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**20TH NATIONAL SYMPOSIUM
ON
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PREFACE

Dear all,

You hold in your hands the proceedings of the 20th National Symposium on Applied Biological Sciences (NSABS 2015).

The first edition of this symposium dates back to 1995, when the 'PhD symposium' was initiated in Ghent. Since 2002, it has been organized in a joint collaboration between Ghent University and KULeuven. The Universiteit Antwerpen, Université de Liège and Université catholique de Louvain were welcomed as official partners in 2012, the Université Libre de Bruxelles (Brussels) joined us in 2014. It is clear that, over the years, NSABS has become a true national event and the doctoral schools in the North and the South of Belgium are now contributing significantly.

NSABS provides an ideal opportunity for PhD students to develop and enhance their presentation and communication skills, a key requirement for their doctoral training. For this 30th edition, we are happy to give the floor to the young researchers presenting in total 29 oral presentations and 66 poster presentations. This edition introduces a new presentation category, namely "My Research in 3 minutes" with 5 short presentations. We hope the presenters will also profit scientifically from the feedback they will get from the audience, as well as from the oral presentation assessments made by the reviewers in each session.

We are looking forward to broaden our research horizons and to exchange ideas with our colleagues from other departments and other universities. We hope you will enjoy the symposium, as well as this edition of the communications.

PROF. XAVIER DRAYE

ON BEHALF OF THE ORGANIZING COMMITTEE

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M01

Bioimaging, phenotyping

EXTRACTION OF FUNGAL GROWTH FEATURES: COMBINING IMAGE ANALYSIS AND GRAPH THEORY

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KEYWORDS

Fungal growth, mycology, image Analysis, graph theory

ABSTRACT

Fungi are present in and affect most environments. Wooden material and plants are subject to their attack, causing economic losses in construction and agronomy industries. In contrast, there are also industries that profit from fungi, as they are used to create products ranging from alcohol and bread to industrial enzymes and antibiotics.

For these reasons, fungi have been studied extensively over the years. They are characterized by their unique structure, which allows for an efficient internal transportation of nutrients and a rapid expansion in a multitude of different environments, even those with extreme conditions. There have been many attempts to model and understand fungal growth dynamics.

Given the increasing availability of image capturing techniques, a new approach resides in image analysis, i.e., the extraction of significant information from images. Capturing images is easy and does not require expensive machinery. Most previous image analysis studies involve manual labeling of the fungal network or invasive techniques, which prevent the tracking of network evolution, and are time-consuming and tedious.

This work presents an automated tool combining image analysis and graph theory to monitor fungal growth. It is highly versatile since it can be tuned for different fungal species and image resolutions and can also be adapted for different growth scenarios and devices. This analysis permits the extraction of the most commonly studied parameters of fungal growth.

EFFICIENT AMPEROMETRIC THIRD-GENERATION BIOSENSOR BASED ON HEMOGLOBIN-MAGNETIC NANOPARTICLES

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KEYWORDS

Third-generation biosensor, haemoglobin, magnetic nanoparticles, direct electron transfer

ABSTRACT

Direct electron transfer (DET) of proteins has been a research focus for many years because of their ability to function as a model for mechanistic studies of biological systems and important in the field of developing third generation biosensors [1, 2]. Hemoglobin (Hb) is probably the protein whose DET is the most extensively studied. However, redox proteins show a slow rate of electron transfer on a conventional electrode due to the deep bury of the electroactive prosthetic group, the adsorptive denaturation and the unfavorable orientations when directly adsorbed on the electrode surface. Therefore, to optimize the electron transfer between the heme center in the large three-dimensional structure of Hb and the electrodes is challenging. Finding ideal electrode materials and suitable protein immobilization methods is highly demanded. Entrapment or encapsulation of a protein within a biocompatible material by using simple procedures, especially physical entrapment of biomolecules without the need of complicated covalently attachment or time consuming step, is certainly desirable. Here, we combined the unique properties of magnetic nanoparticles (MNPs) and biocompatible mixed hemi-micelles film for magnetic entrapment of Hb at the surface of screen printed electrode to develop a rapid and efficient hydrogen peroxide biosensor (Hb@mixed hemi-micelle@MNP/SPE).

POTENTIAL OF HIGH RESOLUTION THERMAL IMAGING SYSTEM TO EVALUATE STOMATAL CONDUCTANCE IN THE FIELD

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KEYWORDS

Thermal imaging, stomatal conductance, drought tolerance

ABSTRACT

Stomatal conductance and transpiration are critical variables for the assessment of drought tolerance of crops, but are barely used in genetic improvement programs for practical difficulties. Our projects evaluates the potential of high resolution thermal imaging systems which provide accurate measurements of leaf temperature, from which stomatal conductance and transpiration can be computed. Because such systems may require several hours for the acquisition of an entire evaluation trial, we have specifically investigated the amplitude of the temporal variation of leaf temperature in the field during the establishment of drought and for a small number of genotypes. Our results reveal that the amplitude of variation of maize leaf transpiration proxies during the day for a given genotype, induced by variation of stomatal conductance and environmental variables, is considerably larger than the genotypic variation of leaf temperature measured at any time. We have then used a statistical approach to model the daily evolution of leaf temperature in order to allow the inference on genotypes that are measured at different time. Interestingly, we have found that the temporal pattern of leaf temperature changes considerably with the position in the field and with the time during the establishment of drought, and that these variations were genotype-specific. This suggests that the interpretation of leaf temperature also requires a careful examination of soil conditions.

