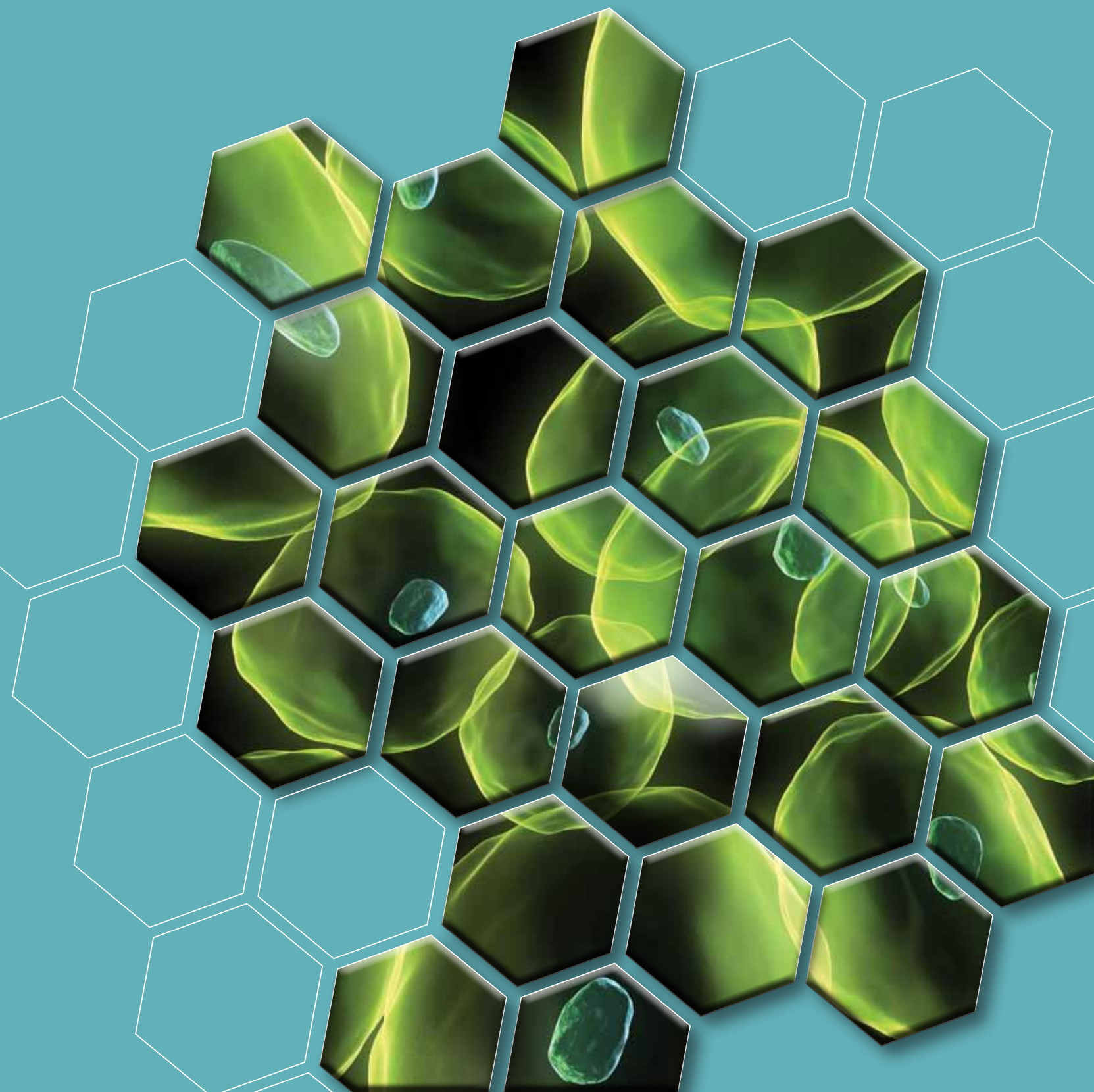


BIOTECHNOLOGY/MICROBIOLOGY



Desiré Barnard

University of the Western Cape

Mentor: Prof D Cowan

Broad research area: Biofuels

Specific research field: Elucidated the 2,3-butanediol pathway in a novel thermophilic organism

Purpose of study:

2,3-Butanediol has applications in various industries making it a very relevant and commercially applicable research topic. Applications include the synthetic rubber industry and the food industry. Additionally, the levo-2,3-BDL form is a potential antifreeze agent due to its low freezing point and the similarities between 2,3-BDL and other liquid fuels such as ethanol and methanol indicate 2,3-BDL serves as an effective fuel additive. While *K.oxytoca* is able to yield high concentrations of 2,3-BDL, it is unable to utilise polysaccharides. In comparison, *B.polymyxa* is able to ferment starch directly yielding 2,3-BDL and ethanol in almost equal amounts. This project aims at identifying the genes involved in the production of 2,3-BDL in 'in-house' strains. Once identified and characterised, *Geobacillus* would be targeted for transformation with these genes. *Geobacillus* is a model thermophilic bacterium containing thermostable enzymes capable of hexose catabolism into fermentable sugars substrates, requiring no temperature control during possible fermentation associated temperature spikes.

