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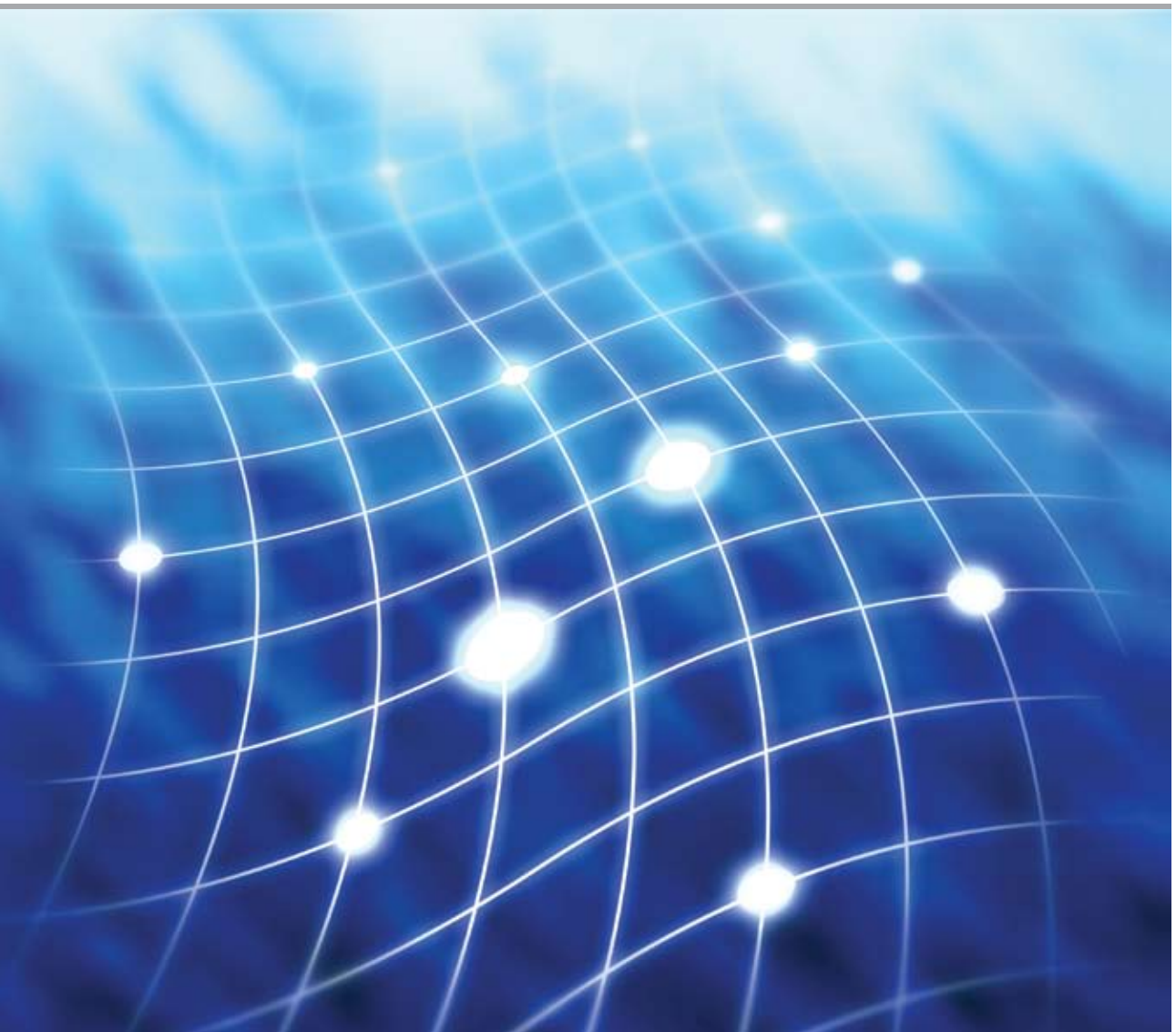

Ana Casanueva

University of the Western Cape

Mentor: Prof D Cowan

Broad research area: Gene discovery and metagenomics

Specific research field: Identification of oxidative enzymes for lignocellulose degradation

Purpose of study:

The majority of the world's energy supply is dependent on fossil fuels, such as coal, natural gasses and oil. All of these sources are non-renewable, finite and at the current rate will be consumed in only a few decades. An alternative source of energy is needed.

