AUSTRIAN INSTITUTE OF TECHNOLOGY
TOMORROW TODAY

BOKU

AIT-BOKU Student Seminar 2012

20TH JUNE 2012

ABSTRACTBOOK

PROGRAMM: AIT-BOKU STUDENT SEMINAR 2012

20TH JUNE 2012

BOKU LV Nummer: 941092, Seminar, 2ECTS credit points
Titel: "Bioresources exploitation and management"

Universitäts- und Forschungszentrum Tulln,
Seminar Room 14
Konrad-Lorenz Straße 24,
3430 Tulln

OPENING

9.00 – 9.20	Michaela Fritz	Head of Health & Environment Department, AIT
	Josef Glößl	Vice Rector for Research and International Research Collaboration; BOKU
	Wolfgang Knoll	AIT Managing Director

1ST SESSION: CHAIR: ANGELA SESSITSCH

9.20 – 9.50	Novel approaches for fungal biotechnology: Understanding and adjusting signal transduction pathways and light response Keynote Lecture: Monika Schmoll (Vienna University of Technology)	1
9.50 -10.10	Testing the ability of bacteria to colonize the rhizosphere of *Salix caprea* grown in contaminated soil Katharina Fallmann	2
10.10 -10.30	Role of plant growth promoting bacteria and soil amendments in phytoremediation of heavy metal contaminated sites Balakrishnan-Ravindran Vivek	3
10.30 -11.00	Coffee Break	

2ND SESSION: CHAIR: JOSEPH STRAUSS

11.00 -11.20	The role of oxidative regulation in the signal transduction of nitrate activation Andreas Elek	4
11.20 -11.40	Phytoremediation of trichloethene - Balance study of the accumulation, metabolisation and votalization of dissolved TCE in the groundwater by plants as well as the quantification of microbial degradation via stable isotopes. Sarah Quast	5

POSTER SESSION: CHAIR: JOSEPH STRAUSS

Conclusions & lunch

NOVEL APPROACHES FOR FUNGAL BIOTECHNOLOGY: UNDERSTANDING AND ADJUSTING SIGNAL TRANSDUCTION PATHWAYS AND LIGHT RESPONSE

Monika Schmoll

Vienna University of Technology
Institute of Chemical Engineering

Abstract

The filamentous ascomycete Trichoderma reesei (anamorph of Hypocrea jecorina) is predominantly known for its efficiency in cellulase production. However, despite decades of cultivation in the lab this biotechnological workhorse still preserved its evolutionary heritage, which can be exploited for biotechnological applications. Our research revealed intriguing interconnections between the pathways for nutrient signalling, light response and sexual development.

The important group of genes encoding glycoside hydrolases shows considerable regulation by light and the light response machinery. Accordingly, environmental signals perceived by the pathway of heterotrimeric G-proteins are transmitted in a light dependent manner to eventually adjust cellulase gene expression to the current nutritional and light condition. In this interconnection between nutrient and light signalling, the phosducin like protein PhLP1 and the light regulatory protein ENV1 act as a mutually regulated pair and hence constitute an important node between the two pathways. However, the function of ENV1 is not limited to this transcriptional interaction, but also extends to regulation of transcript levels of G-protein alpha subunit genes, cAMP levels and development of T. reesei. Investigation of the impact of the light signalling pathway on sexual development in T. reesei revealed a negative regulation of pheromone precursor and pheromone receptor genes of the photoreceptors BLR1 and BLR2. The strong upregulation of these genes caused by lack of ENV1 even results in female sterility in the respective mutants. In accordance with the interrelationship of light response with nutrient signalling, also PhLP1, the G-protein beta and gamma subunit as well as the downstream pathway of cAMP signalling are important for sexual development, which reflects a interconnected network of signal integration for light, nutrients and developmental signals in T. reesei.

TESTING THE ABILITY OF BACTERIA TO COLONIZE THE RHIZOSPHERE OF *SALIX CAPREA* GROWN IN CONTAMINATED SOIL

Katharina Fallmann

University-supervisor: Doz. DI Dr. Angela Sessitsch, University of Natural Resources and Life Sciences Vienna, Institute of Soil Research
AIT-Supervisor: Doz. DI Dr. Angela Sessitsch, H&E Department, Bioresources

Introduction

For the application of bacteria as plant inoculants for bioremediation or agriculture, the ability of the bacteria to colonize the rhizosphere and to compete with other rhizosphere microorganisms is a prerequisite for successful use of their beneficial properties. In this study we tested a screening approach for selecting efficient and competitive root colonizers out of a collection of 74 heavy metal resistant strains.