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Michael Bester

Stellenbosch University

Mentor: Prof F Bauer

Broad research area: Genetics, molecular biology

Specific research field: Regulation of yeast cell adhesion

Purpose of study:

Cellular adhesion of *Saccharomyces cerevisiae* significantly impacts on the fermentation process involved in the production of beer, wine and bio-ethanol. Of specific importance is the adhesion phenotype of flocculation, defined as the non-sexual, reversible and calcium (Ca²⁺)-dependent aggregation of yeast cells to form flocs that rapidly sediment in a liquid environment. This adhesion phenotype tends to naturally occur at the end of fermentation thus serving as a simple and cost effective way to separate yeast from the fermented product upon the completion of fermentation. However, strains display great variability in flocculation potential. Being able to

understand and better control flocculation has obvious importance for the fermentation industry. Another collection of adhesion phenotypes that are of great importance specifically to the medical field includes plastic adherence, invasive growth into agar media and the formation of pseudohyphae. These behaviours are associated with pathogenic yeasts such as some *Candida spp.* that causes infections in humans. Thus *Saccharomyces cerevisiae*, for which extensive molecular analysis tools have been developed, can serve as a model pathogenic system in order to better understand pathogenicity of other virulent yeast species.

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Maria Elizabeth Cawood

University of the Free State

Mentor: A Marston

Broad research area: Genetics, molecular biology

Specific research field: Bioactive metabolites from plants

Purpose of study:

In a general screening programme of South African Amaryllidaceae, the genus *Nerine* was found to be a good source of alkaloids with acetylcholinesterase-inhibitory activity. Fresh bulbs were extracted with 90% ethanol and tested for the inhibition of acetylcholinesterase in a rapid TLC benchtop bioassay. Isolation of the active compounds was principally achieved by high-performance centrifugal counter current chromatography (HPCCC). This is an all-liquid separation method, which unlike solid-phase separations, gives quantitative recovery of the sample injected. As a result crinane-type alkaloids with acetylcholinesterase-inhibitory activity were isolated from *Nerine laticoma*. The same species from different locations showed a wide variation in content of active natural products.

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Jude Chinedu Chukwujekwu

University of KwaZulu-Natal

Mentor: Prof J van Staden

Broad research area: Ethnobotany

Specific research field: Bio-evaluation of selected South African antimalarial and antimicrobial medicinal plants; isolation and structural elucidation of bioactive compounds

Purpose of study:

The development of drug resistance to currently available drugs

has necessitated the search for new classes of antimalarial and antimicrobial drugs. The current project involves the assessment of efficacy, safety and conservation status of selected medicinal plants used by traditional healers in the treatment of malarial and other infectious diseases. Following the preliminary screening of the plant extracts, different chromatographic methods are being employed for the bioassay guided isolation and purification of antiplasmodial and antimicrobial compounds from highly bioactive plants.

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Gareth John Everest University of Cape Town

Mentor: Dr P Meyers

Broad research area: Microbiology

Specific research field: Actinomycete molecular taxonomy

Purpose of study:

Currently 16S rRNA gene sequence analysis is the only molecular marker used in the phylogenetic characterisation of most actinomycete strains and DNA-DNA hybridisation (DDH) is considered the gold standard method for the definitive taxonomic delineation of a bacterial species. However, both have certain drawbacks. The 16S rRNA analysis cannot be used to differentiate between closely related species, due to its highly conserved nature; while DDH is time consuming, expensive (requiring specialist equipment) and results cannot be used to create a comparative database. Thus alternative phylogenetic marker genes that can allow differentiation between closely related strains would be highly valuable, as would be the development of an alternative, preferably sequenced-based, method to allow a rapid assessment of the novelty of an isolate, thereby replacing the need for DDH. My project aims to investigate the feasibility of multilocus sequence analysis (MLSA) that uses gene sequence comparisons of different housekeeping genes to estimate genome similarity, which can possibly be used to replace DDH for taxonomic delineations in the 25 genera belonging to the actinomycete family *Pseudonocardiaceae* (containing several genera that are well known for their ability to produce a range of antibiotics). The initial phase of this project involves determining the *gyrB* gene sequences from the 175 type strains within the family and evaluating the effectiveness of this gene to, firstly determine the phylogenetic relationships between the strains and genera in the family and, secondly to determine if a sequence analysis-based method can replace the need for DDH in the family. This would allow the quick

determination of the potential novelty of a new isolate and whether it would be worth undertaking further study (given that the best chances for finding novel antibiotics occur with novel strains).

