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ABSTRACTS

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Mitochondrial deletions and chromosomal abnormalities in human embryonic stem cells

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ABSTRACT

Human embryonic stem cells (hESCs) are derived from the inner cell mass of the blastocyst. HESCs are capable of indefinite undifferentiated proliferation in vitro while maintaining the capacity of giving rise to all derivatives of the three embryonic germ layers. Chromosomal aberrations and mitochondrial deletions mark deviations from a normal karyotype, giving rise to a problematic in the putative clinical applications of hESCs.

The present thesis is divided in two main topics: mitochondrial DNA (mtDNA) deletions and the analysis of gene expression within the context of a chromosomal duplication. To investigate mitochondrial aberrations in VUB cell lines we amplify and sequenced a mitochondrial region of 8 kilobases. We found 3 deletions in one VUB cell line and we propose a mechanism for determining the mutation load in hESC lines.

To assess a possible selective advantage arising from the duplication in chromosome 20 in hESC lines, we undertook a three way approach: reverse transcriptase PCR for comparison of transcript concentration of BCL2L1 isoforms, Real Time PCR for gene expression analysis and TUNEL stainings for detection of apoptotic cells. Based on knowledge a priori concerning the state of the samples in terms of 20q duplication, all techniques were performed in early and late passages of VUB cell lines. The results showed did not show a direct relation between BCL2L1 and the reported selective advantage.

Genetic analysis of the Osteopetrotic op/op rat

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ABSTRACT

Bone is a highly specialized and mineralized tissue which in connection with cartilage forms the skeleton. It acts as a support for muscle attachment, storage facility for ions growth factors and cytokines and protects vital organs. It undergoes growth, modeling and remodeling during life. Remodeling which is important for the maintenance of bone mass, repair of skeletal microdamage and mineral homeostasis depends on a balanced bone resorption followed by bone formation. If the two processes are quantitatively unequal, unbalanced remodeling occurs which may lead to decreased bone mass as in osteoporosis or increased bone mass as in osteopetrosis. Although osteoporosis is highly prevalent with 50 to 85% heritability compared to the rare osteopetrosis, it is polygenic. Most of the current knowledge of bone homeostasis has been obtained by the study of monogenic diseases such as osteopetrosis. Osteopetrosis represents a family of bone diseases characterized by osteoclast failure and impaired bone resorption. Many spontaneous and induced animal models of osteopetrosis exist. We studied the osteopetrotic *op/op* rat whose underlying genetic defect is unknown. We performed segregation analysis using SNPs within and in the vicinity of a previously delineated candidate region and we were able to decrease the distal region by 0.11Mbps and exclude the important functional candidate gene Atp6voc. We also performed a whole genome expression analysis. We were able to identify 11 interesting candidate genes, 8 of them based on their differential gene expression between differentiated *op* and differentiated normal littermate samples. Pdiff value of <0.05 was used for selection. The additional three genes were selected based on ontology. Expression analysis performed on 5 genes also showed that two of them are expressed in osteoclasts of which one was selected for mutation analysis. We further performed mutation analysis on exons and exon/intron boundaries of these 12 genes but we couldn't find any mutation. Based on this, we recommend further analysis of the genes in the candidate region by whole genome or exome sequencing using next generation sequencing platform.

Key words: Osteopetrosis, gene, segregation analysis, microarray, mutation analysis

Generation of *in vivo* Matured Nanobodies in High Troughput Mode

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ABSTRACT

The aim of this study was to generate and select nanobodies against a large number of antigens in high throughput, compatible with higher throughput approaches in proteomics, structural genomics and diagnostics. For this purpose, 94 purified antigens (including 8 membrane proteins) were obtained from the Structural Genomics Consortium at 1 mg/protein to immunize animals, to enrich binders by panning and to screen for antigen specific nanobodies against all targets.