Materials and Methods

To reduce the high experimental effort of testing every strain separately, we divided the bacteria in three groups and mixed cell suspensions of equal density from all strains in the group. The three suspensions were applied to Zn and Cd accumulating *Salix caprea* plantlets potted in a contaminated, sterilized soil followed by re-isolation and identification of the bacteria after five weeks. Only for strains which could be detected in the rhizosphere or xylem sap, the ability to colonize the roots as a single inoculant in non-sterile soil was evaluated in a second pot experiment.

Results

Fifteen percent of the strains could be re-isolated in the first screening experiment. In contrast, in the second experiment the selected bacterial strains were detected in the rhizospheres of two-thirds of the inoculated plants.

Conclusion

These results show that only a small fraction of previously isolated bacteria colonized the rhizosphere well under competitive conditions. On the other hand, the described approach was shown to be suitable to find strains with a feasible rate of rhizosphere colonization and is considered to have promising potential to select bacteria for field applications.

ROLE OF PLANT GROWTH PROMOTING BACTERIA AND SOIL AMENDMENTS IN PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SITES

Balakrishnan Ravindran Vivek

University-supervisor: Doz. DI Dr. Angela Sessitsch, University of Natural Resources and Life Sciences Vienna, Institute of Soil Research
AIT-Supervisor: Doz. DI Dr. Angela Sessitsch H&E Department, Bioresources

Abstract
On a global basis, soil contamination with cadmium, lead, and zinc is one of the most pervasive environmental problems. In the surroundings of a former Pb/Zn smelter in Arnoldstein (Austria) heavy metal concentrations exceed thresholds for food and feedstuffs. Phytoremediation (in our case the combination of immobilization and phytoexclusion) could be a cost-effective system for improvement of the use of contaminated areas. The aim was to study the effects of plant growth-promoting bacteria (PGPB) and immobilizing soil amendments on heavy metal tolerance of plant and uptake. Pot experiments were performed whereby two maize cultivars were cultivated in varying contaminated soil and treatments (Burkholderia phytofirmans strain PsJN with and without amendment). Inoculation with strain PsJN significantly improved the root and shoot biomass of maize. Rhizosphere and leaves were analyzed for heavy metal content. Results indicated that immobilizing amendments had significant effects on the reduction of ammonium nitrate extractable Zn (< 80%) and Pb (<50%) compared to the controls. Concentration of Zn and Pb in plants was reduced by combined immobilizer and PGPB up to 65% and 40%, respectively. Three different media allowed the selection of 500 isolates based on colony morphology from contaminated soil and plants. For characterization of bacteria, 16S rDNA genes were sequenced from the isolates. Furthermore, the plant growth-promoting potential will be analysed by screening for the production of 1-aminocyclopropane-1-carboxylic acid deaminase, siderophores and indole acetic acid. Selected strains will be further tested for heavy metal mobilization and plant growth-promoting effects in interaction with the plant.

THE ROLE OF OXIDATIVE REGULATION IN THE SIGNAL TRANSDUCTION OF NITRATE ACTIVATION

Andreas Elek

University-supervisor: Prof. Dr Joseph Strauss, University of Natural Resources and Life Sciences Vienna, Department of Applied Genetics and Cell Biology

Introduction

Nitrate is the most important source for assimilation of nitrogen by *Aspergillus nidulans*. There are also other nitrogen sources like ammonia or arginine. Nitrate induced the transcription factor NirA (specific nitrogen transcription factor), which activates together with AreA (global nitrogen transcription factor) the transcription of the gene of nitrate reductase (niaA), nitrite reductase (niiA) and nitrate transporter (nrtA). Arginine doesn't induce NirA, because the active form of NirA a dimer cannot form and NirA shuttles between nucleus and cytosol. Ammonia repressed AreA and NirA is not active.

Our interest is the nucleus export sequence (NES) and activating domain (AD) of NirA, which are important for the induction of NirA. NES has a methionine residue at the position 169, which is in the non induced form oxidised to methionine sulfoxid and in the induced form reduced to methionine. Between both forms AD changes its position in the structure of NirA. It seems to be a switching function in the oxidative regulation. But it is still unknown how the methionine sulfoxid in NirA is reduced to methionine. Either it is reduced e.g. by methionine sulfoxid reductase or by NirA itself.

Materials and Methods

To know more about the induction of NirA we made first a screening growth test with different organic and inorganic nitrogen sources combined with inorganic salts without nitrogen but with the same structure. We used for the growth test a modified *Aspergillus nidulans*, which has a reporter gene -galactosidase coupled to niaD. Parallel to the growth test we did a qualitative X-gal test. It showed whether NirA is induced or not induced by the combination of the substances in the growth test.
In the next step we did a screening test with confocal fluorescence microscopy (CFM). For this we used a modified *Aspergillus nidulans*, which expresses the transcription factor NirA coupled with the green fluorescence protein (GFP). For the induced form or not induced form of NirA we screened with inorganic or organic compounds with and without nitrogen sources.
Finally to quantify the activity of NirA we did an ONPG test with the modified *Aspergillus nidulans* of the growth test. For this test we used selected compounds with different oxidation number of nitrogen or chlorine from the tests before.