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Cornelius Jacobus Franken Stellenbosch University

Mentor: Prof F Bauer

Broad research area: Molecular cell biology

Specific research field: Using yeast as a genetic model system to investigate the role of L-carnitine in oxidative stress

Purpose of study:

L-carnitine plays a well documented role in eukaryotic energy homeostasis by acting as a shuttling molecule for activated acyl residues across intracellular membranes. This activity, supported by carnitine acyl-transferases and transporters, is referred to as the carnitine shuttle. However, several pleiotropic and often beneficial effects of carnitine in humans have been reported that appear to be unrelated to shuttling activity, but little conclusive evidence regarding molecular mechanisms exist. We have recently demonstrated a role of carnitine, independent of the carnitine shuttle, in yeast stress protection. Here we show that carnitine specifically protects against oxidative stress caused by H₂O₂ and the superoxide-generating agent menadione. Surprisingly, carnitine has a detrimental effect on survival when combined with thiol-modifying agents. Central elements of the oxidative stress response, specifically the transcription factors Yap1p and Skn7p are shown to be required for carnitine's protective effect, but several downstream effectors are dispensable. A DNA microarray-based analysis identifies Cyc3p, a cytochrome c heme lyase, as being important for carnitine's impact during oxidative stress. These findings establish a direct genetic link to a carnitine-related phenotype that is independent of the shuttle system and suggests *Saccharomyces cerevisiae* should provide a useful model for further elucidation of carnitine's physiological roles. Current efforts are focused on exploiting this system in order to characterise and identify the molecular mechanisms involved in the oxidative stress related phenotypes that have been linked to carnitine. The upstream regulators of these processes are primary targets of present strategies that employ yeast genetic screens, biochemical affinity studies and also systems biological approaches to effectively unravel the genetic networks involved.

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Mpitloane Joseph Hato

Nha University

Mentor: Prof HJ Choi

Broad research area: Nanotechnology

Specific research area: Electro/magnetorheological characteristics and their applications

Purpose of study:

Soft magnetic carbonyl iron (CI) based magnetorheological (MR) fluids containing three different loadings of submicron-sized organoclay were fabricated. The MR characteristics were measured via rotational and oscillatory tests, in which the flow curves exhibited a non-Newtonian behaviour for all investigated samples. The dynamic yield stress change was measured as a function of magnetic field strength by adopting a linear fit of the relation of $\log(T_y)$ proportional to $\log(H)$, which was originally applied for electrorheological fluids. The viscoelastic performances of the pure CI suspension and the CI/organoclay suspensions showed the existence of a solid-like character. The sedimentation ratio was also investigated to confirm the role of sub-micron organoclay particles on the MR properties, in which the dispersion stability of pure CI was improved by increasing the content of organoclay in the CI suspension.

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Ju-Chi Huang

University of Johannesburg

Mentor: IA Dubery

Broad research area: Biological sciences

Specific research field: Analysis of microbe-associated molecular pattern (MAMP)-inducible RLK- and R-gene expression in *Arabidopsis thaliana*

Purpose of study:

Plants respond to stress conditions by the activation or repression of certain genes. The regulation of the expression of these genes is controlled by DNA-binding regulatory proteins, also known as transcription factors (TFs). TFs consist of various families grouped by their characteristics. NAC (no apical meristem, ATAF, cup-shaped cotyledon) proteins are one of the larger families of plant TFs. A function of the NAC TFs is that they regulate stress perception and share an N-terminal NAC domain. The conserved target DNA sequence has a CGT[GA] core. By performing a differential display on *A. thaliana callus* that was treated with several microbe-

associated molecular pattern (MAMP) molecules we isolated an EST of a gene encoding, a protein that exhibits similarity with NAM domain proteins. The gene encoding this putative NAM protein is currently being characterised by performing expression studies using elicitors at various time intervals. This could better elucidate the mechanism involved in pathogen perception.