M02

**(BIO)CHEMICAL ANALYSIS
AND SYNTHESIS**

DIVERSITY AND COMPOSITION OF BENTHIC MACROINVERTEBRATE COMMUNITIES IN TROPICAL ALBERTINE RIFT RIVERS IN KASESE DISTRICT (SOUTH WESTERN UGANDA)

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KEYWORDS

Bio-monitoring, biotic index, Gini-coefficient, macro-invertebrates, Uganda

ABSTRACT

Biodiversity in Ugandan aquatic ecosystems is threatened by extinction and needs to be assessed to prioritize conservation campaigns.

Data obtained from 71 sites along Albert Nile headwaters in Kasese were analyzed to explain taxa composition and structure in macroinvertebrate communities at a spatial scale. Four diversity indices were used; Shannon-wiener, Simpson, Pielou's evenness and Gini-coefficient.

32,579 individuals were identified to family level. Insecta was dominant (45 families); Oligochaeta (2), Gastropoda (5) and Turbellaria (1). Site classification indicated 41 natural, good (15), moderate (7) and poor (8). All sites had sensitive taxa with Ephemeroptera most abundant (11,359); Plecoptera (24) and Trichoptera (3196). Gini coefficient among the site classes exhibited high inequality. Good and natural sites had highest diversity while moderate and poor the least. Lorenz curves showed a relatively high inequality and unevenness with values between 0.75 to 0.94. However, Pielou's index values ranged from 0.49 to 0.58. The natural sites had the highest taxa richness (41) while poor sites the lowest (24). Upstream sites had the highest number of pollution sensitive taxa like *Baetidae*, *Caenidae* and *Hydropsychidae*, which are collector-gatherers and collector-filterers while downstream locations had high numbers of *Chironomidae*.

Findings showed benthic macroinvertebrate composition, spatial distribution and pollution trends.

THE INTERACTION BETWEEN MALONDIALDEHYDE AND PROTEINS IN AN OIL-IN-WATER EMULSION

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KEYWORDS

Lipid oxidation, malondialdehyde, protein-malondialdehyde interactions, oil-in-water emulsion

ABSTRACT

Oxidative damage to food proteins can occur through an indirect mechanism involving the production of lipid oxidation-derived aldehydes such as malondialdehyde (MDA) and 4-hydroxyhexenal. MDA has been widely used as a marker compound to measure the extent of oxidative deterioration of lipids in food and biological systems. The interaction of various proteins with MDA under physiological conditions has been shown, generating various potential toxic adducts and crosslinking of proteins due to its reactivity towards reactive amino groups. In addition there have been indications that the protein-MDA interactions are involved in the pathogenesis of numerous human diseases including atherosclerosis, diabetics, cancer and Alzheimer. Because of the importance of MDA as a lipid peroxidation product in vivo, most studies have been carried out on its reaction with human proteins in physiological conditions. However, the reaction of MDA with proteins in food products and related food models is also of interest. In this study, the amount of free MDA in fully hydrogenated coconut oil-in-water emulsions stabilized by tween-20 or whey proteins were examined at two distinct temperatures. Our aim was to elucidate the impact of the type of food protein, emulsifier, emulsion matrices and reaction time on the amount of free MDA, in order to understand the interaction between proteins and MDA in an O/W emulsion.

OPTIMIZATION OF *TRICHODERMA REESEI* ENZYME MIXTURES FOR HYDROLYSIS OF AMMONIA PRETREATED MISCANTHUS

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KEYWORDS

Lignocellulose, cellulases, hemicellulases, enzymatic hydrolysis, *Trichoderma reesei*

ABSTRACT

Over the last decades, great efforts have been made to adapt cellulase cocktails to industrial processes. The filamentous fungus *Trichoderma reesei* is used industrially as the main source of cellulolytic enzymes. The parental QM6a strain was used as a starting point to generate highly productive strains, e.g. RUT-C30 and QM9414. Classical mutagenesis and selection on overexpression of cellulases resulted in an altered ratio of hemicellulases and non-glycosidase components in mutant enzyme cocktails when compared with the wild type mixtures.

In this study, hydrolytic performance of the enzyme cocktails secreted by *Trichoderma reesei* strains QM6a and RUT-C30 was compared. QM6a enzymes exhibited higher specific activities on chromogenic substrates and complex hemicellulose fractions. On several lignocellulosic substrates, QM6a cocktails performed better than RUT-C30 cocktails at equal -yet low- enzyme load. When enzyme load was increased, hydrolytic performance varied with the type of C-source used to induce cellulase production.