Results

The growth test combined with the X-gal test and the screening test with CFM showed us several similarities. All compounds which induce NirA had more or less the same acid dissociation constant (pKa) and are strong oxidising agents. These could also show with the ONPG test.

Conclusion

The results show that there is not only the oxidative regulation between methionine and methionine sulfoxid but also a nitrate specific regulation of NirA. In addition to the induced and non induced form of NirA there is minimum one more intermediate step. Combined these results of the tree different tests with results of mutation growth tests can used to explain the interaction of the different proteins in the signal transduction of nitrate activation.

PHYTOREMEDIATION OF TRICHLOETHENE – BALANCE STUDY OF THE ACCUMULATION, METABOLISATION AND VOTALIZATION OF DISSOLVED TCE IN THE GROUNDWATER BY PLANTS AS WELL AS THE QUANTIFICATION OF MICROBIAL DEGRADATION VIA STABLE ISOTOPES

Sarah Quast

University-supervisor: Doz. Mag. Dr. Thomas Reichenauer, University of Vienna
AIT-Supervisor: Dr. Andrea Watzinger, Health & Environment Department, Environmental Resources & Technologies

Abstract
Decomposition of trichloroethene (TCE) through plants (poplar) is examined in a microcosm experiment. As part of this experiment, the plants are hermetically sealed from the atmosphere in a hydroponic pot (~15 l) to which TCE-infused nutrient solution is added. The leafage is separated gas-tight from the groundwater as well as the root zone. The TCE in the water is absorbed by the plants and can be stored, metabolized or transpired into the atmosphere. A mass balance of the different process stages will be made. Water samples will be taken regularly in order to determine the absorbed amount of TCE. In addition to the TCE concentration, the 12C/13C isotope ratio (GC-IRMS) is measured to establish a possible microbial degradation. After completion of the experiment TCE and possible metabolisms are extracted from the plants and their contents is measured by the GC-ECD. Air samples from the gas-filled compartment of the upper leafage part are bound to absorption agents and measured by the P&T-GC-FID.

IN-SITU REDUCTIVE DECHLORINATION OF HALOGENATED ORGANIC CONTAMINANTS USING NANOSCALE ZEROVALENT IRON (NZVI) AND ITS SUITABILITY FOR ALPINE, POROUS AQUIFERS

Philipp Schöftner

Univeristy-supervisor: Univ.-Doz. Mag. Dr. Thomas Reichenauer, University of Vienna
AIT-Supervisor: Dr. Georg Waldner, Health & Environment Department, Environmental Resources & Technologies

Introduction

Halogenated organic solvents like trichloroethene (TCE) have been used in a wide variety of applications causing a large number of long-term contaminants. To dechlorinate those contaminants NZVI is injected into the aquifer where it is transported into and near the dense non aqueous phase liquid (DNAPL). The small size and the high reactivity as well as the improved transportability of nanoscale Fe° particles in the aquifer make them a promising in-situ remediation technology. Beside the desired dechlorination reactions, disadvantageous side reactions like corrosion and formation of hydrogen can occur, depending on particle and hydrogeochemical properties on site. Thus current research focuses on particle modification to improve longevity, contaminant selectivity of the reaction and transport. The aim of this project is to study the suitability of this remediation method under conditions which are representative for Austrian porous aquifers with high carbonate contents and high kf-values.

Materials and Methods

Batch experiments are conducted using various types of water qualities (pH value, groundwater ions, (an) aerobic, different TCE and Fe° concentrations and different reaction periods) and different particles. Beside widely untested commercial NZVI particles (NanoFer) novel composite-particles which are engineered at AIT-Molecular Diagnostics are evaluated.

Preliminary results

First experiments were carried out using commercially available NanoFer 25. Those experiments indicate a rather low reactivity compared to particles which are known from literature. Ethene and ethane were the main endproducts of those reactions. A couple of reaction products are still to be identified and quantified to establish a complete carbon mass balance.

Outlook

Unidentified endproducts will be identified by GC-MS measurements and calibrated on FID.
Further studies will explore a variety of conditions with a focus on the properties which are representative for Austrian porous aquifers:

- Comparison of reactivity under conditions known from literature
- Reactivity under pH-controlled conditions
- Reactivity in carbonate enriched solutions
- Reactivity in aerobic and anaerobic conditions
- Reactivity at varying Fe° and TCE concentrations
- Long-term reactivity

To describe the reactivity and suitability of those NZVI-particles to remediate Austrian porous aquifers surface normalized reaction constants, particle efficiencies and carbon mass balances will be defined.