Such an alternative would be the production of bioethanol from cheap and renewable biomass such as lignocellulose. This is a complex structure composed of cellulose, hemicellulose and lignin. Enzymes such as laccases and peroxidases are needed to break down the different components of lignocellulosics into simple sugars that can then be fermented to bioethanol. Actinomycetes are known to contain these enzymes.

In this study mesophilic and thermophilic actinomycetes were identified containing laccase and peroxidase activities. Large-insert fosmid libraries of the 41 mesophilic actinomycetes were constructed and screened on solid media. No activities were identified, possibly due to the poor expression of actinomycete genes in E. coli. Laccase-specific primers were designed. Out of the 41 mesophilic strains, 7 amplified using the above primers. The PCR product was used as a probe for screening the library by colony blot. Several putative clones have been identified and will be analyzed further.

Large-insert libraries were also constructed in an E. coli-Streptomyces shuttle vector, to screen in Streptomyces for the enzyme activities, circumventing the E. coli expression problem. Transformation into the Streptomyces host is being optimized. Construction of E. coli-Streptomyces libraries of the thermophilic actinomycetes is being undertaken.

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Ayesha Parker

Cape Peninsula University of Technology

Mentor: Prof S Burton

Broad research area: Biocatalysis

Specific research area: Antioxidants

Purpose of study:

Antioxidants are small molecular weight compounds useful in the prevention of oxidative damage due to the free radicals generated by reactive oxygen species (ROS). ROS play a role in some deleterious physiological processes, such as aging and damage to skin by Ultra Violet (UV) radiation.

The laccase enzyme produced by the white rot fungus, *Trametes pubescens* has been successfully used in our research group for the biocatalytic production of novel antioxidant polymers with higher antioxidant properties than the monomeric phenolic starting compounds.

The aim of this research is therefore to assay novel antioxidants produced through biocatalysis reactions, for their antioxidant capacities in vitro and ex vivo by standard antioxidant assays, and to assess their protective properties against the damaging effects of UV radiation on cultured skin cells.

For the production of laccase, *T. pubescens* was grown in airlift reactors. The enzyme was purified from *T. pubescens* culture medium by 80 % ammonium sulphate precipitation and analysed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for the confirmation of the size and purity of the enzyme.

Non-immobilized laccase was used to synthesise polymeric species of ferrulic acid, and the products were analysed by Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-MS (LC-MS). Purified enzyme will be immobilized for the use in the biotransformation of antioxidant monomers in organic media.

Future work includes the immobilization of the purified enzyme for the use in the biotransformation of antioxidant monomers in organic media, the identification and purification of the reaction products as well as assessing the antioxidant properties of the various reaction products in vitro and assessing the protective effects in tissue culture.

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Carlo Bressa

Cape Peninsula University of Technology
Mentor: Prof S Burton
Broad research area: Biotechnology
Specific research field: Biocatalysis

Purpose of study:
The answer scientific researchers gave to the question on how to improve industrial production in terms of quality and quantity and, at the same time, how to become environmental friendly, is biocatalysis.

In the past 30 years the high availability of microorganisms in the environment associated with the possibility to make great scientific discovers drove researchers in biotechnology field to develop fast and safe selection and screening methods. On the other hand during these years investigation shifted progressively from the immediate surroundings to extreme environment and nowadays the emphasis is placed on attempting to redesign enzymes in order to tailor their properties.

Although, in the scientific literature, there are reports of many promising biocatalysts, making them suitable for industrial application takes time: certain industrial parameters must be met in order to implement the biocatalytic steps in production chains.

Our project, started from a successful genetic engineering modification of a thermostable nitrile hydratase from *Geobacillus pallidus*. The double mutant βF52GβF55L *G. pallidus* RAPc8 NHase showed activity towards both aliphatic and aromatic nitriles, while the "wild type" is aliphatic specific.