To improve the saccharification of ammonia pretreated Miscanthus by the RUT-C30 mixture, the enzymes were supplemented with a small amount of QM6a secretome. The enzymatic release of glucose, xylose and galactose increased by 31%, 28% and 10% respectively.

These data provide a first step towards the identification of candidates from QM6a enzyme mixtures to supplement the RUT-C30 enzymatic cocktail for lignocellulose hydrolysis.

CAPILLARY ELECTROPHORESIS FOR THE FUNCTIONAL ANNOTATION OF CARBOHYDRATE-ACTIVE ENZYMES: TWO PRELIMINARY CASE STUDIES

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KEYWORDS

Carbohydrate active enzymes, enzymes discovery, DSA-FACE

ABSTRACT

Nowadays there is a huge demand for greener and cost-efficient industrial processes where the discovery of new and efficient glyco-active enzymes with known specificity and activities may play an important role. For that reason there is a huge effort in developing high-throughput techniques for the correct and fast functional annotation of enzymes.

The present study exploits the DSA-FACE platform (DNA-sequencer aided fluorophore assisted capillary electrophoresis) for the high-throughput qualitative and quantitative analysis of carbohydrates¹. The high-throughput is achieved by the use of multi-capillaries designed for standard 96- and 384-well microtiter plates. Moreover, the technique has a high resolution and sensitivity, which enables to resolve stereoisomers with the same degree of polymerization and to detect products in the fmol range, respectively. The reliable quantitative analyses are particularly attractive for the analysis of glyco-active enzymes kinetics.

Here we describe a case study where the technique is used to elucidate the substrate specificity of arabinofuranosidases from family GH62. This is not fully understood since arabinofuranosidases can hydrolyze arabinose residues from xylose units substituted at C2 and/or C3 carbons.

A case study with amyloglucosidase and maltoheptaose is also presented where it is shown that DSA-FACE is useful to perform enzyme kinetics on a very small amount of enzyme products.

¹ Callewaert et al (2001) *Glycobiology*, 11: 275

BIOCHEMICAL METHANE POTENTIAL OF THREE VARIETIES OF FRESH AND DRY BANANA PEDUNCLES FROM CAMEROON

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KEYWORDS

Anaerobic digestion, banana peduncles, biogas, storage

ABSTRACT

Banana and plantain plants produce significant quantities of post-harvest biomass wastes which can be transformed for bioenergy production. This study aims at evaluating the biochemical methane potential (BMP) of banana peduncles biomass as potential renewable energy source exploitable in Cameroon, where the ratio energy cost versus standard of living for both electric and fossil energy is higher than in USA and EU. In this regard, biomethane can be locally converted into electricity, which could help to meet the local needs. Harvested peduncles samples were stored under two conditions: fresh frozen and air dried. The anaerobic digestion of these samples was monitored for a 60 days in a batch digestion. The obtained biomethane production was in the range of 0.2 l_{CH4}/g_{DM} and the average methane content in the biogas produced was 60%v/v. There were significant differences between the initial biomethane and biogas production rates between air dried and fresh frozen samples: 0.3 and 0.2 l_{CH4}/g_{DM} over the first 8 days. These results confirm that banana peduncles biomass can be used as a source of renewable energy in Cameroon. But sample storage conditions have significant influence on the BMP results.

BIOSYNTHESIS OF SILICA-TITANIA PHOTOCATALYSTS USING DIATOMS

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KEYWORDS

Diatoms, biomineralization, silica-titania photocatalysts

ABSTRACT

Nature provides numerous biomineralized micro-to nanostructures with 3D shapes which have been optimized during earth's evolution. A siliceous material of particular interest is biosilica produced by diatom microalgae. Diatoms are an extremely diverse group of unicellular algae that self-assemble a porous, intricate siliceous cell wall, called frustule. The frustule has an ordered 3D structure which contains pores with nano-to-microscale features.

Since 2008, nanotechnologists have recognized the potential of diatom frustules as biotemplate nanomaterial, which resulted in the new interdisciplinary field of diatom nanotechnology. The hierarchical organization of the porous nanostructures exceeds the capabilities of present day material science engineers and can be used in applications in chromatography, membrane technology and drug delivery. Insertion of foreign elements like titanium can further expand the current application field towards photocatalysis, light harvesting and energy storage. Here, we show that metabolic immobilization of titanium dioxide in the porous 3D architecture of the frustules results in a photocatalytic active material.

PROFICIENCY TESTING ON NATURAL VERSUS ARTIFICIAL MILK SAMPLES

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KEYWORDS

Proficiency testing, *Bacillus cereus*, lactic acid bacteria

ABSTRACT

Proficiency Testing (PT), also called inter-laboratory comparisons, are a widely used quality control tool to assess the laboratories' analytical performances. Most food microbiology PT provide exclusively artificial PT samples, although the samples analyzed in routine by the laboratories are naturally contaminated foods. This raises the question: can we draw reliable conclusions on the analytical competence of laboratories in routine, based only on their results on artificial PT samples?