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Robert John Huddy

University of the Western Cape

Mentor: Dr R Bauer

Broad research area: Biotechnology

Specific research field: Screening a compost metagenomic library for novel lignocellulases

Purpose of study:

Biofuels are currently recognised as the most viable renewable resource to replace fossil fuel derived energy. Agricultural plant waste, also known as lignocellulosic biomass, represents an alternative feedstock for biofuel production and is primarily composed of cellulose, hemicellulose and lignin. The complex and fibrous nature of lignocellulosic biomass makes them highly recalcitrant to enzymatic degradation, without initial high temperature pre-treatment steps. Consequently, the use of thermostable enzymes for the thermophilic pre-treatment of lignocellulosic biomass is attracting interest from a variety of industrial sectors, including those involved in biofuel production. Microorganisms are a promising source of novel biotechnologically important genes and gene products. However, it is believed that less than 1% of the bacterial species can currently be cultured using conventional approaches and methods. Metagenomics is a culture-independent approach to gene discovery that involves the cloning of total DNA from environmental samples into large clone libraries, which are screened and sequenced in order to analyse the genes contained within the library. The aim of this study is to construct and screen metagenomic libraries for the expression of thermophilic hemicellulases. A metagenomic fosmid library was constructed in *E. coli* using DNA extracted from compost samples ranging in temperature from 60–71.1°C. The library is comprised of 150 000 clones with an average insert size of approximately 31 Kb, representing 1 300 prokaryotic genomes. High-throughput screening was employed to screen approximately 140 000 clones for functional lignocellulosic enzymes. Over 100 clones displaying cellulase, xylanase or lipase/esterase activities were

identified during the primary screening phase. The majority of these lignocellulolytic enzymes are thermostable at 60°C. Next generation high-throughput sequencing technology has been used to identify the genes encoding these enzymes. The functional properties of any thermophilic heterologous enzymes isolated in this study will be further investigated for use in the bioconversion of plant biomass into a fermentable feedstock for biofuel production.

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Nobalanda Mabizela

CSIR

Mentor: N Moleleki

Broad research area: Protein expression and purification

Specific research field: Production of therapeutic peptides in *Y. lipolytica*

Purpose of study:

Production and purification of recombinant polypeptides often presents a challenge as these peptides are small in molecular weight. In this study we have taken advantage of Lip2 gene, a highly expressed gene in *Y. lipolytica*, to express proteins to the extracellular. Peptides were fused to Lip2 and expressed successfully in *Y. lipolytica*. Production of the fusion protein was optimised. The protein was purified which was followed by cleavage of the peptide from the reporter gene.

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Inonge Mulako

University of the Western Cape

Mentor: Dr R Bauer

Broad research area: Biotechnology

Specific research field: Identification of lignocellulose degrading enzymes for the purpose of bioethanol production

Purpose of study:

My research focuses on the identification of thermophilic genes that encode enzymes involved in lignocellulosic digestion. The objective of the project is to identify and clone a number of enzymes involved in the digestion of the lignocellulose components (cellulose, hemicellulose and lignin) for the purpose of biofuel production.

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Debra Rossouw

Stellenbosch University

Mentor: Prof F Bauer

Broad research area: Biotechnology

Specific research field: Comparative omics of wine microorganisms

Purpose of study:

The yeast *Saccharomyces cerevisiae* is an important component of the wine fermentation process and determines various attributes of the final product. However, lactic acid bacteria (LAB) are also an integral part of the microflora of any fermenting must. Various wine microorganism engineering projects have been endeavoured in the past in order to change certain wine characteristics, namely aroma compound composition, ethanol concentration, levels of toxic or allergenic compounds etc. Most of these projects focus on a specific gene or pathway, whereas our approach aims to understand the genetically complex traits responsible for these phenotypes in a systematic manner. Our aim is to create a comprehensive metabolic and genetic regulatory map for the modelling and prediction of fermentation parameters in complex winemaking conditions by following a 'systems biology' approach. Such an approach is based on the global analysis of all layers of biological information in a system, and offers the best option to explore the nature and regulation of complex phenotypic traits as well as interactions between various wine microorganisms.