For this purpose, two animals were immunized with varying cocktails of the 94 antigens. After six immunizations, both animals produced a good and diverse humoral response against almost all antigens. Phage display libraries were made from gel purified cDNA of mRNA obtained from B lymphocytes of these immunized animals. Phage particles were produced, libraries were pooled and enriched by high throughput panning against solid phase immobilized antigen. After two rounds of panning, 22 clones selected against each antigen were randomly picked and analyzed in ELISA to be specific for the antigen. Antigen specific antibodies were identified against 72 antigens out of 94 from two consecutive rounds of high throughput selection by panning.

In a control experiment, the individual libraries were also panned against 8 solid phase coated antigens (A9-H9) using two consecutive rounds of conventional panning. Selection outputs were remarkable and showed an overall increasing trend from round I to round II in the separate pannings as seen in the high throughput pannings. Similar to the high throughput experiment, 22 clones selected against each antigen were randomly picked and analyzed in ELISA to be specific for the antigen.

Finally, we also sequenced all antigen specific nanobodies against (A9-H9), selected by conventional pannings or in high throughput panning on the pooled libraries, respectively and grouped all related nanobodies in sequence families. Detailed analysis of all sequences shows that the numbers and the diversity of the antigen specific binders selected by conventional panning on the separate libraries or in high throughput panning on the library pool are comparable.

We conclude that *in vivo* matured nanobodies can be produced in a cost-effective medium throughput approach (96 well standard) compatible with sample sizes of many experiments in proteomics, structural genomics and high throughput diagnostics.

Exploration of bacteriophage polymerases for synthesis of artificial nucleic acids

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ABSTRACT

Nucleic acids are molecules with functional applications such as catalysts, specific binders, and molecular switches. When these nucleic acids are modified, their performance increases. These modified nucleotides still need to be incorporated into growing chain by a polymerase before their improved functions can be carried. Few polymerases have shown to be capable of incorporating these artificial nucleotides and if they do, not to reasonable extend. That is why in this project, two bacteriophages (YuA and LUZ19) were explored to see if their polymerases could be evolved to incorporate these nucleotides. We were able to PCR-amplify the polymerase genes and cloned them in pNIC28-Bsa4 plasmids. BL21 cells were able to express the two polymerases, after the cells were transformed with the recombinant plasmid. The YuA polymerase was expressed more. The exonuclease domain of the YuA polymerase was knocked out by site-directed mutagenesis (though was not confirmed by DNA sequencing yet) and SOE was used to re-clone the PCR mutated fragments in pNIC28-Bsa4 plasmid. In further experiments, the site directed mutagenesis will be confirmed by sequencing and the polymerase activity tested. If the activity of the polymerase to incorporate artificial nucleotides is positive, then molecular evolution of the polymerases will be the next step.

Putative lectins of probiotic *Lactobacillus rhamnosus* GG and their potential for pathogen exclusion

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ABSTRACT

Probiotic bacteria are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001). These bacteria are capable of restoring some imbalances, especially in gastrointestinal tract (GIT). *Lactobacillus rhamnosus* GG (LGG), a prototype probiotic strain isolated from human feces, is clinically well documented. However, the molecular mechanisms mediating health effects are fragmentarily known. LGG can adhere to intestinal mucosa, transiently colonize the GIT. *In vitro* studies have also shown that LGG can inhibit or exclude certain pathogens by the production of lactic acid or competition for adhesion sites. In this study, the role of two lectin-like proteins (Llps) in certain probiotic characteristics of LGG is investigated. Based on the genome sequence, the *llp1* and *llp2* genes, encoding Llp1 (~70 kDa) and Llp2 (~71 kDa) respectively, were selected, since the predicted domain architecture shows similarity with plant and bacteria (*L. plantarum* WCFS1) L-type lectin proteins. Single and double knock-out mutants in the *llp1* and *llp2* genes were constructed. These knock-out mutants were subjected to phenotypic analysis. *In vitro* adhesion and biofilm formation experiments showed a redundant function of Llp1 and/or Llp2 in adhesion to Caco-2 cells. By sugar binding assay, Llp1 and Llp2 showed binding capacity to maltose more than to glucose, mannose and mannan, suggesting a possible recognition of complex sugars. Survival experiments in simulated gastric conditions (pH 2), and in presence of mucin, bile and lysozyme showed that the survival capacity of LGG is not dependent on the *llp1* and *llp2* genes. The *llp1* and *llp2* mutant strains were also shown to induce IL-8 expression in Caco-2 cells at the same level that LGG wild-type (WT), while the double mutant induce less expression compared to LGG WT.

