethanol levels.

Materials and Methods

A *Saccharomyces cerevisiae* strain obtained from Illovo Sugar Limited was subjected to sequential and combined (co-stress) exposure to temperature ranges of 35 - 42^{o}C and ethanol concentrations of 10-15%. This was done sequentially in a stepwise manner and co-stress which consists of simultaneous exposure to ethanol and temperature stress conditions.

The fermentative ability of *S. cerevisiae* mutant strains was carried out in fermentation media consisting of 50 g/L glucose temperatures of 35^{o}C, 40^{o}C and 42^{o}C for 24 hours. Sampling was carried out at three different times, namely, T_0, T_9 and T_{24}. Aliquots of 1 ml were taken, centrifuged and supernatant -20ºC for quantitative analysis of residual sugars, ethanol, acetic acid, glycerol and trehalose using High Performance Liquid Chromatography (HPLC).

Results

Sequential mutants obtained were able to grow at 42°C and 40°C in a maximum ethanol concentration of 15% (v/v). Co-stress mutants grew at 41°C and 16% (v/v) ethanol. The fermentative ability of the presumptive mutants was assessed using shake flask fermentation at 35°C and 40°C. At 35°C the original strain produced higher ethanol levels than the sequential mutants but equivalent and slightly lower ethanol than co-stress mutants. At 40°C the sequential mutants produced higher ethanol levels 34% higher than the original strain. Both co-stress mutants produced 28% lower ethanol than the original strain. At 40°C the original strain experienced a lag during the first 9 hours presumably because the strain was acclimatizing to the high temperature conditions.

Conclusion

Sequential exposure was more effective in the development of robust yeasts that tolerated higher temperature conditions and retained their fermentative ability. This is quite promising for use of these robust *S. cerevisiae* mutant strain in industry.

OP 94

Abstract Number : 178

A Uniquely South African Microalgal Isolate - Powering Brains Since 2010

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Background.

Omega-3 fatty acids are of specific interest due to their role in brain health and development. Omega-3 fatty acids are generally obtained from fish oil extracted capsules purchased from pharmacies. Interestingly, fish acquire the fatty acids by ingesting microalgae capable of naturally synthesising these fatty acids *de novo*. The combination of expensive supplements, decreasing fish stocks and an increase in vegetarianism warrants the need for an alternative source of

omega-3s. In response to this, CSIR Biosciences launched the Microalgal Bioprospecting Program with the objective to screen and select candidate organisms capable of producing omega-3s.

Methods.

Microscopic fluorescent analysis and flow cytometry was used to rapidly screen and isolate for lipid rich isolates. Selected isolates were subjected to a growth (nutrient replete) and stress (nutrient deplete) study in order to compare lipid production during these two phases. Samples were taken on a daily basis for cell count and omega-3 measurements. Fatty acid content was analysed via the fatty acid methyl ester (FAME analysis) method.

Results.

Screening for novel omega-3 producing microalgal isolates yielded 2 potential organisms capable of synthesising the fatty acids. Growth rates of 0.413, 0.166 and 0.366h^{-1} were obtained for CSIR isolates A23.2, A27.2 and *Phaeodactylum tricornutum* (control organism) respectively. Biomass productivities were reported as 5 x 10^6, 1 x 10^6 and 4 x 10^7 cells.mL^{-1}.day^{-1} for A23.2, A27.2 and *P. tricornutum* respectively. Fatty Acid analysis for the stress study revealed specific omega-3 productivities of 23.1, 19.1 and 13.6 pg.$cell^{-1}$.day^{-1} for A23.2, A27.2 and *P. tricornutum* respectively.

Conclusions.

The high growth rate of A23.2 along with specific omega-3 productivity indicates the potential of the organism for substantially higher growth and EPA productivity under appropriate culture conditions in comparison with the control. It is recommended that further studies be conducted on isolate A23.2; this unique organism auto flocculates and can therefore potentially eliminate costly biomass separation processes.

OP 95

Abstract Number : 129

Increasing the Scale of Peroxidase Production by Streptomyces sp. strain BSII#1

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Background

Peroxidases are enzymes capable of oxidising a broad range of organic and inorganic substrates to produce many useful compounds or remove unwanted pollutants in waste streams. However, their potential usefulness has been limited by the relatively high isolation and purification costs of the enzyme in comparison to other commercially available oxidative enzymes. Aside from the basidiomycetous fungi, actinomycetes, which are well-known antibiotic producers, are known to secrete extracellular peroxidases into their natural environment. A major aim of this work was to develop a method for the cheap and reliable production of peroxidase from an actinomycete.