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Natasha Sanabria

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Mentor: Prof I Dubery

Broad research area: Biological sciences, biochemistry, biotechnology

Specific research field: Profiling of plant receptor-like kinase (RLK) genes up-regulated during enhanced immune responses

Purpose of study:

Recognition of pathogen-associated molecular pattern (PAMP) molecules can occur through pattern recognition receptors (PRRs) on the surface of plant cells. Lipopolysaccharides (LPS) embedded in the cell wall of Gram-negative bacteria can trigger defence responses or prime the plant in order to respond more rapidly, following perception of bacterial pathogens. A receptor-like kinase was identified as a putative receptor for LPS. This RLK was

has now been further characterised as an S-domain, designated NS-RLK and has been shown to be LPS-inducible. Analyses of the effects of SA induction on NS-RLK expression have also been conducted in order to investigate the role of SA as a secondary messenger of NS-RLK, as part of the plant defence mechanism. Current studies include expression analyses using other PAMPs (e.g. flagellin and chitosan) as inducers of NS-RLK, by means of qPCR and HRM-qPCR. Preliminary HRM data showed a similar result between LPS and Chitosan treated samples, where the B-lectin domain of NS-RLK appears to be variably expressed. Changes between the expression of exons, of the same gene, indicate an inducible splicing event within the gene sequence. This finding could be pivotal in pathogen recognition and, subsequently, plant defence mechanisms associated with NS-RLK. Therefore, Northern blot analyses are currently being investigated.

The effects that other PAMPs may have on the S-domain RLKs associated with defence responses in other plant species was investigated. Some of the findings were published in a review article (Sanabria NM, Huang J and Dubery IA. 2010. *Self/nonself-perception in plants in innate immunity and defense. Self/Nonself* 1: 40-54). Orthologues of NS-RLK in crop plants have also been identified and are currently being investigated for crop-protection strategies. Specifically, tomato plants were treated with LPS and the orthologue gene expression was monitored. Future prospects include expression analyses in grapevine.

The identification of genes whose transcription is altered locally by LPS treatment will allow the development of screens to identify plant mutants that are insensitive to LPS. Resistance may then be achieved either by genetic modification or by using breeding programmes with a direct selection for these specific genes. Additional analyses to determine the sub-cellular localisation of NS-RLK protein and to determine whether or not LPS directly binds to the NS-RLK protein are also being investigated. An NS-RLK-GFP-fusion protein has been designed for transient expression using the Gateway system (Invitrogen). Future prospects include binding studies of the NS-RLK-GFP-fusion protein to labelled LPS via flow cytometry analyses.

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Dawn Elizabeth Stephens Technology Innovation Agency

Mentor: J Sakwa

Broad research area: Molecular biology

Specific research field: High-throughput technologies at the National Genomics Platform

Purpose of study:

The National Genomics Platform (NGP) is an initiative of the Technology Innovation Agency (TIA) and the Department of Science and Technology (DST). The NGP was founded with an objective of providing a high-throughput genomic research facility based on a partial cost recovery business model for South African researchers. To achieve these objectives the strategy at NGP is two-pronged, namely aligning our services with the research objectives of each client, and provision of an integrated basket of services to our clients that include assistance with business development of IP generated from each project. Scientists at the NGP are also actively involved in TIA and NRF mentorship and capacity development initiatives.

The NGP offers a cutting edge facility for research in genomics. At the core of our facility is the Roche Genome Sequencer FLX, Luminex 200 multiplex bioassay system and real time PCR light cyclers. The Roche GS FLX forms the backbone of the technologies housed at the NGP. The system uses novel pyrosequencing technology, which enables ultra-fast, accurate, cost-effective, high-throughput sequencing. Applications include *De novo* sequencing of whole genomes, massively parallel amplicon sequence generation, ultra deep amplicon sequence generation for detecting low frequency mutations, complete transcriptomics (tags, ESTs, full length cDNA), metagenomics and microbial diversity, etc. The Luminex200 system is a multi-analyte bioassay detection system capable of multiplexing up to 100 bioassays simultaneously in a single well. This system combines internally coloured microspheres, lasers, optics, fluidics, and advanced digital processing into a single, integrated system that increases assay specificity and throughput. The system delivers fast and cost-effective bioassay results on many assay formats including nucleic acid assays, receptor-ligand assays, immunoassays and enzymatic assays.

Core projects at the NGP are largely HIV- and TB-based, but also encompass environmental and agricultural research.

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Francesca Stomeo
University of the Western Cape

Mentor: Prof D Cowan

Broad research area: Molecular ecology

Specific research field: Microbial diversity of Antarctic dry valley soils.

Purpose of study:

My current project involves the use of molecular techniques to investigate the microbial diversity and structure of soil samples collected from the McMurdo Dry Valley in Antarctica. The samples were taken along an established vertical transect from both the north and south facing side of the McMurdo Dry Valley.

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