KP 2

Three decades of developments in our understanding of the microbiology of biomining

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A selection of significant discoveries concerning the microbiology of biomining over the past thirty three years with which the author has been involved will be reviewed. Early steps in the development of the Biox® stirred tank process for the recovery of gold from arsenopyrite ores from laboratory work carried out in the early 1980s up to construction of the first full-scale processes will be described. This will include the development and adaptation of microbial inocula for such processes and that besides selection for arsenic resistance, selection for rapid growth in a continuous-flow system was almost certainly an important, though little recognized, time-consuming adaptation requirement. How the use of molecular techniques led to the discovery that *Acidithiobacillusferrooxidans* did not play a major role in continuous-flow biooxidation tanks and why this is so will be discussed. Molecular techniques also clarified our understanding that some of the microorganisms that were initially recognized as being major role players in many bioleaching operations, such as *At. ferrooxidans*, *Acidithiobacillusthiooxidans* and *Leptospirillumferrooxidans* consisted of more than one species and that the species initially identified were, in many cases, not the main role players. The track record for the development of genetic systems for some of the main microbial role players in bioleaching will be briefly described as well as some of what has been learned during this development. This includes evidence that families of plasmids with a common DNA backbone exist within isolates of certain bacterial species even though the isolates originated from different continents. However, the accessory genes that they carry may be very different. The view that genetically manipulated microorganisms are likely to play a very limited role in tank and heap leaching operations that are open to the environment will be defended. In conclusion, some of the history of the debate concerning the direct vs indirect mechanisms of bioleaching will be briefly reviewed as well as how the idea of 'contact leaching' has helped to resolve that debate. The presentation will cover many aspects of basic and applied microbiology using research on biomining as a theme.

KP 3

Biological Consequences of Functional Amyloids in Yeast Adhesins

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Yeast cell adhesion proteins have conserved amyloid-forming sequences, including *C. albicans*Als proteins and *S. cerevisiae* Flo flocculins. Exposure of these amyloid sequences leads to assembly of the adhesins into cell surface clusters called amyloid nanodomains. Nanodomains have been detected *in situ* on fungal cell surfaces within abscesses in autopsy sections from candidiasis victims. Amyloid nanodomains also activate anti-inflammatory responses in *C. elegans* and humans. The adhesins in nanodomains show greatly increased avidity for ligands, a result of the clustering of the adhesins on the surface. Nanodomain formation is triggered by force-induced unfolding of amyloid domains under turbulent flow in a vortex mixer or under laminar flow. The resulting strengthened binding mediates fungal aggregation, flocculation, and biofilm formation. Conversely, perturbation of the amyloid formation inhibits nanodomain formation and activation of cell adhesion. Thus the amyloid sequences in yeast adhesins serve as force-sensitive switches to activate cell adhesion and biofilm formation.

KP 4

Emerging challenges of microbial water quality in South Africa: a clarion call for the review of current guidelines and problem of shortage of skilled manpower in the water sector

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ABSTRACT

Water is the most abundant compound on our planet earth. Its usefulness cuts across every facet of our existence, from domestic usage through to world economy and security. Though water constitutes 70% of our planet, only 1% of this is available as freshwater and is recognized as a scare resource in South Africa. However, protection of this important resource has become a major global challenge especially amongst developing countries including South Africa, more so with a growing population and increased urbanisation, coupled with the apparent inability of most local authorities to effectively treat urban and industrial wastewaters. Even so, the problem of inadequate skilled manpower in the water sector is a recurring decimal suggesting the urgent need for collaboration between the water sector, government and academia. Also, the increasing incidences of emerging and re-emerging microbial pathogens in water; their survival strategies in conventional treatment processes; evidences suggesting increasing incidences of resistance to regular disinfection regimes; bioprospecting aquatic microbial diversity for new bioactive compounds in water treatment to replace current compounds that are being implicated in hazards; and the need for review of existing water quality guidelines to capture new emerging trends such as wastewater effluents as reservoirs of antibiotic resistance becomes imperative and will be discussed at length in this presentation.