Methods

Streptomyces sp. strain BSII#1 was identified from a previous screening program as a potentially good peroxidase producer (1.30±0.04 U ml^{-1} in 10 ml culture volume) and scale-up investigations were implemented. *Streptomyces* sp. strain BSII#1 was grown in different culture volumes using different bioreactor systems: in 100 ml and 400 ml (baffled shake flasks), in 500 ml (bubble column bioreactor), 3 L (airlift bioreactor) and 6 L (stirred tank bioreactor). Peroxidase activity was assessed daily using the 2,4-dichlorophenol assay.

Results

Highest peroxidase production was achieved with the airlift bioreactor (4.76±0.46 U ml^{-1}) employing a complex production medium (pH 8) and incubating at ambient temperature. Maximum peroxidase production was lower for the other fermentation configurations tested, ranging from 0.26±0.08 U ml^{-1} (400ml baffled flasks) to 1.71±0.49 U ml^{-1} (bubble column bioreactor).

Conclusion

This study shows, to the authors' knowledge, the highest peroxidase production by an actinomycete recorded. Good aeration and gentle mixing were recognized as important requirements for successful production of peroxidase by *Streptomyces* sp. strain BSII#1. The study also shows that peroxidase production by an actinomycete can be accomplished on a scale feasible for industrial application.

OP 96

Abstract Number : 176

Biopreservative activity of bacteriocins of lactic acid bacteria isolated from spontaneously fermented milk (Amasi)

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Background

Biopreservation is a sustainable and a more natural way of food preservation as compared to the use of synthetic chemical food preservatives. Bacteriocins of lactic acid bacteria are perceived to be potential food preservatives due to their antimicrobial activity and the GRAS status of the lactic acid bacteria. This study investigated the biopreservative activity of bacteriocins of lactic acid bacteria isolated from fermented milk.

Methods

Bacteriocins were partially purified from the lactic acid bacteria cell free supernatants utilising saturated ammonium sulphate purification. Biopreservative activity of the bacteriocins was investigated against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* and *Klebsiellapneumoniae* in fresh milk food system. Data was analysed through independent-samples T test. The 16S rRNA gene sequence analysis was used to identify the lactic acid bacteria isolates.

Results

The lactic acid bacteria isolates were found to be in the *Lactobacillus* genus. The following isolates exhibited biopreservative activity with $p < 0.05$; LAB 1 was active against *Escherichia coli, Staphylococcus aureus*, LAB 3 was active against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiellapneumoniae*, LAB 4 was active against *Salmonella,* LAB 10 was active against *Escherichia coli*, LAB 12 was active against *Escherichia coli, Staphylococcus aureus, Salmonella* and *Klebsiellapneumoniae* and LAB 14 was active against *Escherichia coli.*

Conclusions

Bacteriocins of lactic acid bacteria isolates had potential to act as biopreservatives in a food system and can be used as substitutes for synthetic chemical preservatives in future.

Key words: Biopreservation, Lactic acid bacteria, Bacteriocins, fermented milk, food preservation

OP 97

Abstract Number : 65

Characterization of Lactobacillus fermentum Kh09, a bacterioncin producing strain isolated from sour milk (madila).

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Background

Minimally processed foods are increasingly gaining favour from health conscious consumers because of the possible adverse health effects of food treated with chemical additives/preservatives. Bacteriocins produced by Lactic Acid Bacteria (LAB), are natural preservatives or bio-preservatives that are considered to be safe. Therefore, the objective of the study was to identify a bacterial strain isolated from *madila* (traditionally fermented sour milk) and characterise its bacteriocin producing potential.

Methods

Standard microbiological methods as well as the API 50 CHL V 5.1 system (bioMérieux, France) were used to identify the isolate. Identification of the bacterial strain was confirmed by partial sequencing of 16S rRNA. The Bacteriocin was partially purified using ammonium sulfate precipitation and the Hitrap ion exchange column. The spectrum of activity of

the bacteriocin and sensitivity to heat and various enzymes was determined using agar diffusion bioassay. Plasmid curing was done by treating the strain with different concentrations of novobiocin. The molecular size of the bacteriocin was elucidated using sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Results

The bacterial isolate from sour milk (*madila*) was identified as *Lactobacillus fermentum* and given the strain number Kh09. The bacteria was found to produce a bacteriocin with antimicrobial activity against *Bacillus cereus,* a food borne pathogen, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Bacillus subtillis*. The antibacterial activity of the supernatant was inhibited by the following proteases, pepsin and trypsin, revealing the bacteriocins protein nature. The bacteriocin was found to be approximately 6.5kDa in size, thermo-stable and genes chromosomally encoded.