A PT scheme involving 14 laboratories was organized in 2014 to compare the performances on an "artificial" sample (sterile milk) and on an "authentic" sample (raw milk), both spiked at identical levels, for the enumeration of 8 parameters.

For several parameters, like the enumeration of lactic acid bacteria and *B. cereus*, the performances of the laboratories were good in the UHT milk but completely incoherent in the raw milk. The enumeration was, indeed, much more arduous in the raw milk sample due to the variety of colony types and to interfering flora. The artificial PT sample provided a systematic underestimation of the analytical variability.

When the performance of the participants is assessed exclusively on the basis of artificial PT samples, several analytical issues cannot be detected. To avoid disregarding these matrix-related analytical issues, PT providers should include at least one naturally contaminated sample in their PT schemes.

IDENTIFICATION AND QUANTIFICATION OF PHENOLICS FROM *PISTACIA LENTISCUS* L. (*ANACARDIACEAE*) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIODE-ARRAY DETECTION

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KEYWORDS

Pistacia lentiscus, flavonoids, HPLC-DAD

ABSTRACT

Pistacia lentiscus L. (*Anacardiaceae*), common shrub in the Mediterranean Basin and dispersed along the Algerian coast. It contains several families of extractable compounds (flavonoids and tannins). It is used in traditional medicine to treat such diseases as eczema, diarrhoea, and inflammatory diseases.

Initially, we performed a selective extraction with different solvents on two parts of the plant (leaves and seeds). The extracts obtained are then assayed to quantify rates of various phenolic compounds and then successively analyzed, identified and quantified by HPLC-DAD methods.

The assay results show that the aqueous extract of chloroform is the richest in phenols (1104.603 ± 0.8 , 366.04 ± 9.85) EC/100g powder, for leaves and seeds successively. After the optimisation of the HPLC-DAD method, 12 different compounds were identified, in *P. Lentiscus* leaves and seeds extracts. The majority of the compounds were quantified. Flavonoids and phenolic acids were the most abundant compounds, those with the highest concentrations being gallic acid (23360,68 $\mu\text{g/g}$ of PF-B extract), 3,4 dihydroxy cinnamic acid (712,28 $\mu\text{g/g}$ of PF-B extract), syringic acid (526,671 $\mu\text{g/g}$ of PF ethanolic extract) and catechin (317,934 $\mu\text{g/g}$ of PF ethanolic extract). In light of the results obtained, it appears that *Pistacia lentiscus* is a natural source of biologically active substances.

PUBLIC HEALTH RISK ASSOCIATED WITH THE CO-OCCURRENCE OF MYCOTOXINS IN SPICES CONSUMED IN SRI LANKA

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KEYWORDS

Mycotoxins, spices, Sri Lanka, margin of exposure, risk assessment, hepato cellular carcinoma

ABSTRACT

A quantitative risk assessment of mycotoxins due to the consumption of chilli (*Capsicum annum* L.) and black pepper (*Piper nigrum* L.) was performed in Sri Lanka. A food frequency questionnaire was administered in order to collect the data on consumption of spices by households in the Northern and Southern region (n=249). The mean chilli consumption in the North was significantly higher ($p < 0.001$) compared to the South. Mean exposure to aflatoxin B1 (AFB1) in the North (3.49 ng/kg BW/day) and South (2.13 ng/kg BW/day) have exceeded the tolerable daily intake due to chilli consumption at the lower bound scenario, while exposure to OTA was small. Dietary exposure to other mycotoxins, fumonisin B1, fumonisin B2, sterigmatocystin and citrinin due to spices were estimated. Margin of exposure estimations at the mean exposure to AFB1 were remarkably lower due to chilli (45-78) than for pepper (2315-10,857). Moreover, the hepato cellular carcinoma (HCC) risk associated with the mean AFB1 exposure through chilli at the lower bound was 0.046 and 0.028 HCC cases/year/100,000 based on the North and South consumption, respectively. AFB1 exposure via chilli should be considered as a great public health concern in Sri Lanka due to both high mycotoxin concentration and high consumption.

BIOGENIC SILICA WITH A GREAT POTENTIAL IN CATALYSIS

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KEYWORDS

Diatom, silica, catalysis

ABSTRACT

Diatoms are a diverse group of unicellular, eukaryotic microalgae that self-assemble dissolved silica into a specialized cell wall structure or frustule. The frustule is composed of amorphous silica with an ordered pore structure leading to a hierarchical material. Interesting properties are the high mechanical and thermal resistance, the high surface area and the 3D ordered pore structure with micro and macro pores.

In the last decade several applications in electronics, optics and chromatography have been explored, but the intrinsic properties like high surface area and great mechanical and thermal resistance offer a great potential for catalytic applications. Here we disclose the surface area and pore size distribution of frustules from different diatom species which are key properties for catalytic processes. Additionally, the adsorption capacities for acetaldehyde in a gas phase reactor are presented.