Our research focuses on the double mutant NHase properties and its potential in new suitable applications. We started studying the nitrile-degrading reaction with a range of substrates to define its chemio-, regio- and stereo- specificity.

A second step has been to investigate its feasibility as an industrial biocatalyst reporting the characterisation of the enzyme kinetics, respectively, in the production of amides fine chemicals or in the degradation of polluting nitrile compounds. The results we obtained add new information to develop further understanding of the active site mechanism of the nitrile hydratase.

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Dirk Stephan

Stellenbosch University
Mentor: Prof J Burger
Broad research area: Plant virology, molecular plant pathology, plant biotechnology
Specific research field: Virus induced gene silencing, resistance

Purpose of study:
In the presented work a grapevine (*Vitis vinifera*) infecting plant virus will be evaluated for its use as molecular tool for grapevine gene function studies by virus-induced gene silencing (VIGS) and delivery tool for antimicrobial peptides (AMPs). For these two different approaches, the grapevine infecting virus, Grapevine virus A (GVA, family *Flexiviridae*, genus *Vitivirus*) was modified in the laboratory to allow the integration of non-viral sequences.

For the GVA-based VIGS system the partial sequence of the grapevine phytoene desaturase (PDS) gene was integrated into the viral genome. After optimisation of the grapevine agro-inoculation procedure, the recombinant virus successfully silenced the PDS gene, visible by a photobleaching phenotype. The photobleaching was restricted to leaf veins, which reflects the movement limitation of GVA to the phloem tissue.

In a similar attempt to avoid the generation of transgenic grapevine plants and to allow the screening of more candidates in a short period of time, the grapevine infecting plant virus was modified to allow the expression of foreign proteins. This will give the possibility to pre-screen AMPs for their efficiency against several grapevine pathogens before the selection of an AMP expressing transgenic plant line is started. This will be the first attempt to express AMPs in plants by a viral vector. First results of the use of GVA as a protein expression vector and VIGS system for grapevine will be presented and discussed.

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Hanel Sadie-van Gijsen

Stellenbosch University
Mentor: Dr W Ferris
Broad research area: Biochemistry
Specific research field: Cell biology

Purpose of study:
Our group utilises a system of cultured rat adipose-derived stromal cells (ADSCs). These cells can differentiate (develop) into several other cell-types, including osteoblasts (bone-forming cells) and adipocytes (fat cells), and can therefore be used as a model system in which to study osteoblast and adipocyte differentiation, as well as the balance between these two processes.

In culture, osteoblastic differentiation can be induced by supplementing the standard culture media with ascorbic acid, glycerol-2-phosphate and 10 nM of the glucocorticoid dexamethasone (OM). Adipocytic differentiation can be induced by supplementing the standard culture media with insulin, indomethacin, isobutylmethylxanthine (IBMX) and 1 µM dexamethasone (AM).

The primary markers used to determine osteoblastic and adipocytic differentiation are extracellular matrix mineralisation (Alizarin Red S staining) for osteoblasts and intracellular lipid accumulation (Oil Red O staining) for adipocytes.

In addition, the expression of phenotype-specific transcription factors, including Cbfa1, Mx2 and osterix (for osteoblasts) and PPARgamma2 (for adipocytes) is also measured. Our current work focuses on differences in osteoblastic and adipocytic differentiation potential between ADSCs harvested from subcutaneous (scADSCs) and perirenal visceral adipose tissue (vADSCs).

We also study ADSCs harvested from rats that have been fed on a high-caloric diet. These rats become hyperphagic (over-eating), and as a result develop visceral obesity, hyperinsulinaemia and hyperlipidaemia, which are hallmarks of Western-type life-style diseases. Our results demonstrate that scADSCs have in vitro osteogenic potential, while vADSCs do not. scADCS from hyperphagic animals have reduced osteogenic potential, compared to scADSCs from lean animals. In addition, we have also found that vADSCs have greater adipogenic potential than scADSCs.