KP 5

Abstract Number : 40

Monitoring comparative transcriptional changes in a susceptible and tolerant cultivar of cassava infected with South African cassava mosaic virus using next- generation sequencing

Prof. C Rey[1] ,F Allie[1] ,M Okoniewski[2] ,E Pierce[1]
1 - Wits 2 - ETHZ

Background

Transcriptional reprogramming following virus infection takes place at a global level, both temporally and spatially within the plant leaves and other organs, and depending on the outcome, a resistance or susceptible response is initiated. Cassava mosaic disease is caused by *several distinct geminivirus species, including South African cassava mosaic virus-[South Africa:99](SACMV).*

Methods and Results

A global transcriptome profiling (RNA-seq) study over the infectivity time course (12, 32 and 67 days post inoculation), using the ABI SOLiD platform, was performed in order to monitor comparative transcriptional responses to SACMV in a susceptible (T200) and tolerant (TME3) cultivar. Infectivity assays showed significantly higher viral loads (quantitative RT-PCR) in susceptible T200 compared with tolerant TME3, at 32 and 67 dpi. Viral DNA was not detected in TME3 at the pre-symptom stage (12 dpi), but was detected at 32 dpi (full systemic infection), and declined at 67 dpi, correlating with recovery (symptomless leaves). Paired-end NGS sequencing run produced an average of 634,081,313 paired end reads from both susceptible and tolerant libraries. Of this, approximately 51.89% of the T200 reads and 52.81% of TME3 reads mapped to the cassava reference genome available at Phytozome (www.phytozome.net). Using a log2-fold cut-off ($p < 0.05$), comparative analysis between the six cDNA libraries identified a total of 4181 differentially expressed transcripts in T200 (compared to mock inoculated) across 12, 32 and 67 dpi, whereas TME3 had a total of only 1008 SACMV-responsive transcripts across the same time course of infection. Dynamic gene expression changes were observed in both T200 and TME3 at different time points post-infection where a limited number of genes were expressed across all time points, while uniquely up- or down-regulated transcripts occurred at different infection phases for both cultivars. Gene ontology annotation analysis identified alterations in several functional groups, the top three being subcellular localization, protein binding function and metabolism.

Conclusions

Differences in patterns and levels of transcriptome profiling between T200 and TME3 with susceptible and tolerant phenotypes, respectively, supports the hypothesis that viruses rearrange their interactions in adapting to hosts with different genetic backgrounds.

KP 6

Abstract Number : 168

Engineering yeast expression systems and optimizing cellulase synergy to improve yeast strains for cellulose conversion

Dr. R Den Haan[1] ,S Van Zyl[1] ,Mr. J van Zyl[1] ,H Kroukamp[1] ,Prof. W van Zyl[2] ,T Harms[1]
1 - Stellenbosch University 2 - University of Stellenbosch

Background:

Lignocellulose is an abundant, renewable feedstock for the production of commodities such as fuels and chemicals, if low-cost technologies can be developed to overcome its recalcitrance. Hydrolysis of cellulose is achieved by the synergistic action of endoglucanases, exoglucanases and β-glucosidases. Most cellulolytic microorganisms produce a varied array of these enzymes and the relative roles of the components are not easily defined or quantified. Various cellulases have been produced heterologously in the yeast *Saccharomyces cerevisiae* but complete conversion of crystalline cellulose was not achieved. A greater understanding of the interaction of the cellulases as well as improved levels of secretion is imperative to enabling complete cellulose conversion by this yeast.

Methods:

In this study we have used partially purified cellulases produced heterologously in *S. cerevisiae* to increase our understanding of the roles of some of these components. CBH1 (Cel7), CBH2 (Cel6) and EG2 (Cel5) were separately produced in recombinant yeast strains. Binary and ternary mixtures of the enzymes at loadings ranging between 3 and 100 mg/g Avicel were assayed. Strains were also engineered to allow for higher levels of secretion of the heterologous cellulases through genome shuffling and rational design strategies.