THE INTERPLAY BETWEEN CARBOXYLESTERASES FROM MONOCOTYLEDONOUS PLANTS AND FUSARIUM IN TRICHOTHECENE METABOLISM

Clemens Schmeitzl

University-supervisor: Ao.Univ.Prof. DI. Dr. Gerhard Adam,
University of Natural Resources and Life Sciences Vienna

Introduction

Trichothecenes are a large family of mycotoxins, produced by numerous, mostly phytopathogenic fungi and are strong inhibitors of protein synthesis by interacting with eukaryotic ribosomes. Therefore, trichothecenes can lead to severe health issues of animals and humans.

Isolates of Fusarium graminearum that produce the trichothecene Deoxynivalenol (DON) can be subdivided into the chemotypes 3 acetyl-DON (3-ADON) or 15-acetyl-DON (15-ADON), based on the metabolite that initially predominates in axenic cultures. Both types of strains initially produce 3,15 diacetyl-DON. It depends on the allelic variant of the Tri8 esterase which intermediate is accumulated. The order of toxicity for wheat ribosomes is 15 ADON > DON >> 3-ADON.

Currently there is a worldwide shift from 15-ADON strains to 3-ADON strains, but the evolutionary advantage remains still unknown. Deacetylation reactions by carboxylesterases (CXEs) are most likely highly relevant in the early stage of plant pathogen interactions and are not only catalysed by fungal enzymes and acetylation of the C3-OH is considered to be an important mechanism of self-protection during toxin biosynthesis.

Materials and Methods

All Fusarium genes tested were cloned and tested in yeast. *In vivo* assays by incubating dense yeast cultures for a certain time in toxin containing medium were performed and samples were analyzed by HPLC-MS at the IFA-AZ by Franz Berthiller and Mehrdad Shams.
Possible CXEs from *B. distachyon* were identified via a bioinformatical approach and performing a semi-quantitative expression analysis. All putative *B. distachyon* CXEs were cloned codon optimized in yeast and also tested in vivo. The samples were analysed at the IFA-AZ by Franz Berthiller.

Results

Several alleles of TRI8 from *Fusarium* were cloned, analyzed and tested on substrate specificity. Additionally paralogue and homologue genes were cloned and analyzed in the same way. The 3-ADON allele of Tri8 deacetylates at the C-15 position, while the 15-ADON allele is decatetylating at the C-3 position. The Tri8 Gene of *F. asiaticum* did not show any activity against 3- or 15-ADON as well as all para- and homologues tested.
Tri8 from F. graminearum was modeled by homology modeling and threading programs.
It was shown that CXEs from monocotyledonous plants are capable of deacetylating 3-ADON to DON. A set of 9 putative carboxylesterases of B. distachyon 9 were tested for activity against 3-ADON, 15 ADON and *Fusarenon X*. Pilot assays indicated that they might show activity agains 3 and 15 ADON.

Conclusion

The reason for the strong chemotype shift of *Fusarium* strains (15-ADON to 3-DON) remains still unknown, especially as 3-ADON is far less toxic as 15-ADON on a ribosomal level. In this study it was shown that monocotyledonous plants are capable of deacetylating trichothecenes. This leads to an interplay between host and pathogen concerning trichothecene metabolism and potentially could influence which *Fusarium* chemotype is predominating. Preliminary results suggest that several putative CXEs of B. distachyon are capable of deacetylating ADONs.

FUNKTIONAL GENOMICS OF UDP-GLUCOSYLTRANSFERASE: A YEAST ASSAY TO TEST FOR THE CAPABILITY OF DEOXYNIVALENOL DETOXIFICATION AND BRASSINOSTERIOD GLYCOSYLATION

Maria Paula Kovalsky Paris

University-supervisor: Ao.Univ.Prof. DI. Dr. Gerhard Adam,
University of Natural Resources and Life Sciences Vienna

Introduction

The plant pathogen *Fusarium graminearum* is capable of colonizing nearly all types of crops including wheat, barley and maize leading to massive losses in grain yield and quality. The fungus produces a series of mycotoxins including the trichothecene deoxynivalenol (DON). This protein biosynthesis inhibitor has been shown to be a virulence factor in wheat required for spread of infection. Consequently, resistance to the spreading of *Fusarium* is mediated by the ability of the plant to glucosylate DON into the less-toxic conjugate DON-3-O-glucoside by plant UDP-glucosyltransferases (UGT). Searching for these genes in crop plants is not a trivial task. UGTs constitute a large multifunctional genetic family with broad substrate specificity. Diploid grass genomes contain about 150 UGT genes and there are potentially hundreds present in hexaploid wheat. UGTs seem to undergo rapid evolution, and due to different copy numbers in gene clusters it is difficult to identify true orthologues.