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Hussaini Makun

University of Johannesburg
Mentor: Prof M F Dutton
Broad research area: Biochemistry (toxicology)
Specific research field: Mycototoxicology

Purpose of study:
In a previous study [PhD project] Fusarium verticillioides (Sacc.) Nirenberg was demonstrated to be the novel, most toxic metabolites producing fungi contaminating rice in Niger State, Nigeria.

Mice and chicks were susceptible to the crude extract of the fungus and the organs injured were the liver, kidney and intestinal tracts of the animals. Total fumonisins quantified in the culture material of the fungus was 8.233 ppm, this quantity of toxin is not known to be acutely toxic to rodents as shown in the above studies which is indicative of the presence of other toxins.

This therefore necessitates the cytotoxicity evaluation and complete elucidation of the mycotoxins elaborated by this strain of fungus. Another comprehensive mycotoxins survey of rice from same state using PCR and DNA sequencing

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and high performance liquid chromatography will be more informative than the previous studies that employed basic techniques of macroscopic and microscopic methods for fungi identification and thin layer chromatography for mycotoxin detection.

This Postdoctoral Fellowship project will therefore be of two parts. The first is the cytotoxicity and elucidation of mycotoxins in the crude extract of *Fusarium verticillioides* while the second is the fungal and mycotoxin survey of rice in the Niger State, Nigeria.

The extract of the isolated *F. verticillioides* has been shown to have stimulatory effects on human lymphocytes and contains fumonisins B1, B2 and B3. In the second survey, various toxigenic fungal species and major mycotoxins (aflatoxins, ochratoxin A, deoxynivalenol, T-2 toxin zearalenone fumonisins) were found in Nigerian grown rice.

The projects will generate incidence data and profile of fungi and mycotoxins of rice grown in Nigeria, with a view to demonstrating the types of animal and human diseases expected from our diets and the extent of risk to these mycotoxicoses.

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John Stenson

University of Cape Town
Mentor: Prof S Harrison
Broad research area: Yeast cell wall structure
Specific research field: Understanding yeast cell wall structure to inform structure – function relationships in biological response of yeast to processes stresses and for manipulating intracellular product release

Purpose of study:
The yeast cell envelope is of critical importance in bioprocesses, affecting the impact of process stresses on yeast physiology, defining yeast resilience and controlling the release of intracellular products on altered cell permeability and cell breakage. The construction of the cell wall is a tightly regulated process where the polysaccharide composition, structure and wall thickness can vary considerably depending on the environmental conditions and the period during the cell cycle.

This work investigates the effect of the population age on the mechanical properties of the yeast cell. *Saccharomyces cerevisiae* reproduces asexually by budding that leads to a chitinous ring structure being formed at the point of cytokinesis in both the mother and daughter cells. In the mother cell this ring is a persistent bud-scar structure and the number of scars on a cells surface is indicative of the cell age. The birth scar is a much weaker structure appearing to increase in size with cell age and eventually becoming indistinguishable from the surrounding cell wall.

Age synchronised cell populations of the budding yeast *S. cerevisiae* were prepared using cell separation by rate zonal sedimentation in sucrose density gradients. Using this procedure it is possible to produce high yield populations up to the eighth generation with high purity. Fluorescence and confocal microscopy techniques have been used to accurately determine the yeast cell generation as well as providing information on the cell size and scar dimensions.

Cell breakage studies will be conducted on these separated populations using sonication and high-pressure homogenisation. The resultant protein and enzyme release profiles will be used to determine the influence of the generation age on the cell mechanical properties.

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Kark-Heinz Storbeck

Stellenbosch University
Mentor: Prof P Swart
Broad research area: Biochemistry
Specific research field: Cytochromes P450

Purpose of study:
South Africa is the world leader in mohair production, supplying approximately 55% of the global demand. South African Angora goats (*Capra hircus*) are the most efficient fibre producing, but least hardy small stock breed in Southern Africa. The industry is hampered by the severe loss of young, newly shorn Angora goats, which occur during cold spells. Investigations into cold-stress related deaths at first implicated the adrenal cortex and subsequently a single steroidogenic enzyme, cytochrome P450 17βhydroxylase/17,20 lyase CYP17, as the cause of the problem.