Subsequent pathogen exclusion experiments indicate that Llp1 and Llp2 are not involved in preventing adhesion of *Salmonella enterica* serovar Typhimurium to Caco-2 cells while *Candida albicans* adhesion to VK2 cells was prevented by LGG wild-type, experiments with the mutant strains indicate that Llp1 and Llp2 are not involved. Finally, both proteins were overexpressed in *E. coli* strains BL21 (DE3) pLysS and BL21 (DE3).

Function of elastin gene in chronic progressive lymphedema in Belgian Draft horses

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ABSTRACT

Horses like other farm animals are affected by different diseases. Among the diseases which affect draft horses including Belgian draft horse, is chronic progressive lymphedema. This disease has been identified in three breeds; these are Clydesdales, Shires and Belgian draft horses, suggesting a genetic cause.

The disease affects the lower limbs of horses, and characterised by progressive swelling of the lower limbs associated with development of the thick skin folds, ulcerations, fibrosis and marked hyperkeratosis, this result to severe discomfort often requiring euthanasia (De Cock *et al,* 2006, Young *et al*, 2007).

Many efforts have been done to identify the aetiology of this condition including evaluation of different candidate genes which may be associated with this disease in draft horses, such as *FOXC2* gene (Young *et al,* 2007) but at present there is no actual cause of the disease has been identified.

In this study elastin gene was evaluated as a candidate gene to identify polymorphisms between three CPL positive. Belgian draft horses and three Vlaams paard CPL negative horses which may be associated with CPL in Belgian draft horses. Since its product elastin is essential component of the elastic fibers which support the lymph vessels and enable them to function efficiently.

Genomic DNA was extracted from blood samples of six horse, three CPL positive Belgian draft horses and three CPL negative Vlaams paard horses. Primers were designed for the elastin gene, six exons were amplified by PCR, these were exon one, exon four, exon five, exon six, exon eight and exon nine. DNA was purified from gel sequencing reaction was done and sequenced by using ABI PRISM 3100 genetic analyzer. Sequences from each exon for CPL positive and CPL negative horses were aligned with reference sequence using ChromasPro program to form a consensus sequence in which the presence of polymorphism in those sequences was evaluated and blasted to NCBI to compare them with the elastin gene sequence in database.

Results show that there was no polymorphism in sequences from CPL positive Belgian draft horses and CPL negative Vlaams paard horses. This was for all horses from all sequences of amplified exons. Although there is no polymorphism identified in this study, it does not rule out the presence of polymorphism since the gene has twenty one exons and only six exons were amplified. It is possible that there is a polymorphism in the remaining exons, hence the status of elastin gene polymorphism between the CPL positive Belgian horses and CPL negative Vlaamse paard horse will be established when all the exons have been sequenced.

Antagonism of *Pseudomonas savastanoi* by *Pseudomonas putida* RW10S2 induced by the phytopathogen

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ABSTRACT

Each year, a significant percentage of crop yield loss happens due to the destruction by plant diseases. Hence increasing crop yield by protecting plants from pests and disease is necessary to assure food supply especially for poor regions.

One of the approaches that is proposed to possibly increase agriculture production, specifically crop yield, is the so called biotechnology which involves the use of any living entities and or their derivatives with or without modifying them. Nowadays, living microorganisms like Plant Growth Promoting Rhizobacteria (PGPR) are becoming an interesting research area in view of using them as bio-control agents for plant disease. This is not only considering the issue of safety of human and environment but also reduces the use of synthetic chemicals. PGPR are soil bacteria capable of colonizing plant roots with useful effects on plant growth either directly or indirectly. One of the bio-control mechanisms of PGPR exploited so far by researchers is the production of antimicrobial molecules like lipopeptides which have broad-spectrum action against several plant pathogens affecting crops.