Results:

Binary and ternary mixtures of the enzymes allowed us to illustrate the relative roles of the enzymes and their levels of synergy. A mathematical model was created to simulate the interactions of these enzymes on crystalline cellulose, under both isolated and synergistic conditions. In addition, yeast strains were created where secretion levels could be increased up to 4-fold.

Conclusions:

The model can be used to predict the optimal synergistic mixes of the enzymes. This information can subsequently be applied to help to determine the minimum protein requirement for complete hydrolysis of cellulose. These advances will be greatly beneficial for the design of better enzymatic cocktails and processing organisms for the conversion of cellulosic biomass to commodity products.

KP 7

Structural Details of Human Tuba Recruitment by InlC of *Listeria monocytogenes* Elucidate Bacterial Cell-Cell Spreading

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Summary

The human pathogen *Listeria monocytogenes* is able to directly spread to neighboring cells of host tissues, a process recently linked to the virulence factor InlC. InlC targets the sixth SH3 domain (SH3-6) of human Tuba, disrupting its physiological interaction with the cytoskeletal protein N-WASP. The resulting loss of cortical actin tension appears to slackens the junctional membrane allowing protrusion formation by motile Listeria. Complexes of Tuba SH3-6 with physiological partners N-WASP and Mena reveal equivalent binding modes but distinct affinities. The interaction surface of the infection complex InlC/Tuba SH3-6 is centered on phenylalanine146 of InlC stacking upon asparagine1569 of Tuba. Replacing Phe146 by alanine largely abrogates molecular affinity and *in vivo*mimics deletion of *inlC*. Collectively, our findings indicate that InlC hijacks Tuba through its LRR domain, blocking the peptide binding groove to prevent recruitment of its physiological partners.

Keywords: Molecular mechanism of infection, engineering of protein-protein interactions

KP 8

Abstract Number : 132

Biodiversity and evolution of magnetotactic bacteria

Dr. C Lefèvre[1]
1 - None Yet

There are only few examples of protein- and lipid-bounded organelles among prokaryotes that are encoded by conserved gene clusters and lead to a specific function. The magnetosome chain represents one of those rare examples and is responsible for magnetotaxis in magnetotactic bacteria (MTB), a behavior thought to aid in finding their optimal growth conditions in aquatic environments. The origin and evolution of the magnetotaxis is still a matter of debate. Recent breakthrough in isolation, cultivation, single-cell separation and whole genome sequencing has generated abundant data that give new insights into the biodiversity and evolution of MTB. In this presentation, I will introduce the biodiversity, origin and evolution of this unique group of prokaryotes and show how they control the formation of their organelle responsible for magnetotaxis.

KP 9

Abstract Number : 304

Carbon utilization profile of a thermophilic fungus, Thermomyceslanuginosus, using Phenotype and DNA microarray

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[2] Advanced Studies in Genomics, Proteomics and Bioinformatics, University of Hawaii, Honolulu, Hawaii, USA
[3]Centre for Chemical Biology, UniversitiSains Malaysia, Bayan Lepas, Penang, Malaysia

Background:

The thermophilic filamentous fungus, *Thermomyceslanuginosus* produces the highest amount of xylanase reported. In addition to this, it expresses high levels of other enzymes that have been used industrially or have academic interest. Thus, this fungus has the potential to be applied for biomass conversion to produce biofuel or in other applications.

Methods:

In this study, the Biolog system was used to characterise utilisation and growth of *T. lanuginosus* on 95 carbon sources. For DNA microarray analysis, probes were designed for the 5105 genes found in T. lanuginosus with the coverage of over 80 times per genes using a 360 000 microarray slide.

Results:

The carbohydrate based compounds (sugars and oligosaccharides) showed the best utilisation profile, with xylose inducing the highest growth, followed by trehelose, raffinose, mannose turanose, fructose and glucose. Among the oligosaccharides, sucrose had the highest mycelium formation followed by stachyose, maltose, maltotriose, glycogen and dextrin. Interestingly the fungus also grew well on cellobiose suggesting that this fungus can produce ß-glucosidases. D-alanine promoted fungal growth while the other amino acids tested were similar to the control. To identify which genes were involved in the utilisation of xylose and glucose DNA microarray analysis was done using these compounds. Several known proteins were identified including xylanase and xylosidases but also relatively unknown genes were also involved.