The first gene encoding an enzyme with the ability to glucosylate DON from a monocotyledonous plant was recently described (HvUGT13248, Schweiger et al. 2010). However, since the genomes of both wheat and barley have not yet been fully sequenced, we decided to investigate the homologues of HvUGT13248 in the genome of the monocot species *Brachypodium distachyon* (Bd). *Brachypodium* has a small sequenced genome which is very closely related to cereals and should therefore provide an insight into the architecture of crop UGTs.

Overexpression of a DON glucosylating UGT should increase *Fusarium* resistance in plants. However, UGTs have also been shown capable of altering the activity of plant hormones, such as brassinosteroids, by glucosylation which in turn leads to undesired dwarfing of the plant. The main goal of this study is to characterize candidate UGTs with the ability to deactivate DON in crop plants and to investigate possible unwanted dual activity towards both mycotoxins and brassinosteroids.

Materials and Methods:

Microarray data generated from *Fusarium* and DON challenged barley was the first step to identify relevant UGT genes. The functional characterization of the candidate UGTs was conducted in a yeast bioassay by cloning the open reading frame of the UGT behind a c-myc tag under the control of the strong constitutive ADH1 promotor in a yeast expression vector. A genetically engineered toxin-sensitive yeast strain was transformed with the resulting plasmid. Yeast strains expressing each gene respectively were spotted onto plates containing different concentrations of DON for qualitative analysis or incubated with the toxin in liquid culture for quantitative analysis. Within the 177 predicted BdUGTs six of these genes have the highest amino acid sequence similarity to HvUGT13248 and are located on a cluster on chromosome 5g. All six candidate Bd UGTs and HvUGT13248 were tested for their ability to glucosylate both DON and the brassinosteroid castasterone in a liquid yeast bioassay.

Results:

Out of a total of 11 barley UGTs which were up-regulated upon *Fusarium* and DON stress, only one of the gene products was capable of deactivating DON in the yeast bioassay (HvUGT13248, Schweiger et al. 2010). Additionally, two of the candidate Bd UGTs were able to glucosylate DON to DON-3-O-glucoside to the same extent as HvUGT13248 in a liquid assay. These three genes were tested for their ability to glucosylate the brassinosteroid castasterone and no castasterone-glucoside formation was detected.

Conclusion:
It was observed that multiple UGTs are induced by DON, however only few confer resistance to the toxin. Closely related isozymes, such as the 6 clustered candidate *Brachypodium* UGTs, do not necessarily share DON substrate specificity. A combination of sequence and transcriptional data is useful for successful identification of UGTs, nevertheless, validation is essential.
Additional work conducted in our group showed that the overexpression of the barley HvUGT13248 gene in Arabidopsis thaliana led to increased DON resistance of seedlings without unwanted side effects such as dwarfing. The candidate barley and *Brachypodium* UGTs could therefore be potentially attractive candidates to increase DON and Fusarium resistance in transgenic crops. These genes should provide an insight into *Fusarium* resistance.

BIOCHAR APPLICATION TO TEMPERATE SOILS-EFFECTS ON SOIL FERTILITY AND CROP YIELD

Stefanie Kloß

University-supervisor: PD Dr. Franz Zehetner, University of Natural Resources and Life Sciences, Vienna Institute of soil research
AIT-supervisor: PD Dr. Gerhard Soja, Health & Environment Department, Environmental Resources & Technologies

Introduction

Biochar (BC) application to soil as a potential soil amendment is currently intensively explored. Depending on feedstock and highest treatment temperature (HTT), BC application to soil may contribute to the soil nutrient status by directly adding nutrients to the soil as well as by increasing pH, cation exchange and water holding capacity. These parameters are known to play an important role in the soil nutrient status and nutrient availability. A positive effect on plant growth after BC application to tropical soils has been observed repeatedly; however, the effect of BC application to soils in temperate climate regions is much less explored.

Materials and Methods

We investigated the effect of BC to temperate soils and crop yield using a randomized pot experiment in a greenhouse with three agricultural soils (Planosol, Cambisol, Chernozem) and four BC types (from straw, mixed woodchips and vineyard pruning, all pyrolyzed at 525°C). In order to analyze the effect of pyrolysis temperature, we additionally applied vineyard pruning BC pyrolyzed at 400°C. Selected treatments were planted with mustard (Sinapis alba L.), followed by barley (Hordeum vulgare). Soil sampling was carried out after barley harvest. Investigated soil parameters included pH, electrical conductivity (EC), C/N ratio, cation exchange capacity (CEC), CAL-extractable P and K, as well as nitrogen supplying potential (NSP). Biomass production of the two crops was determined as well as its elemental composition.