Pseudomonas is one of the most important and well known PGPR bacteria. The main objective of this study was to investigate and characterize the antagonistic activity of *Pseudomonas putida* RW10S2 (a rice rhizosphere isolate) against *P. savastanoi*, causative agent of olive knot disease, via purification of the active compound(s). This antagonism is observed only when both bacteria are co-cultured. An NRPS (non-ribosomal peptide synthetase) gene and a novel gene cluster consisting of the RW10S2 QS system *PmrR-PmrI* and seven ORFs, are involved for this antagonism. Several liquid co-culture possibilities were tested to find best condition for high production of antimicrobial compound. The active compound(s) was extracted by solid phase extraction procedures and then separated by High Pressure Liquid Chromatography (HPLC). The major peak was collected and subjected to mass spectrometry analysis. Several amino acids were identified in the structure of the active compound, but further analysis is required to elucidate its structure.

Purification and characterization ofBeta-alanine responsive Regulator (BarR) protein from *Sulfolobus tokodaii*

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ABSTRACT

BarR protein is a member of the Lrp family of transcription regulators found in the hyperthermoacidophilic archaeon *Sulfolobus tokodaii* strain 7. Little information is known about the structure, physiological role and the target genes of the regulator protein hence a lot of information has to be elucidated still. Previously, *β*-alanine has been identified as the co-factor of the regulator. In this thesis report, many experiments have been conducted to try to elucidate some of the processes and provide new information on aspects such as: expression of the recombinant protein in *E.coli* as an expression host; purification, denaturing and refolding of the protein expressed as inclusion bodies; oligomeric state in solution in the presence and in the absence of *β*-alanine as a co-factor; binding activity to its own control region and to new target genes in the presence and in the absence of the co-factor; the bending angle induced by the protein to its own control region; footprinting of the protein to the control regions of the new targets genes; crystallization of the protein in the presence and in the absence of the co-factor and creating a construct for BarR protein gene (*Saci2136*) knock out in *S.acidocaldarius*. From the experiments, it has been determined that the best refolding and purification procedure for BarR protein from inclusion bodies is by on-column refolding in the presence of 400mM of L-arginine. Further results have shown a higher binding affinity of the protein to its own control region as compared to the new target genes. Furthermore, the cofactor has been shown to interfere with the protein-DNA binding interaction in a concentration-dependent manner for both the control region and to the new targets. The IC_{50} of □-alanine inhibition for binding to the own control region was determined to be 1 □M. The oligomeric state of the protein was shown to be a tetramer. The DNA deformation induced by the protein to its own control region was determined by circular permutation assay and the bending angle was found to be 61.36°. Some of these experiments, such as the study of the BarR crystal structure and the creation of a *Saci2136* gene knockout strain, have to be continued to decipher more information on the protein and gene functions. The protein is encoded by the *barR* gene and may be responsible for the regulation of acetyl coA biosynthesis in the archaea. In the biosynthetic process of the acetyl-coA, *β*-alanine is an important intermediate metabolite that also binds to the regulator protein as an effecter and hence is hypothesized to regulate the acetyl-coA synthesis.