Conclusions:

These results demonstrate the ability of this fungus to grow relatively well on most plant-based compounds. Thus making this fungus an ideal candidate for plant biomass conversion for a number of biotechnological applications, including biofuel production.

KP 10

Abstract Number : 200

Are antimicrobial peptides of LAB new age antibiotics? A look at their stability and possible delivery systems

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Background

The emergence of multi-drug resistant bacteria such as methicillin-resistant strains of *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum β-lactamase (carbapenemase)-producing Enterobacteriaceae is a serious threat. New generation antimicrobial agents and novel drug delivery systems need to be developed. Antimicrobial peptides of lactic acid bacteria, encapsulated in nanoparticles or nanofibers and directed to sites of infection, is a viable option.

Methods

Antimicrobial peptides were electrospun into nanofibers consisting of poly(vinyl alcohol) (PVA) and poly(D,L-lactide) (PDLLA). Skin wounds were inflicted on mice, colonized with a bioluminescent strain of *S. aureus* and covered with nanofiber dressings. Some wound dressings were modified by co-spinning with 2,3-Dihydroxybenzoic acid (DHBA). Changes in the metabolic activity (bioluminescence) of *S. aureus* were recorded by *in vivo* imaging. Cell numbers of *S. aureus* were recorded in dressings, and wounds were examined for histological changes. In another study, *S. aureus* infection was treated by incorporating an antimicrobial peptide into self-setting orthophosphate-based bone cement (CPC).

Results

Antimicrobial peptides were released from the nanofibers with an initial burst, but continued to diffuse from nanofiber dressings for at least 4 days *in vitro.* The metabolic activity and cell numbers of *S. aureus* from infected areas decreased drastically and the nanofiber dressings stimulated wound closure, without adverse changes in histology. Nanofibers containing 50 mg/ml DHBA inhibited the growth of *S. aureus*, but also *P. aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella typhimurium*. Lantibiotic-impregnated bone cement killed all cells of *S. aureus* and prevented infection.

Conclusions

Antimicrobial peptides are protected by the polymer scaffold in nanofibers and bone cement. This is the first step to the design of a target-specific drug delivery system. Coating of nanoparticles with ligands that recognises specific receptors on target cells is the next step into target-directed drug development.

KP 11

From cell biology to food security: Trending Interactions

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Causal effects for virusemergence have been difficult to establish in many cases but events related to human effects on the world's climate and ecosystems reduction in biodiversity, and increased frequency of contact between potential hosts and reservoir species due to increased global trade and travel have all been implicated in the emergence of plant, animal and human viruses. Like zoonotic animal viruses, plant viruses, particularly those with arthropod vectors, share the ability to jump species barriers, which results in their "emergence" into new populations of potential hosts, often with socioeconomic consequences. Additionally, mixed infections in plant hosts and selection pressures imposed by the use of genetic or engineered resistance in modern cultivars drives viral recombination andreassortment. Thus, the agricultural sector faces ever-greater challenges in preventing the spread exotic pathogens. Conventional means to control viraldiseases will increasingly integrate novel control strategies derived from the understanding of the molecular basis of plant-pathogen interactions. With this in mind, we have employed high-resolution *Nicotianabenthamiana* yeast two-hybrid and live-cell imaging technologies to develop a model for the cell-to-cell movement of plant-adapted rhabdoviruses. Comparisons of protein and membrane dynamics employed by closely related viruses are beginning to identify themes and variations in protein and membrane interactions as well as nuclear import pathways employed by closely related rhabdoviruses.

KP 12

Abstract Number : 112

The bacterial magnetosome, a versatile tool for biotechnological applications.