Results

Biochar application (3% wood-based BC) caused a considerable pH increase for the acidic Planosol. The effect of BC application on CEC was dependent on the original status of the soil, notably soil pH and texture. 3 % BC application (wood) decreased CEC by 3.5 % and 10 % for the Chernozem and Cambisol, respectively, but increased CEC by 35 % for the acidic, sandy Planosol, which may be due to the strong liming effect found for the Planosol. BC application significantly raised CAL-extractable K for all soils. CAL-extractable P only increased in the Planosol and Cambisol at 3% application rate. Mustard yield decreased by 67% for vineyard pruning BC if nitrogen deficiency was not compensated for, straw-derived BC only caused a 2 % decrease of mustard yield. Barley yield was still significantly lower in most BC-treated pots compared to the controls, however, plant yields were less reduced for the second crop. Only straw-derived BC treatments showed a significantly higher barley yield (1955 ± 40 g m-2) compared to the control (1837 ± 70 g m^{-2}). Especially mustard composition showed that all BC treatments caused decreased micronutrient uptake (Cu, Fe, Mn, Zn).

Conclusion

BC application to temperate soils seems to have more complex effects on soil. The actual extent is dependent on the original soil status such as pH. BC application on low pH soils caused decreased micronutrient uptake that is partly reflected in the reduced plant yields, which must be considered when applying BC to the soil.

ENDOPHYTIC COLONIZATION OF BACTERIA INDUCE DROUGHT-STRESS TOLERANCE IN MAIZE

Naveed Muhammad

University-supervisor: Doz. DI Dr. Angela Sessitsch, University of Natural Resources and Life Sciences Vienna, Institute of Soil Research
AIT-Supervisor: Doz. DI Dr. Angela Sessitsch, H&E Department, Bioresources

Introduction

Drought is a potential major constraint to maize production in all areas where it is grown. Global warming, deforestation, and urbanization will all increase the severity and frequency of drought in the future, leading to a possible decrease in global food production at the same time that increasing human population demands an increase in food supplies. Therefore, there is an urgent need to improve crop plants to bear up drought stress and ensure yield optimally.

Materials and Methods

The study was planned to investigate the effect of microbial inoculation on growth of maize cvs under drought stress. Two plant-growth promoting bacteria B. phytofirmans strain PsJN and Pantoea sp. FD17 were tested on two different maize cultivars (Kaleo and Mazurka). Surface-sterilized maize seeds were primed in bacterial suspension for 4 hours. Control seed were soaked in sterile broth. The seeds were planted in pots filled with 15 kg soil and recommended dose of NPK fertilizers were applied. Drought stress was applied by withdrawing water after 48 days of planting. Agronomic and physiological data were recorded before/after harvesting. Plant colonization by the applied bacteria was monitored by microscopy and plate-counting assays.

Results

The bacterial inoculation improved plant growth parameters and photosynthesis of maize cultivars Mazurka and Kaleo compared to uninoculated control under normal and drought stress conditions. However, B. phytofirmans strain PsJN inoculation response was more prominent compared to Pantoea sp. The PsJN inoculation resulted in up to 55% increase in the biomass of maize as compared to the untreated control under drought stress. The photosynthesis activity was up to 67% higher in inoculated plants than in the untreated control. Similarly, inoculation also improved the chlorophyll fluorescence (photochemical efficiency of PsII) content of maize plant.

Conclusion

The study clearly demonstrates that bacterial inoculants could be used to minimize the negative effects of drought stress on growth and photosynthesis of maize.

STUDY OF THE COMBINATION OF CHEMICAL AND BIOLOGICAL METHODS FOR DEGRADATION OF PETROLEUM HYDROCARBONS IN SOIL

Juliane Hörnig

Univeristy-supervisor: Univ.-Doz. Mag. Dr. Thomas Reichenauer, University of Vienna
AIT-Supervisor: Dr. DI Katharina Pirker, Health & Environment Department, Environmental Resources & Technologies

Petroleum hydrocarbons are beside chlorinated hydrocarbons one of the common pollutants, which can be detected at contaminated sites. In the past contamination of soil and groundwater with petroleum hydrocarbons often was treated by excavation and followed by landfilling or off-site treatment. However excavation of polluted soil is problematic both from an ecological and a financial point of view.

The so called in-situ remediation methods are a possible alternative. With these methods it is possible to degrade the pollutants in the underground directly. Since two decades people develop different in-situ methods, which are based on both: biological and chemical principles. But only a few papers could be found describing a combination of these two methods.