In mammals, CYP17, which is encoded by a single gene, plays a critical role in the production of mineralocorticoids, glucocorticoids and androgens by the adrenal cortex. Two CYP17 isoforms with unique catalytic properties have been identified in this study. A real-time-based genotyping assay was used to identify the distribution of the two CYP17 alleles in the South African Angora population.

These data revealed that the two CYP17 isoforms were not the product of two alleles of the same gene, but two separate CYP17 genes encoding the two unique CYP17 isoforms. This novel finding was subsequently confirmed by quantitative real-time PCR. Goats were divided into three unique genotypes which differed not only in the genes encoding CYP17, but also in copy number. Furthermore, in vivo assays revealed that the identified genotypes differed in their ability to produce cortisol in response to intravenous insulin injection.

This study clearly demonstrates the presence of two CYP17 genes in the South African Angora goat, and further implicates CYP17 as the primary cause of the observed hypocortisolism in this subspecies.

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Margaretha van der Merwe

Stellenbosch University
Mentor: J Kossmann
Broad research area: Natural sciences
Specific research field: Biotechnology

Purpose of study:
Small molecule regulation of plant growth and development results in a great deal of phenotype plasticity and metabolic redundancy. My work, concerned with the regulation of metabolism, will focus here on the role of primary metabolites and phytohormones, and how they attenuate growth responses. As an example, I will firstly discuss *Acacia longifolia* gall formation as a result of the encapsulation of the pteromalid wasp *Trichilogaster acaciaelongifoliae*. These galls result in carbon and nitrogen constraints in plant growth, reducing plant fitness and results in premature senescence.

This work will highlight the progress made to understand how this occurs. Secondly, novel plant growth promoting substances and/or phytohormones are constantly discovered. Here I will report on one such novel player in plant growth promotion and the molecular network that it participates in.

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Nuraan Khan

Cape Peninsula University of Technology
Mentor: Prof S G Burton
Broad research area: Biotechnology
Specific research field: Oxidative enzyme production from Actinobacteria

Purpose of study:
Peroxidases catalyse the peroxide-dependent oxidation of a wide range of organic and inorganic compounds. They are ubiquitous in nature, and are widely distributed intracellularly in plants, animals and microorganisms. Extracellular peroxidases, however, have only been identified in a few specific microorganisms including ligninolytic fungi and actinobacteria.

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The class *Actinobacteria* comprises a heterogenous group of bacteria that are widely distributed in natural environments. These organisms secrete a range of extracellular enzymes, including peroxidases that are believed to play a role in the degradation of complex organic compounds such as lignin. Unlike fungal peroxidases, extracellular peroxidases produced by actinobacteria are not as widely described in terms of either their structure or function.

In this study, actinobacteria have been isolated from a wide variety of environments including the Antarctic Dry Valleys, alkaline lakes in Ethiopia, hotsprings in Zambia, Namibian desert soils, termite guts, sea sponges and sea squirts from Algoa Bay and Marion Island, geothermal areas in New Zealand and a wide variety of other environments in South Africa. These isolates have been screened for peroxidase activity and the top peroxidase producers have been identified. The current focus of this project is to induce and optimize extracellular peroxidase production in the organisms identified.

The next step in the project is the purification and characterisation of these enzymes, which will be optimally produced under bioreactor conditions. Purified peroxidases will also be used in studies involving the degradation of lignin in agricultural feedstocks for biofuel production and in biocatalytic reactions for the production of useful antioxidants.

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Natasha Sanabria

University of Johannesburg

Mentor: Prof I A Dubery

Broad research area: Biological sciences, biochemstiry, biotechnology

Specific research field: Plant defense responses

Purpose of study:

This study investigates the profiling of plant receptor-like kinase (RLK) genes up-regulated during enhanced immune responses.

Current models regarding plant: pathogen interactions assume that 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 characterized as an S-domain, designated NS-RLK.