Dr. N Ginet[1]
1 – CNRS

Magnetosomes are perfectly crystalline magnetic nanoparticles that are biomineralised by a diverse group of prokaryotes, the magnetotactic bacteria (MTB). This organelle is made of a lipid vesicle loaded with a single magnetite or greigite crystal. Aligned within the cell, the magnetosome chain acts like a compass needle allowing the orientation of MTB along geomagnetic field lines. These biogenic lipid-coated permanent nano-magnets display high chemical purity, narrow size range, species-specific crystal morphology (from 35 to 120 nm). Purified suspensions of magnetosomes are readily obtained after cellular disruption and magnetic separation. Cells of magnetite-producing MTB and their magnetosomes have been envisioned in a variety of scientific, commercial, and other applications. This potential is substantiated by the possibility to genetically functionalise the magnetosome surface with peptides or proteins. In this presentation I wish to illustrate the potential of these biogenic nanoparticles with two examples of magnetosome-based biotechnological applications developed in our research team, namely the bioremediation of pesticides with suitably functionnalisedmagnetosomes and the development of a new class of sensitive contrast agents for high-field magnetic resonance molecular imaging *in vivo* in preclinical studies.

KP 14

Abstract Number : 35

Minor differences in sand physicochemistry lead to major differences in bacterial community structure and function after exposure to synthetic acid mine drainage

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1 - CPUT 2 - University of Pretoria 3 - Cape Peninsula University of Technology

Background

Acid mine drainage (AMD) emanates from sulphide-rich ores of abandoned mines flooded with groundwater, or mine tailings oxygenated with rainwater. AMD is characterized by a low pH and high concentrations of sulphate, iron and other dissolved metals. Neutralization of this waste can be accomplished by physicochemical and/or biological means. Systems requiring minimal intervention have been dubbed "passive" treatment systems and include treatment (constructed) wetlands, permeable reactive barriers and other bioreactors designed to enhance microbial sulphate reduction.

Methods

Synthetic acid mine drainage was added to pre-equilibrated, sand-filled experimental and control mesocosm replicates (1 m x 2 m) containing two different batches of silica-dominated (quartz) sand from the same quarry site. Glucose was added as a carbon source for microbial iron and sulphate reduction. Composite core sand samples were taken for bacterial DNA fingerprinting using terminal restriction fragment length polymorphism (T-RFLP), elemental chemistry and mineralogy using XRF and XRD, respectively and concentrations of micro- and macronutrients using ICP-OES. In addition, pH and Eh, chemical oxygen demand (COD), concentrations of ferric and ferrous iron, sulphate, sulphide and sulphite and concentrations of glucose and organic metabolites were determined in weekly effluent samples from each mesocosm.

Results

Complete neutralization of highly acidic (pH 2) AMD took place in all experimental mesocosms. No carbonates or aluminoslicates were detected in the sand, precluding physicochemical and supporting biological iron- and/or sulphate reduction as the primary neutralization mechanism/s. Significant differences related to sand batch were noted in the contribution of sulphate- and iron reduction to the biological neutralization of AMD. In mesocosms containing one batch of sand, the sand itself and the influent AMD had similarly significant effects on the bacterial community structure, while in the remaining mesocosms the effect of the physical substrate was more pronounced.

Conclusions

The importance of the physical substrate on the selection of functional microbial communities in systems remediating AMD should not be under-estimated. In treatment systems relying on microbiological processes, the physical substrate should be carefully selected and it may be prudent to include small-scale comparative studies in each particular setting prior to full-scale implementation.

KP 15

Abstract Number : 123

Bacterial taxonomy for the unintentional taxonomist.