The goal of this work is to study the combined effect of biological and chemical methods on the degradation of petroleum hydrocarbons in the soil. The work with contaminated material from an existing contaminated site is performed in the lab and the greenhouse. We started degradation of petroleum hydrocarbons in a batch experiment with different chemical (addition of oxidative amendments) and biological (biostimulation, i.e. addition of nutrients) methods followed by a phase of rhizodegradation in the greenhouse. In addition we are investigating possible radical-mechanisms of contaminant degradation using Electron Paramagnet Resonance (EPR) Spectroscopy.

Batch experiments showed that the petroleum hydrocarbons are degradable by biostimulation. Preliminary results of spin trap experiments with the EPR spectrometer indicate the production of hydroxyl radicals by iron containing oxidative agents like nano-iron and magnetite.

PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF P-GLYCOPROTEIN FUNCTION AT THE RAT AND HUMAN BLOOD BRAIN BARRIER STUDIED WITH POSITRON EMISSION TOMOGRAPHY

Julia Müllauer

University-supervisors: Ao.Univ.-Prof. Mag Dr. Wolfgang Birkfellner, Medical University Vienna, Center for Medical Physics and Biomedical Engineering
AIT-supervisor: PD DI Dr. Claudia Kuntner, Health & Environment Department, Biomedical Systems

Introduction

Pharmacokinetic (PK) modeling is the common method of choice to quantitatively analyze data obtained with positron emission tomography (PET) and to verify, that the PET signal indeed represents the underlying physiological, biochemical and pharmacological functions studied. PK modeling software is usually "in-house" developed software and therefore presents a low level of validation. Additionally, based on the individual PK model parameter estimates population averages and variability is generated after each subject is analyzed separately. In this study we used population mixed effect modeling (in NONMEM VI – acronym for Nonlinear Mixed Effect Modeling), which is used in standard PK pharmacodynamic (PD) modeling but is not yet routinely used for PET data analysis to overcome these drawbacks. A population approach analyses all subjects simultaneously, and gives a description of the PK in the typical subject as well as the variation in the study population.

Materials and Methods

Data obtained from preclinical (naïve and 48 h post status epilepticus) and clinical (healthy subjects) (R)-[11C]verapamil (VER) PET used to study in-vivo P-glycoprotein (Pgp) function at the rat and human blood brain barrier before and after inhibition with the Pgp inhibitor tariquidar (TQD) was analysed. A population mixed effect model was developed, which allows predicting the rate constants describing the pharmacokinetics of VER and the effect of TQD on VER pharmacokinetics. The effect of tariquidar was studied on both, the influx rate constant from the plasma compartment to the first brain compartment Qin and on the efflux rate constant from the first brain compartment to the plasma compartment Qout in order to investigate if TQD is enhancing the brain uptake of VER by increasing the influx or decreasing the outflux. Additionally, the influence of epilepsy was tested on all model brain parameters. Finally, the model developed based on the rat dataset was applied to the human dataset to identify potential species differences.

Results

There is still an ongoing debate, whether tariquidar is enhancing the brain uptake of VER by increasing the influx or decreasing the outflux. Our model indicated, that tariquidar enhances brain uptake of VER by decreasing the outflux of the tracer (VER). Thus we were able to show that the efflux rate constant from the first brain compartment to the plasma compartment Qout was decreased drastically after TQD administration. Additionally the influence of epilepsy was found to be on the first brain compartment Vbr1. Under physiological considerations this efflux enhancement of Pgp implies that substrates could be transported from the cytoplasm into the blood. This model indicates similar increases in volume of distribution VT as observed with the PK modeling approach. The developed model described the rat data set rather well, but attempts to directly use the model together with clinical data proved difficult. In particular, the model did not fit the human data obtained immediately after Pgp inhibition.

Conclusion

Population mixed effect modeling (in NONMEM) is a potent and powerful alternative and supplement to PK modeling and should be promoted for PK/PD modeling of PET data.

EVALUATION OF SERUM-AUTOANTIBODY-BIOMARKERS FOR EARLY DIAGNOSTIC TESTING OF COLON CANCER

Johana Luna-Coronell

University-supervisors: DI. PD. Dr. Andreas Weinhäusel, University of Natural Resources and Life Sciences Vienna. Institute of Applied Genetics and Cell Biology

AIT**-supervisor:** DI. PD. Dr. Andreas Weinhäusel. Health & Environment Department, Molecular Diagnostics

Introduction and Aim

Cancer has become a serious health problem in the last decades. Early diagnosis can improve survival, thus, there is great need and anticipation to identify novel biomarkers for cancer diagnostics at the earliest stage as possible. The aim of this project is to identify tumor autoantibodies (TAA) and to develop a test which enables early identification of colon cancer patients

Materials and Methods

We have produced a protein-chip from 16,000 human cDNA expression clones. On these 16k protein chips we have performed a candidate marker screening for identifying autoantibody profiles suitable for distinction of 4 groups, namely carcinoma patients, patients with polyps (low risk and high risk groups) and healthy controls. Biomarker screening was performed with isolated IgG from serum samples from a test-set of 134 samples derived from 99 serum patients between the 4 defined groups. Biostatistical microarray data was performed. A literature review was also made to check the reported colon TAAs.