Key mechanisms of systemic RNAi (interference) in insects

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ABSTRACT

This research was aimed to investigate the dsRNA-uptake mechanisms in the desert locust *S. gregaria*. Two mechanisms for dsRNA uptake in animals have been described. One pathway is a transmembrane channel-mediated dsRNA uptake exemplified by the ***Caenorhabditis elegans*** SID-1 protein and the other is a Scavenger receptor mediated Clathrin dependent endocytosis discovered in *Drosophila melanogaster*. Although the Scavenger receptor dependent dsRNA-uptake has been described for *D. melanogaster*, the fruit fly is also known to be rather insensitive toward a systemic response and this pathway is only capable of importing small amounts of dsRNA. The main focus of this thesis was investigating the possible role of these two dsRNA-uptake cascades in *Schistocerca gregaria,* an insect that can respond very robustly to extracellular dsRNA. In order to investigate the potential involvement of SID-1 and/or the Clathrin dependent endocytosis we could rely on sequences found in the available EST-database of *S. gregaria*. These genes were silenced by RNAi, followed by investigating the effects on the RNAi response. However, no sequence information was available for the Scavenger receptor family. Therefore, we worked with a protein inhibitor of the entire Scavenger receptor family. Phenotyping the RNAi response by means of quantitative real-time RT-PCR (qRT-PCR) after silencing sid-1 and/or blocking the Scavenger receptors suggested that both pathways are functionally involved in dsRNA-transport in the desert locust. This would be in correspondence with the mechanisms occurring in *C. elegans.* Recently, it has been proven that also nematodes use endocytosis to transport dsRNA, this in addition to the SID-1 mediated transport.

On the role of Fis in the expression of the *car*AB operon of *Escherichia coli*

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ABSTRACT

E.coli carAB operon encodes the carbamoylphosphate synthase that is the enzyme responsible for synthesis of carbamoylphosphate (CP). CP is a precursor common to the *de novo* biosynthesis of arginine and pyrimidine nucleotides, therefore CP occupies an important position at the intersection of these two pathways. The production of carbamoylphosphate synthase is tightly regulated by the transcriptional regulation of *carAB* operon. *E.coli* is the best suited model organism to unravel complex transcriptional regulatory pattern. *E.coli carAB* operon constitutes a particularly intriguing model system comprising a regulatory network of different complex mechanisms, involving different forms of interactions i.e. Protein-DNA, protein-protein and protein-ligand interactions. The complex regulation of *E.coli carAB* operon is not completely understood. Transcription of *carAB* start at two tandem promoters P1 and P2 , and is mainly under the control of several transcription factors (IHF, PepA, PyrH, PurR, ArgR, RutR) and effector molecules. P1 promoter activity is under the control of architectural protein IHF, PepA and sensor module UMP kinase (PyrH) and they exert pyrimidine (uracil) specific repression effect on *carAB* transcription. RutR seems to have uracil dependent positive effect on P1 promoter activity. PurR exerts purine specific repression effect on the activity of P1 promoter. Repression at P2 promoter is exerted by arginine bound ArgR (arginine repressor). The regulatory mechanisms of *carAB* transcription are characterized to a certain degree at the molecular level but the global pictures stays unclear. More recently, the global regulator Fis has been implicated in *carAB* transcription regulation and several binding sites have been predicted *in silico*.

Here we wanted to study the interaction of Fis with the *carAB* P1 and P2 control region as well as the effect of binding of Fis on *car* P1 and P2 promoter activity and repressibility. To identify the Fis binding sites on *carAB* promoters control region, we constructed a $(His)_6$ tag *fis* plasmid construct using plasmid pET28(a+) which possesses T7 inducible promoter. The pET28(a+)-*fis* plasmid construct was transformed into DH5α strain to amplify this plasmid construct. The amplified plasmid construct was then purified from DH5α strain and transformed into BL21 (DE3) strain to overexpress Fis. The Fis protein was over expressed by culturing of BL21 (DE3) with IPTG induction. The overexpressed Fis protein was then purified and eluted by His trap Ni-NTA affinity chromatography and preserved at -20°C for DNA binding experiments. By PCR amplification we synthesized P1+P2 (560 bps), P1 (436 bps) and P2 (138 bps) promoters control region DNA fragments by using ^{32}P labeled primer and *E.coli* genomic DNA as the template. Protein-DNA binding experiments (EMSAs) were performed with Fis and these radiolabelled P1, P2, and P1+P2 DNA fragments. The autoradiograph of the EMSAs were performed and analyzed. It revealed the formation of several complexes with a different migration velocity suggestive of the existence of several binding sites (≥8) for Fis on *car* P1 promoter control region and several binding sites (≥8) on *car* P2 promoter control region. We attempted to identify the position of the binding sites and hence we performed Cu-phenanthroline in gel footprinting. However, it was not possible to identify the position of Fis binding sites on *carAB* control region by footprinting. This suggests

that a band with a distinct migration velocity is heterogenous and consists of complexes with Fis bound to various sites in different DNA molecules.