Extensive analyses are required in order to determine the exact role NS-RLK plays within an interactive signal transduction pathway, via the innate immunity or basal resistance models expressed in literature. Current studies include expression analyses using other PAMPs (e.g. flagellin or peptidoglycan) as inducers of NS-RLK. In addition, the effects that these other PAMPs have on the S-domain RLKs already reported to be associated with defence responses in other plant species, is being investigated.

Orthologues of NS-RLK in crop plants have also been identified and are currently being investigated for crop-protection strategies. 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 (using a fusion protein), and to determine whether or not LPS directly binds to the NS-RLK protein (using ELISA) are also being investigated.

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Samantha Gildenhuys

University of the Witwatersrand

Mentor: Prof H W Dirr

Broad research area: Protein biochemistry.

Specific research field: Protein structure-function and folding.

Purpose of study:

Functional three-dimensional proteins are produced during a process of folding form the one-dimensional amino acid sequence. Key amino acids direct folding while others play pivotal roles in structure and function. The role of these residues is assessed by site directed mutagenesis, following identification of the residues as conserved within the sequences of structurally and functionally related proteins.

Glutathione transferases (GST's) are one such group of proteins, consisting of monomeric members as well as dimeric detoxification enzymes. The dimeric enzymes conjugate the addition of glutathione to xenobiotic compounds within an active site composed of two binding sites a G- (glutathione) and H- (hydrophobic) site, each subunit possess one such active site. Arg15 a key residue situated at the interface of the G- and H- sites impacts on catalysis of the conjugation reaction through transition state stabilization and the binding of substrates (predominantly a the H-site not the G-site).

Mutation of Arg15 to Leu results in substantially diminished CDNB-GSH (1-chloro-2,4-dinitrobenzene to glutathione) conjugating activity of the enzyme, 3-fold decrease in affinity at the H-site for anionic non-substrate ligand 8-anilino-1-naphthalene sulphonate (ANS) and reduced affinity for anionic βcomplex formed between GSH and 1,3,5-trinitrobenzene (an analogue of the anionic transition state of the SNAr reaction between CDNB and GSH).

This mutation however has little effect on glutathione binding and protein structure and stability. Domain-domain interactions between the thioredoxin like N-terminal domain and alpha helical C-terminal of monomeric GST's produces cooperative folding. Domains are compact substructures within proteins that can play a role during folding of a protein to its native structure, once folded the interface between domains can produce active sites of enzymes. Folding of monomeric GST members such as glutaredoxin-2 where potential domain-domain interaction disrupting mutations have been created will provide information on key residues involved in folding.

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Sharath Govind

Stellenbosch University

Mentor: Prof M Vivier

Broad research area: Grapevine biotechnology.

Specific research field: Grape berry suspension cultured cells – a model to study grape berry ripening changes – a protemic approach

Purpose of study:

In addition to their economic importance, the consumption of grapes has numerous nutritional and health benefits for humans. There are more than 200 polyphenolic compounds that are anti-oxidants.

Therefore, for a variety of reasons there is a great scientific interest in manipulating grape berry development. The grape berry has a double sigmoid pattern of growth. Surge in genomic data led to a large scale tissue specific proteomic studies on berries sampled at single time point during ripening.

However, these studies mentioned above were limited to berries collected during the growing season. Thus, a more simplified model system which be used for the study of ripening related changes and also for large scale production of favorable compounds needs to be established.

Use of suspension cultured cells established from grape berries is therefore a viable alternative. The results we have obtained in terms of proteins identified and their expression profiles in part confirm proteomic studies carried out on whole berries.

These studies have also observed that most dramatic changes in terms of relative expression

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occur at Veraison. The data presented here from three different ripening stages and two growth stages suggests that berry cell cultures actually mimic berry related processes.

These studies have also observed that most dramatic changes in terms of relative expression occur at Veraison. Several other studies using iTRAQ labelling coupled with nanoLC/MS has proved to be more sensitive than gel–based electrophoresis with regards to quantitation but there was limited replicable detection of proteins among biological samples.