Results

Class prediction between carcinoma vs. control samples gave a mean percent of correct classification of 89% of the samples, and cross-validation receiver operating characteristic (ROC) area under the curve of 0.938. Correct class prediction classification between controls vs. low risk samples resulted in 89%; low risk vs. high risk samples in 86%; and high risk vs. carcinoma samples 68%. A literature review was also made from published TAAs, and the classifier proteins which were found in the 16k human cDNA expression clones were added to the resulting clone list.

Conclusion

An optimized protocol for the 16k protein microarray was established and implemented. Biostatistics results showed that the samples could be differentiated between the defined groups. In the end, a total of 729 candidate markers were selected and will be used to generate targeted micro-arrays. Validation of these previous results will take place using a validation-set of serum samples from 100 individuals per group and tumor entity (n=400).

RAPID MULTIPLEX DETECTION OF BACTERIA, TOXINS AND ANTIBIOTICS

Thomas Hahn

Univeristy-supervisor: Dr. Agnes Grünfelder, Fachhochschule Tulln
AIT-supervisor: Dr. Claudia Preininger, Health & Environment Department, Bioresources

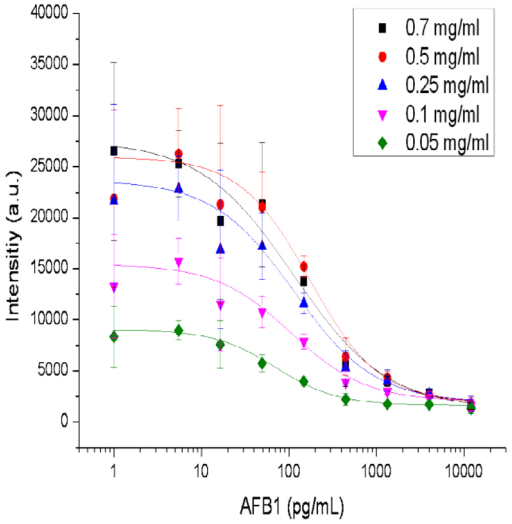
Introduction

Pathogenic bacteria, antibiotics and mycotoxins present in food are of major public health concern. In general, bacteria are analysed with cultivation-based methods, while small non-biological contaminants are determined by either ELISA or chromatographic techniques like HPLC or GC/MS. In order to simultaneously detect both pathogenic bacteria and hazardous chemicals we have developed a rapid multiplex screening tool based on a protein microarray.

Methods

The protein array for parallel detection of Aflatoxin B1, Penicillin G, Enrofloxacin and enterotoxigenic Escherichia Coli was established by using an inhibition assay format for the chemical compounds and a sandwich format for the bacteria.

For the inhibition assay antibiotic-BSA- and aflatoxin-BSA conjugates were spotted on ARChip Epoxy slides, while for the sandwich assay an equimolar mix of four aptamers was used. The BSA-conjugates were prepared using the mixed anhydride and EDC/NHS method and characterized spectroscopically. Detection of the bacteria was done using an anti-K88 detection antibody. Spiked milk was tested in order to investigate interferences from the sample matrix.



Results

The figure above shows a typical assay curve as obtained for aflatoxin spiked in milk, diluted 1:1 with assay buffer. As can be seen from the figure the signals as well as the slope, which is a measure for the assay sensitivity increase with increasing concentration of spotted BSA-conjugate. The dynamic range of the curve when using 0.5 mg/mL spotted BSA-conjugate is 50 pg/mL to 1300 pg/mL which perfectly covers the MRL of aflatoxin (MRL= 500 pg/mL)

Similar results were obtained for Penicillin G and Enrofloxacin. The best assay performance was achieved using 0.01 M PBS with 1 % highmolecular weight PVA as blocking reagent. The best suited conjugation method for carboxyl containing compounds was the EDC/NHS method.

Conclusion

Herein we demonstrate that the rapid multiplex protein array for simultaneous detection of bacterial pathogens, antibiotics and toxins represents an attractive screening tool compared with expensive chromatographic techniques and traditional biological binding assays, is much faster than conventional microbiological tests and even more important meets the requirements set by health authorities.

NOTES

AIT Austrian Institute of Technology GmbH
Donau-City-Straße 1 | 1220 Wien, Austria
T +43 (0) 50550-0 | F +43 (0) 50550-2201
office@ait.ac.at | www.ait.ac.at