To analyze the effect of binding of Fis on the *carAB* transcription we performed assays (β-galactosidase assay) on *in vivo* expression of reporter gene *lacZ* in both wild type and *fis* deletion mutant strain of *E.coli.* To do this assay, we first constructed a *fis* deletion mutant strain of *E.coli* (FW102Δ*fis*) using the wild type FW102 strain. The CSH100 *E.coli* strain transformed with the plasmid pFW11 constructs contains *car* P1, P2 and P1+P2 fused with reporter gene *lacZ*. The *car* P1-*lacZ*, P2-*lacZ* and P1+P2-*lacZ* construct is integrated in the episome of CSH100 strain. The CSH100 strains containing these episomal constructs were provided from this laboratory. We performed conjugation experiments between wild type FW102 and CSH100/F'-*car*P1, CSH100/F'-*car*P2 and CSH100/F'-*car*P1+P2 separately and generated FW102/F'-*car*P1, FW102/F'-*car*P2 and FW102/F'-*car*P1+P2 strains. In the same way by conjugation with *fis* deletion mutant (FW102Δ*fis*) we generated FW102Δ*fis*/F'-*car*P1, FW102Δ*fis*/F'-*car*P2 and FW102Δ*fis*/F'-*car*P1+P2 strains.

The wild type and *fis* deletion mutant transconjugants were grown on different media (Minimal, minimal supplemented with uracil, adenine, uracil + adenine for P1-*lacZ* constructs, minimal and minimal + arginine for P2-*lacZ* construct, and Minimal, minimal + uracil, minimal + arginine + uracil for P1+P2-*lacZ* constructs) and cells were collected in the exponential growth phase (at OD 0.5 to 0.6 at 600nm). The cell-free enzyme extracts from wild type and *fis* deletion mutant strains were analyzed by β-galactosidase assay to measure the expression of reporter gene *lacZ* from the P1-*lacZ*, P2-*lacZ* and P1+P2-*lacZ* episome borne constructs in the presence of Fis (in wild type) and in the absence of Fis (in *fis* deletion mutant strains) in different growth conditions. Therefore, we calculated the β-galactosidase enzyme specific activity for wild type and *fis* deletion mutant strains for every condition and analyzed the data statistically. We found significantly higher expression of *lacZ* gene in *fis* deletion mutant strain than in wild type strain in case of P1-*lac*Z and P1+P2-*lac*Z constructs but no quit significant difference in *lacZ* expression in case of P2-*lacZ* construct. Thus, it indicates that, Fis has significant repression effect on *car* P1 promoter activity but little/no effect on *car* P2 promoter activity.

Characterization of maize lines transgenic for *PARP1* silencing construct, *HUB1* over-expression construct and *Brachypodium* promoter-*GUS* reporter gene construct

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ABSTRACT

Transgenic research in maize is a key biotechnological tool aimed at boosting maize productivity and increasing tolerance to abiotic stresses among other advantages. In this thesis maize lines transformed with either *Poly(ADP-ribose)polymerase* (*PARP1*) silencing construct, *Arabidopsis HISTONE MONOUBIQUITINATION1* (*HUB1*) over-expression construct or *Brachypodium* promoter-*GUS* reporter gene construct were characterized.

Silencing of endogenous maize *PARP1* gene was based on a novel strategy for improving abiotic stress tolerance in plants by maintaining energy homeostasis under stress conditions, as demonstrated in *Arabidopsis* and *Brassica*. Two *PARP1* hairpin constructs for silencing maize *PARP1* gene (homolog of the *Arabidopsis PARP2* gene) were transformed into B104 maize genotype. In this study, T-DNA stability of the *PARP1* hairpin constructs was analyzed by PCR and phosphinotricin acetyl transferase (PAT) assay in T2 progenies of the *PARP1* maize lines. In addition, QPCR analysis was used to determine the expression level of *PARP1* hairpin construct and down regulation of endogenous maize *PARP1* gene. We observed mild over-expression of the hairpin construct and mild down-regulation of the endogenous *PARP1* gene.