Conclusions

The bacteriocin produced by *Lactobaccillus fermentum* Kh09 has potential application as a biopreservative, and the strain can possibly be used as a probiotic formulation in indigenious or commercial fermented food products such as, sour milk (*madila*/*amasi*), fermented porridge (*ting*), sour maize beverage (*mageu/mahewu*) and yogurt.

OP 98

Abstract Number : 250

The impact of yeast and lactic acid bacteria interactions on malolactic fermentation and wine flavour

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Background

Malolactic fermentation (MLF) is a secondary fermentation occurring during wine production that confers desired microbial stability, softens the palate and contributes to the aroma and flavour of wine. Malolactic fermentation is conducted by lactic acid bacteria (LAB) and is necessary for the production of red, certain sparkling and white wines. In addition to using the standard wine yeast, *Saccharomyces cerevisiae*, the use of non-*Saccharomyces* yeasts to add complexity and improve wine quality is increasing in popularity. The objective of this study was therefore to investigate the interaction between *Saccharomyces*, non-*Saccharomyces* yeasts and LAB, and the resulting impact on MLF and wine flavour profile.

Methods

The interactions between *Metschnikowiapulcherrima,Candida zemplinina*, *Hanseniaspora uvarum,Torulaspora delbrueckii* and *Lanchancea thermotolerans* and a commercial MLF culture, Viniflora oenos, were investigated in Chardonnay, Shiraz and synthetic wine under standard winemaking conditions. Juice and wine samples were analysed and microbial counts were also performed. Chardonnay and Shiraz wines were sensorially evaluated by descriptive analyses.

Results

The effect different non-*Saccharomyces* yeast species and strains have on the progression of MLF in Chardonnay, Shiraz and synthetic wine, vary. The yeast strains had an effect on the growth of LAB during fermentation and on the progression of MLF in Chardonnay and Shiraz wines. *L. thermotolerans* strain, Y0560 delayed MLF in Chardonnay and Shiraz wines when used as a co-inoculum. Wines produced with *Saccharomyces* and non-*Saccharomyces* yeasts not having undergone MLF were sensorially different from wines having undergone MLF. There were also differences between wines that were co-inoculated for MLF and wines that were sequentially inoculated. Shiraz wines were significantly different in 'colour', 'berry' and 'vegetative' aroma, and 'body/mouthfeel'.

Conclusions

These results highlight the importance of yeast and LAB selection, and how this choice can affect the progress of MLF and wine flavour.

OP 99

Abstract Number : 297

Performance Evaluation of New Molecular Technology for the Detection of E. coli O157 in Food

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*Escherichia coli*is a member of the *Enterobacteriaceae* family, which comprises some of the most important enteric pathogens. Although most *E. coli* do not cause gastrointestinal illnesses, certain groups can cause life-threatening diarrhea and severe disability. Many innovative technologies are being used for the microbiological analysis of foods. Unlike traditional methods that rely on specific media to select and grow pathogens, developments in molecular biology have led to innovative methods that are easier and more rapid to perform, requiring only minutes to complete. The 3M™ Molecular Detection System (MDS) combines two technologies - isothermal DNA amplification and real-time bioluminescence detection - in a robust platform that is expected to be less prone to matrix interference. This study was conducted to evaluate the performance of the new system following recommendations from the AOAC guidelines for validation of microbiological methods. Samples of foods were spiked with *Escherichia coli* O157 (at 1-10 CFU/25g) and with *Escherichia coli* O103 and *Enterobacter aerogenes* (10-100 CFU/25g). Several food categories, including meat, fish and seafood, fruits and vegetables, dairy products and miscellaneous were evaluated. Each food product was divided into four 25g samples: (1) negative control, (2) spiked with *E. coli* O157, (3) spiked with *E. coli* O103 and *E. aerogenes*, and (4) spiked with all three organisms. All 120 samples were enriched and incubated according to the manufacturer's recommendation. After incubation, the samples were analyzed in duplicate using the 3M MDS. No matrix interference was observed for any of the food products tested. From 240 tests conducted, the new technology demonstrated 99% repeatability and 99% accuracy. The 3M MDS was found to be not only a suitable, but also a practical, rapid, and sensitive method for the detection of *E. coli* O157 even in the presence of related organisms.