SOLAR LIGHT PHOTOCATALYSIS: USING THE SUN TO ITS FULL POTENTIAL

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KEYWORDS

Photocatalysis, surface plasmon resonance (SPR), titanium dioxide (TiO₂), visible light

ABSTRACT

Photocatalysis is an attractive technology in which a semiconductor is used to degrade environmental pollutants through the absorption of a sufficient amount of light energy. The most encountered photocatalyst, titanium dioxide (TiO₂), experiences a large drawback due to its wide bandgap, which limits photon absorption to the UV range of the spectrum. As this UV region represents at most 5% of the solar spectrum, TiO₂ is not efficient when sunlight is used to drive photocatalytic reactions.

It is possible to tailor the photoresponse of TiO₂ to match the solar spectrum through the use of Au-Ag alloy nanoparticles. These metallic nanostructures display surface plasmon resonance in the visible light region of the spectrum, which leads to improved light absorption and thus higher photocatalytic efficiency using free sunlight¹.

¹ S. Verbruggen, M. Keulemans, M. Filippousi, D. Flahaut, G. Van Tendeloo, S. Lacombe, J. Martens and S. Lenaerts, Appl. Catal. B-Environ. 156-157, 116 (2014)

M03

SURVEY, DATA MANAGEMENT, STATS

OPTIMIZING HABITAT PREFERENCE MODELS OF *AZOLLA FILICULOIDES* (LAM.) [AZOLLACEAE] FOR REDUCING ECOLOGICAL MODELLING COMPLEXITY

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KEYWORDS

Ecological modelling, exotic species, habitat preferences, wetland management

ABSTRACT

An ecological study was carried out in Selkeh Wildlife Refuge (Anzali wetland, northern Iran) with the main objective of applying ecological modelling techniques for predicting habitat requirements of an exotic aquatic fern species (*Azolla filiculoides*, Lam.). The database consisted of measurements from 7 different sampling sites in the wetland during the 2007-2008 period. The measured variables consisted of water quality and structural habitat variables with cover percentage of *A. filiculoides* as output variable. We attempt to explore and compare the use of greedy stepwise (GS) and genetic algorithms (GA) in combination with classification trees (CTs) and support vector machines (SVMs) to select the most relevant input variables for predicting habitat preferences of *A. filiculoides*. The predictive power of models was assessed by the percentage of Correctly Classified Instances (CCI %) and Cohen's kappa statistics (k). Results show that after variable selection, the predictive performances were improved only for CTs. However, GA outperformed GS in both CTs and SVMs, leading to reliable prediction. Results also show that the dependence of the exotic fern on structural habitat parameters was more important than water quality variables. The outcomes of our study can help wetland managers and decision makers in order to set up successful wetland restoration/conservation and management programs.

STATISTICAL DATA TREATMENT OF N₂O FLUX MEASUREMENTS WITH CLOSED DYNAMIC CHAMBERS

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KEYWORDS

N₂O flux measurement, dynamic closed chamber system, statistical data treatment

ABSTRACT

Experimental campaigns of n₂o concentration measurement with a system of closed dynamic chambers have been performed at the GX-ABT field site of Loncée, during five months (June to October 2014).

From the results of these experimental campaigns, statistical data analyses are performed to determine the emitted fluxes during the period of measurement.

Fitting of the n₂o concentration vs. time in the closed chamber provides a way to compute the corresponding fluxes. Several regressions are compared (linear, quadratic and exponential). It turns out of this study that the linear fitting performs poorly compared to the two other ones (i.e. it most often gives a lower coefficient of determination) and tends to underestimate the emissions.

Statistical analyses are further performed to discriminate between relevant and irrelevant measurements. These analyses use, among others, the size of the 95% confidence interval for the regression parameters.

A Graphical User Interface is under development for the quick visualization of the results (fittings, goodness of fit and corresponding fluxes).

Once the fluxes have been determined, a study is carried out, aiming at identifying the environmental driving factors (e.g. soil moisture and temperature) accounting for the dynamics of these fluxes.

DRIVEN FACTORS OF CONSUMER CHOICE FOR IMPORTED EUROPEAN BEER IN CHINA

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KEYWORDS

China, consumer, European beer, driven factor

ABSTRACT

The demand for European alcoholic beverages in China has increased tremendously in recent years. However, there is still a lack of understanding of the behavior of Chinese consumers towards European alcoholic beverages. This study explores the important product attributes driving consumer choice for imported European beer in China. The data are collected in two cities: Shanghai and Xi'an. Participants (n=541) indicate the importance of thirteen product attributes for their beer choice. Additionally, their consumption experience for imported European beer has been explored. Results show that 'origin' and 'price' are the most important product attributes for Chinese consumer's choice for imported European beer. The consumption of imported European beer is positively associated with 'brand', 'origin', 'assortment', 'color', 'texture' and 'smell' and it is negatively linked to 'price' and 'alcoholic content'. These findings are helpful for European alcoholic beverage producers to better understand consumers in China and to develop effective marketing strategies for this huge market.