However, to get a tissue specific understanding of ripening processes in the berry cell cultures it is important to delve deeper by sub cellular fractionation including cell wall and the secretome. Detailed proteomic analysis with berry suspension cells and their sub fractions at different stages of ripening is the next step towards a complete understanding of protein expression related to berry ripening processes in model cell suspension culture.

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Algasan Govender

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Broad research area: Microbiology

Specific research field: Microbial bioremediation.

Purpose of study:

Chlorinated hydrocarbons are some of the most widely used and produced chemicals of the modern world. These carcinogenic compounds are used as solvents, chemical intermediates as well as fuel additives and are toxic to both terrestrial and aquatic ecosystems.

Accidental spills, poor handling and illegal dumping have resulted in contamination of the environment. Thus far several bacteria have been identified that are capable of utilising these compounds as a sole carbon and energy source.

Our study focuses on the isolation and characterisation of bacterial isolates from a chlorinated hydrocarbon contaminated site as well as a metagenomic analysis of the site. Samples obtained from this site are batch cultured in minimal medium containing various chlorinated hydrocarbons present in this site and repeatedly enriched.

A halogen release assay is performed in order to determine whether individual isolates possess the ability to cleave carbon-halogen bonds. Enzyme activities are confirmed with cell free extracts using all of the intermediates in proposed chlorinated hydrocarbon degradative pathways and compared to known chlorinated hydrocarbon degrading microorganisms.

Total DNA was isolated from contaminated borehole water and primers based on genes involved in both hydrolytic and reductive degradation of chlorinated hydrocarbons were synthesized. PCR analysis indicated the presence of both hydrolytic and reductive dehalogenases.

Future work involves the use of denaturing gradient gel electrophoresis (DGGE) of the 16SrRNA regions to determine the identity of the bacterial isolates. The metagenome will be sequenced and analyzed in order to identify novel gene sequences involved in degradation of chlorinated hydrocarbons. This overall aim of this study is to identify novel genes and bacteria involved in the degradation of chlorinated hydrocarbons as well as to provide insight into the overall strategy to be used to bio-remediate chlorinated hydrocarbon contaminated sites.

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Marilize Le Roes-Hill

Cape Peninsula University of Technology

Mentor: Prof S Burton

Broad research area: Microbiology and biocatalysis

Specific research field: Oxidative enzymes in actinobacteria

Purpose of study:

Unique environments and their microbial populations are constantly being exploited for novel secondary metabolites or enzymes with potential application in industrial processes. In the past 20 years there has been an increased interest in the oxidative enzymes produced by actinobacteria. Oxidative enzymes encompass a vast group of enzymes with varied catalytic abilities. Among them, the enzymes laccase, peroxidase and tyrosinase are known for their ability to catalyze oligomerisation, depolymerisation and hydroxylation reactions. These enzymes have successfully been used in our research group in various biotransformation reactions, notably using enzymes of fungal origin.

The aim of this study was to devise a screening program to isolate actinobacteria from unique environments and to screen them for potentially novel oxidative enzymes suitable for industrial application. A multi-faceted screening program was designed involving the use of direct assays, solid media assays and liquid media assays. The existing culture collections screened included actinobacteria isolated from deep-sea environments and those isolated from the intestinal tracts of an indigenous lower-order termite, *Microhodotermes viator*.

Others were isolated from soil samples collected from various areas in South Africa, copper-contaminated soil in the Zambian copperbelt region, the Bwanda and Gwisho hot springs in Zambia, from the dry Miers Valley, Antarctica and from the Namib Desert, Namibia. Symbiotic actinobacteria were also isolated from marine sponges and sea squirts collected from Algoa Bay and Marion Island. 16S rRNA analyses have revealed that laccase producers are ubiquitous in nature and are mostly represented by members of the genus *Streptomyces* and members of "rare" actinobacteria genera including *Actinomadura, Micromonospora* and *Verrucosispora*. Peroxidase producers (mostly *Streptomyces* spp.) were identified amongst the isolates from termite guts and the copper-contaminated soil, while the Antarctic strains were the predominant tyrosinase producers. Optimisation studies for enzyme production by the top enzyme producers are currently underway.

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