Dr. C Bull[1]
1 - USDA, Agricultural Research Service

The three branches of taxonomy (Nomenclature, Classification, and Identification) are intimately intertwined though the work for each is accomplished through very different processes. Nomenclature is governed by a strict set of rules, which are applied to the naming of new or emended taxa. In contrast, Classification is accomplished through the scientific method and is governed by peer review and adoption is based on scientific opinion. Likewise, Identification is a scientific discipline governed by the scientific method but regulatory agencies can nullify scientific neutrality and dynamic scientific opinion by codifying methods required for identification of certain organisms. Microbiologists may first encounter the intricacies of taxonomy when they need to identify an organism they have begun to research. The experiments needed for an accurate identification are different than those needed if the organism is not neatly allocated to a previously classified taxon. Classification results in the proposal of novel taxa only after extensive comparison of the new organisms to related type or pathotype strains. The rules of nomenclature are applied if classification results in taxa needing names. The primary goal of bacterial taxonomy is to provide a universal means to communicate about bacteria. Although stability is a primary principle of taxonomy, the rapid changes in bacterial classification and the resulting associated name changes make identification and communication challenging to the casual user. Scientific neutrality dictates that each researcher must determine whether to continue to use the former classifications and nomenclature or to adopt newly published names given to taxa in novel classifications. Thus, each of us contributes to the scientific opinion about competing classifications through the use of the corresponding nomenclature for each classification.

KP 16

Abstract Number : 208

Disease Control in a Post-Antibiotic Era - Bacteriophage Therapy

Prof. R Bragg[1] ,W van der Westhuizen[2] ,Miss. J Lee[2] ,E Coetsee[2] ,C Boucher[2]
1 - None Yet 2 - University of the Free State

The increasing problems with antibiotic resistance can lead to a post antibiotic era. In animal production, this will occur sooner than later. Some antibiotics have already been banned for use in animal production and soon, antibiotics will not be used as growth promoters. Large scale production of poultry is currently highly dependent on the use of antibiotics, and if the use of antibiotics is banned, this will devastate the industry world-wide.

Options for disease control in a post antibiotic era include 1) improved biosecurity and 2) the use of bacteriophages as a treatment option.

Before the discovery of antibiotics, research was undertaken on the use of bacteriophages as a therapeutic option. With the discovery of antibiotics, research on bacteriophage therapy declined, but with the impending post antibiotic era, interest is once again turning to the use of bacteriophages for the control of bacterial diseases.

Results

This research groups have been investigating the use of bacterophages for the control of Avian pathogenic *E. coli*. In order to undertake this study, the first objective was to gain an understanding of the virulence genes. A multiplex PCR to detect 18 different virulence genes was set up. Using this test, the virulence profiles of various *E. coli* isolates were tested. Once this was done, the *E. coli*collection were screened with a collection of bacteriophages. Bacteriophages were identified by molecular techniques as well as by EM studies. It was established that bacteriophages are highly specific for different APEC strains. This could potentially hamper the long term use of bacteriophages. There would be a need for highly specific diagnostic services to ensure that the correct phages are used for any infections with *E. coli*. In order to overcome the very high specificity of the phages, attempts have been made to express various phage genes.

Conclusions

Bacteriophages show possibility of being used for treatment of bacterial diseases in poultry. The high level of specificity of bacteriophages could hold advantages as well as disadvantages. The use of expressed phage enzymes could hold the key to the use of bacteriophages in the future.

KP 17

Abstract Number : 161

The wine microbial consortium and its evolution during spontaneous fermentation

Dr. M Setati[1] ,B Bagheri[1] ,F Bauer[1]
1 - Stellenbosch University

Background:

The grape berry surface habours a diverse microbial community. The diversity and density of the population is affected by agricultural and viticultural practices. The natural yeasts on the grapes are the active biological agents that drive spontaneous fermentation and their dynamics is dependent on various factors including microbial interactions, SO_2 and ethanol tolerance. Our research evaluated the impact of pruning strategies and farming systems on grape associated diversity. In addition, the diversity and dynamics of autochthonous yeasts was monitored throughout fermentation.

Methods:

Grape must derived from a conventional, biodynamic and integrated vineyards, as well as shaded and exposed grape bunches were used for microvinifications. Yeast diversity was evaluated by direct plating followed with PCR amplification and sequencing of the ITS1-5.8S rRNA-ITS2 region. In addition, metagenomic DNA was extracted from grape must and sequenced using the Roche GS-FLX 454 platform.