WILDFLOWER STRIPS FOR CROP PROTECTION: WHAT DO WE KNOW? WHAT SHOULD WE KNOW?

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KEYWORDS

Wildflower strips, conservation biological control, functional diversity, mowing regime, ecosystem services, Wallonia

ABSTRACT

Wildflower strips (WFS) are known to support the conservation of a large diversity of insects and thus natural enemies (predators and parasitoids) that can control pests. However, the conclusions of studies looking at the efficiency of WFS to control pests are not unanimous. Indeed, the enhancement of pest control seems to depend on (1) the ability of flowers to attract the natural enemies at the right moment and (2) the capacity of natural enemies to migrate into the adjacent crops to attack pests. Therefore, constituting appropriate flower mixes may be an essential lever to enhance the efficiency of pest control. In this context, using functional diversity is promising. To our knowledge, few studies have tested the impact of the functional diversity of a flower mix on insect abundance and diversity and the control of pests.

Additionally, the management of the WFS, such as mowing, seems to be another lever that may affect pest control. Indeed, perennial WFS should be regularly mown to maintain initial flora. However, mowing may affect insect populations by temporarily destroying the habitat. Previous studies already assessed the impact of the mowing regime on insect diversity but knowledge on its impact on the trophic relations between these insects seems to be very limited.

Through this contribution, the insect diversity and abundance found to be associated with the different kinds of WFS and management applied will be discussed, as well as the further research needed.

LINKING BEES AND FLOWERS: MUTUALISTIC INTERACTION NETWORKS TO STUDY ECOSYSTEM FUNCTIONING

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KEYWORDS

Pollination networks, pollinators, food web, ecosystem functioning

ABSTRACT

In recent years, much attention is going to pollinators, as they are important for the increasing food production while being threatened by agricultural intensification and other environmental drivers. While honeybees (*Apis mellifera* L.) are generalist according to their flower preferences, wild pollinators' flower preferences can go from generalist to very specialist. The same goes for plant species, which can be generalist or specialist in the pollinator species they need to get pollinated. The combination of a set of generalist and specialist plant species with a pollinator community consisting of generalists and specialists in a certain habitat, results in an interaction network between pollinators and the visited plant flowers. Studying this mutualistic interaction network is a time-consuming method, but can provide valuable data to calculate indicators of ecosystem functioning. One of the important conclusions that came already out of studies applying this method is that pollination systems are often more generalized than thought based on pollination syndromes. This poster will explore the method of studying mutualistic interaction networks with a focus on the sampling protocol, the indicators that can be derived from the data and their meaning.

ADSORPTION BEHAVIOR OF RHODAMINE 6G AND RHODAMINE B ON SDS-COATED MAGNETIC NANOPARTICLES

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KEYWORDS

SDS-coated magnetic nanoparticles, mixed hemimicelle/admicelle, dye adsorption, response surface methodology, central composite design

ABSTRACT

Rhodamine B (RB) and rhodamine 6G (RG) are derivatives of the xanthene dyes which are used as colorant in textiles, foodstuffs, water tracer, petroleum products dyeing, paper printing, color photography and cosmetic products. They are injurious if swallowed by humans and they may be harmful if inhaled or in contact with the skin and eye. Also, their carcinogenicity, reproductive, developmental, neuro- and chronic toxicity have been proven [1]. However, due to the fact that RB and RG are soluble in water, they are easily released into the environment. Therefore, making efforts to develop an efficient and reliable method for removal and determination of RB and RG in different aqueous samples is inescapable.

In this work, we studied the behavior of adsorption of rhodamine B and rhodamine 6G on SDS-coated magnetic nanoparticles as mixed hemimicelle/admicelle nanocomposites. The important parameters affecting on adsorption such as SDS/MNP ratio and amount of MNPs were optimized by response surface methodology. Also, adsorption kinetic and adsorption isotherm studies were performed. Finally, the method was successfully applied to the adsorption of RG and RB from real samples.

M04

IN VITRO TECHNIQUES

IMPACT OF POLYETHYLENE GLYCOL-INDUCED DROUGHT STRESS ON IN VITRO HYPHAL NETWORK DEVELOPMENT AND GERMINATION OF *RHIZOPHAGUS IRREGULARIS*

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KEYWORDS

Monoxenic culture, drought stress, hyphal network, spore germination, polyethylene glycol (PEG)

ABSTRACT

Water stress is a major issue in most subsaharan countries impacting crop development and yield. Most plants are associated to arbuscular mycorrhizal fungi (AMF). These obligate root symbionts have been reported to mitigate drought stress in plants. They could thus be considered in crop management practices. However, before application there is a need for a thorough evaluation of the impact of water shortage on their growth and development to select the most efficient strains. Here, we present preliminary results on the impact of polyethylen glycol (PEG)-induced drought stress on the development of hyphal network and on spore germination of *Rhizophagus irregularis* MUCL 41833 under in vitro culture conditions. Using monoxenic culture of the fungus, different water potentials (ψ) were achieved in bicompartmented plates by adding different concentrations of PEG in a solid growing medium. Our first outcomes tend to show a negative effect of the addition of PEG at 50g.l⁻¹ on hyphal length (-49%) and spore production (-67%) as well as a significant effect for [PEG] \geq 50g.l⁻¹ on the germination rate (-70 to -100 %) compared to the control.