HUB1, an *Arabidopsis* RING E3 ligase protein with a function in organ growth has been proposed to have a role in the regulation of cell cycle during early organ growth in plants, seed dormancy and flowering time. Over-expression of *HUB1* cDNA in rice resulted in positive phenotypes such as enhanced emergence vigor, root growth, number of flowers and seed size. *Arabidopsis HUB1* cDNA was over-expressed in B104 maize lines using a maize ubiquitin promoter. A preliminary growth analysis was done on the *pUBIL-HUB1* maize lines as well as a segregation analysis using PAT and ammonium assay. Additionally, QPCR analysis was carried out to determine over-expression of the *HUB1* transgene in T1 transgenic maize lines. The fact that we did not see a significant over-expression of *HUB1* transgene in maize correlated well with the lack of faster seedling emergence in *HUB1* transgenic lines.

The current limited number of promoters for regulating heterologous gene expression has created a need for identifying more promoters for transgenic maize research. In this study, three putative constitutive *Brachypodium distachyon* (*Bd*) promoters (*pBdSAMDC*, *pBdEF*1α and *pBdUBI10a*), selected based on *in silico* expression analysis and QPCR, were analyzed in various maize lines throughout development using *GUS* reporter gene constructs. Lines co*n*taining either *CaMV 35S-GUS* or *ubiquitin-GUS* constructs were analyzed and used as reference lines. *pBdEFIα* and *pBdUBI10a* were shown to be constitutive promoters with GUS expression visible in all the tissues and organs tested at different stages of maize plant development while *pBdSAMDC* was shown to be a vascular-specific promoter.

Genetic diversity within/between *Trypanosoma congolense* populations : Validation of a new method based on microsatellite analysis

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ABSTRACT

African Animal trypanosomosis is a major constraint to the livestock industry development in Africa. The disease is caused by several species of trypanosomes but the most pathogenic one is *Trypanosoma congolense.* The genetic diversity of *T. congolense* has not extensively been studied at the molecular level although this parasite is present in very different biotopes. Microsatellite markers have been used to study genetic diversity in different animals including other species of trypanosomes that affect humans. In this study, microsatellite analysis was used to determine the genetic diversity of *T. congolense* and the main aim was to validate the efficiency and repeatability of microsatellites in this parasite. Nested PCR was used to amplify the microsatellite markers using TCM1, TCM2, TCM3 and TCM4 primer pairs. The precise fragment size of each PCR products was determined by capillary electrophoresis and the genotype of *T. congolense* isolated from animals within the same herd, animals from different herds, animals with disease before and after treatment with trypanocides and finally *T. congolense* clones was established. The results obtained indicate that most *T. congolense* isolates from Adamaoua region in Cameroon are composed of triploid and tetraploid lines for TCM1, TCM2 and TCM3 markers. Due to this polyploidy, it is difficult to assess how the alleles are inherited from parent population to the offspring population based on these microsatellite markers. As a result the validation of microsatellite markers as new method in studying the genetic diversity of *T. congolense* was not achieved. Further studies are required to investigate and confirm the results obtained in this study and to understand the mechanism(s) of polyploidy development in *T. congolense*.

Influence of Bacteriophage P22 on the behaviour of *Salmonella enterica*

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Characterization of a new broad spectrum lipopeptide antibiotic from *Serratia plymuthica* RVH1

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Initial characterization of two candidate Black Sigatoka responsive banana genes and their promoters

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Defense responses of the two peptides of *Arabidopsis thaliana* against necrotrophic pathogens.

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Development of xylose-utilizing and inhibitor-tolerant yeast strains for bioethanol production

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