Results:

The grape must from different vineyards exhibited high yeast diversity with more than 9 different species often isolated. Phylogenetic analysis of the metagenome from one of the vineyards revealed 84 fungal species comprising 22 yeast species including known wine yeasts. More than 50% of these were retrieved by cultivation. Amongst the cultivable non-*Saccharomyces* yeasts, members of the genera *Hanseniapsora* and *Candida* were found to be dominant and persisted until the middle of fermentation. *Saccharomyces cerevisiae* was below detection at the beginning of fermentation but rapidly increased and remained dominant until the end of fermentation. Different strains of *S. cerevisiae* were found present throughout fermentation.

Conclusion:

Our data demonstrate that farming systems and viticultural practices have a significant impact on fungal diversity. Although culture-dependent methods may underestimate the yeast diversity in the wine microbial consortium it could successfully be used in conjunction with other methods to provide a broad overview of yeast dynamics.

KP 18

Abstract Number : 22

Modern bioenergy and its potential role towards enabling a sustainable future for southern Africa

Prof. W van Zyl[1] ,Dr. R Den Haan[2] ,S Rose[1] ,M Viljoen-Bloom[1] ,A Chimphango[1] ,J Gorgens[1] ,J Knoetze[1]
1 - University of Stellenbosch 2 - Stellenbosch University

Bioenergy, particularly biofuels, have played a pivotal role in Africa in the past and could help address the need for energy expansion in the future, especially when considering up to 80% of African countries rely on traditional firewood to meet their energy needs for that Africa has to embrace modern bioenergy technologies with higher efficiencies. Lignocellulose is globally recognized as the preferred biomass for the production of a variety of fuels and chemicals that may result in the creation of a sustainable chemicals and fuels industry, with significant benefits in agricultural development, also avoiding the food versus fuel debate that is of particular importance in the African context. Within the African context bioenergy/biofuels production has to be integrated with food production to (i) provide local energy and (ii) promote food security by providing alternative markets, and very important, should be (iii) socially-beneficial to the rural population at large.

The Chair of Energy Research (CoER): Biofuels focuses on the technological interventions required to develop commercially-viable advanced (2^{nd}) generation lignocellulose conversion technologies to biofuels in Southern Africa. The CoER : Biofuels research program undertook to develop both biochemical (CBP yeast development) and thermo-chemical technologies for complete conversion of plant biomass to biofuels. These technologies will be discussed briefly, as well as innovative methods of process integration, in order to minimize the capital investment, maximize energy efficiency and improve overall economics. Some examples for energy integration between lignocellulosic conversion processes and adjacent industrial processes (including existing bio-based industries) to achieve more attractive financial returns, will be discussed.

Finally, the sustainable production of sufficient food and modern bioenergy/biofuels to enable social transformation in southern Africa will be contextualized in a common vision and road map established in close collaboration between Stellenbosch University, NEPAD as political implementation arm of the African Union, and the fast experience from the Bioenergy programme of FAPESP in Brazil, coordinated by the CoER: Biofuels.

KP 19

MIQE Guidelines: minimum information for publication of quantitative Real-Time PCR experiments

Dr. Stephanie Tobollik, Application Specialist Roche Applied Science, Germany

Abstract:

The real-time quantitative polymerase chain reaction (qPCR) has become the benchmark technology for the detection of nucleic acids in areas like microbiology, biomedical research, biotechnology, high throughput screening and in forensic applications. It is possible to achieve accurate and biologically meaningful quantification if meticulous attention is paid to the details of every step of the qPCR assay, starting with sample selection, acquisition and handling through assay design, validation and optimisation. The growing awareness of the need for standardisation, quality control and the significant problems associated with inadequate reporting of the assay has resulted in the publication of guidelines for minimum information for the publication of qPCR experiments (MIQE).