CYTOTOXICITY ASSESSMENT OF INGESTED SILVER NANOPARTICLES ON AN INTESTINAL COCULTURE MODEL

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KEYWORDS

Silver nanoparticles, caco-2 cells, cytotoxicity assays, mucus

ABSTRACT

Due to their anti-microbial properties, silver nanoparticles (AgNPs) are the most common nanomaterial mentioned in consumer products descriptions. In the agri-food sector, they can be found in sprays or anti-microbial coatings of containers and packaging devices or contents. Except from unbrand dietary supplements, they are not directly incorporated in food but can migrate and be ingested. However, in vitro studies on intestinal cells are lacking, compared to lung or liver cells. For this study, suitable cytotoxicity assays were first chosen based on their absence of interference with AgNPs. Toxicity of AgNPs and silver ions (Ag⁺) was then compared through chosen assays on undifferentiated Caco-2 cells, showing that Ag⁺ present in solution of colloidal AgNPs are not responsible of all the toxicity caused by AgNPs. Finally, the assays were applied on cells cultivated on inserts with either monocultures of Caco-2 cells or cocultures of Caco-2 cells and HT-29MTX cells, leading to a mucus layer. A dose dependent toxicity was observed with a succession of viability aspects deterioration. At low concentrations of AgNPs, cytoplasmic ATP decreased dramatically while, at higher concentrations, membranes were damaged and cell detachment was observed. The mucus layer failed to protect cells from a decrease in ATP although protecting their viability estimated by neutral red assay. This lack of convergence can not be explained and further investigations are needed.

COMBINATION OF RHIZOBACTERIA AND RHIZOPHAGUS IRREGULARIS INTO ALGINATE BEADS: A STEP TOWARDS THE FORMULATION OF MICROBIAL BIO-INOCULANTS

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KEYWORDS

Mycorrhiza, rhizobacteria, potato, bioinoculant

ABSTRACT

The aim of the present study was to test the feasibility of using the encapsulation in alginate beads of different rhizobacteria strains with in-vitro produced spores of the Mycorrhizal Fungi: *Rhizophagus irregularis*; and evaluate the effects of bacteria on the symbiotic and symbiotic development of the fungal partner.

Several bacterial strains were isolated and identified from 4 field plots in Ecuador. However, for the in-vitro tests only bacteria belonging to the Firmicutes group were selected due to their capacity to produce resistant spores. In total 30 strains were tested for antagonism against *Phytophthora infestans* and *Rhizoctonia solani* and their capacity to produce IAA (indolacetic acid), P solubilization and ACC deaminase capacity. Further, only ten bacterial strains were selected for the encapsulation with isolated spores of the AMF *Rhizophagus irregularis* MUCL41833. The results showed that one of these rhizobacteria strains identified as *Paenibacillus* sp (R47065) had a positive effect on asymbiotic development of *R. irregularis* spores ($p < 0.001$).

Afterwards, alginate beads containing propagules of *R. irregularis* and *Paenibacillus* sp. (R47065) were inoculated in close contact with the roots of three potato varieties (I-Fripapa, Bintjie and Unica) growing under autotrophic in-vitro systems. The results showed significant positive effects of the bacteria in the performance of *R. irregularis*, associated to the potato plants for most of the evaluated parameters.

YEAST DIVERSITY OF CUBAN COCOA BEAN HEAP FERMENTATIONS AND THEIR ENVIRONMENTS

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KEYWORDS

Cocoa bean fermentation, yeast, Cuba

ABSTRACT

The yeast diversity of spontaneous cocoa bean heap fermentations carried out in the east of Cuba was investigated. Yeasts were isolated from eight fermentation processes and their environments such as surfaces, material tools, insects, flowers and plants. The basic fermentation process parameters temperature and pH were recorded. One hundred fifty-one yeast isolates were grouped by M13-PCR-fingerprinting of genomic DNA. Representative isolates were identified using the ITS and D1/D2 region of the large subunit rRNA gene, as well as partial actin gene sequences were used if required. Preliminary results showed *Pichia kudriavzevii* and *Hanseniaspora opuntiae* as the major members among 24 detected species in the fermentation yeast community. Also *Saccharomyces cerevisiae* was present. *Hanseniaspora opuntiae* was also the most dominant species in the fermentation environments among 20 detected species.