KP 21

Abstract Number : 269

Characterisation of Old Yellow Enzymes from Cryptococcus neoformans

Prof. C Pohl[1] ,R Ells[2] ,D Opperman[2] ,Mr. K Albertyn
1 - None Yet 2 - University of the Free State

Background

The pathogenic yeast, *Cryptococcus neoformans* can produce immunomodulatory eicosanoids, prostaglandin E_2 (PGE_2) and prostaglandin F_{2a} (PGF_{2a}), from exogenous arachidonic acid. A laccase enzyme (Lac1) has been identified to be involved in PGE_2 production in *Crypt. neoformans*, but downstream enzyme(s) responsible for PGF_{2a} production, is unknown (Erb-Downward et al., 2008). These authors speculated that, since it is known that an Old Yellow Enzyme (OYE) is involved in PGF_{2a} production in *Trypanosomacruzi,* OYEs might also play a role in prostaglandin $F_{2\alpha}$ production in *Crypt. neoformans*. This study characterized the OYEs of Crypt. neoformans and tested this hypothesis.

Methods

The three *Crypt. neoformans* genes encoding for OYEs were synthesized and cloned into bacterial expression systems, pET22b(+) and pET28b(+). The three OYEs have been successfully expressed with only two of them being in the soluble form, leading to successful purification of these proteins for further analysis. The optimal pH, temperature, enzyme concentration and substrate concentration were determined using cyclohexenone as substrate and NADH or NADPH as co-factor. In addition, the two purified OYEs were evaluated for activity towards arachidonic acid, PGH_2 and PGE_2 as substrates at the optimum conditions determined.

Results

The enzymes required different co-factors for optimum activity, however both had optimum activity at pH 7 and at 35^{o}C. Although the TLC analyses indicated that PGH_2 may be converted to PGE_2 by both these enzymes, co-factors were not metabolized when prostaglandins were used as substrates, therefore autoxidation of the substrates could not be excluded.

Conclusion

This is the study characterizing OYEs from *Crypt. neoformans* and although further studies are required to determine if they do contribute to prostaglandin production, their characterisation is a first step in obtaining more information regarding these important enzymes

Reference

Erb-Downward J..R., Noggle R.M, Williamson P.R. and Huffnagle G.B. (2008) The role of laccase in prostaglandin production by *Cryptococcus neoformans*. Molecular Microbiology 68: 1428-1437.

KP 23

Abstract Number : 161

The wine microbial consortium and its evolution during spontaneous fermentation

Dr. M Setati[1] ,B Bagheri[1] ,F Bauer[1]
1 - Stellenbosch University

Background:

The grape berry surface habours a diverse microbial community. The diversity and density of the population is affected by agricultural and viticultural practices. The natural yeasts on the grapes are the active biological agents that drive spontaneous fermentation and their dynamics is dependent on various factors including microbial interactions, SO_2 and ethanol tolerance. Our research evaluated the impact of pruning strategies and farming systems on grape associated diversity. In addition, the diversity and dynamics of autochthonous yeasts was monitored throughout fermentation.

Methods:

Grape must derived from a conventional, biodynamic and integrated vineyards, as well as shaded and exposed grape bunches were used for microvinifications. Yeast diversity was evaluated by direct plating followed with PCR amplification and sequencing of the ITS1-5.8S rRNA-ITS2 region. In addition, metagenomic DNA was extracted from grape must and sequenced using the Roche GS-FLX 454 platform.

Results:

The grape must from different vineyards exhibited high yeast diversity with more than 9 different species often isolated. Phylogenetic analysis of the metagenome from one of the vineyards revealed 84 fungal species comprising 22 yeast species including known wine yeasts. More than 50% of these were retrieved by cultivation. Amongst the cultivable non-*Saccharomyces* yeasts, members of the genera *Hanseniapsora* and *Candida* were found to be dominant and persisted until the middle of fermentation. *Saccharomyces cerevisiae* was below detection at the beginning of fermentation but rapidly increased and remained dominant until the end of fermentation. Different strains of *S. cerevisiae* were found present throughout fermentation.

Conclusion:

Our data demonstrate that farming systems and viticultural practices have a significant impact on fungal diversity. Although culture-dependent methods may underestimate the yeast diversity in the wine microbial consortium it could successfully be used in conjunction with other methods to provide a broad overview of yeast dynamics.