INGESTED SILVER NANOPARTICLES MODIFICATIONS THROUGH THE GASTRO-INTESTINAL TRACT

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KEYWORDS

Silver nanoparticles, in vitro pre-colonic digestion, SEM-EDX, DLS, UV-vis spectrophotometry

ABSTRACT

Silver nanoparticles (AgNPs) has anti-microbial properties requested by the food sector to develop food supplements and improve f.i. container coatings and packaging. Therefore, AgNPs could be ingested by consumers and go through their gastro-intestinal tract before reaching systemic circulation and tissues. Nevertheless, AgNPs induce damage for different cell lines and their physico-chemical properties might affect their toxic potential. Moreover, during the digestion process, enzymes, electrolytes and pH might alter the AgNPs before they attain the intestinal cells and could have an influence on their toxicity.

In order to study the fate of AgNPs in the gastro-intestinal tract, we developed an in vitro pre-colonic digestion method for ingested AgNPs < 20 nm divided in 3 steps – i.e. salivary, gastric and intestinal – that were mimicked by the addition of the major digestive enzymes and appropriate pH adjustment. Digesting AgNPs were sampled after each steps and characterized by various methods (UV-vis spectroscopy, SEM-EDX and DLS).

Our results indicate that AgNPs physico-chemical features are modified through the in vitro digestion process, after the gastric step during which nanoparticles agglomerate. However, clusters appear to be disintegrated in the intestinal step where dispersed and nanometric silver particles are recovered. Toxicity assays have still to be performed on intestinal cells to assess if those modifications have an impact on the toxic effect of AgNPs.

SILVER NANOPARTICLES EFFECT ON THE INFLAMMATORY STATE OF THE GUT

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KEYWORDS

Silver nanoparticles, inflammation, Caco-2 cells, oxidative stress

ABSTRACT

Due to their widefield applications, nanomaterials have been used extensively leading to an increase of human exposure with potential impacts for health. Silver nanoparticles (Ag-NPs) are mostly used in food packaging as well as pharmaceutical products, although their potent harmful implications have been investigated and reviewed. In the present study we investigated the role of Ag-NPs and their potential to induce oxidative stress and inflammation in the human intestinal cells. The use of quercetin, a phenolic antioxidant found in vegetables and fruits was also employed. The results revealed that Ag-NPs reduced the IL-8 levels concentration in the apical compartments of intestinal cells and down-regulated the IL-8 mRNA expression, while NO levels were increased in both compartments. In addition, as a result of oxidative stress induced by reactive oxygen and nitrogen species formation, a decrease in reduced thiol groups, increased lipid peroxidation and lysosomal membrane instability was revealed upon exposure to Ag-NPs. These data demonstrate that Ag-NPs influence the inflammatory state of the gut by induction of oxidative stress, while the down-regulation of IL-8 suggests a potential anti-inflammatory role for Ag-NPs. In the case of administration of Ag-NPs with quercetin, the latter protects the gastrointestinal cells from the harmful effect of Ag-NPs via reduction of oxidative stress, cytotoxicity and by preserving the integrity of the cells.

CHARACTERIZATION OF POLYPHENOLS AND ANTIOXIDANT POTENTIAL OF BLACK CARROT (*DAUCUS CAROTA*) BY-PRODUCTS: PEEL AND POMACE

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KEYWORDS

Black carrot, pomace, peel, by-products, polyphenols, anthocyanins, phenolic acids, antioxidant activity

ABSTRACT

Recently, black carrots has gained interest as a natural colorant due to their substantial content of bioactive compounds, especially anthocyanins. Black carrot is often consumed after processing into various products. As a result of processing, large amounts of by-products including peel and pomace are generated. These by-products have the potential to be used as valuable resources for bioactive compounds. Given the above, the aim of the current study was to characterize the polyphenols and antioxidant potential of black carrot peel and pomace. Total anthocyanins, total phenolics, polymeric color and antioxidant capacity were determined using spectrophotometric methods, whereas individual phenolic acids and anthocyanins were identified using HPLC-DAD. The results revealed that the total anthocyanin content of pomace was slightly higher (3%) than black carrot, whereas the anthocyanin content of the peel was 25% lower than whole carrots. Major anthocyanins detected were cyanidin-based with different sugar moieties, among them two were non-acylated (cyanidin-3-xylosyl-glucosyl-galactoside and cyanidin-3-xylosyl-galactoside), and three were acylated with sinapic acid (cyanidin-3-xylosyl-sinapoyl-glucosyl-galactoside), ferulic acid (cyanidin-3-xylosyl-feruloyl-glucosyl-galactoside) and coumaric acid (cyanidin-3-xylosyl-coumaroyl-glucosyl-galactoside). Overall, the current study highlighted black carrot peel and pomace as good sources of polyphenols with high antioxidant activity.

ELICITATION OF THE DEFENCE MECHANISMS AT PLANT CASE OF *CUCUMIS MELO*

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KEYWORDS

Elicitor, Melon, carob, defence mechanisms, priming, prick

ABSTRACT

The judicious use of elicitors could reduce the amount of pesticide necessary to protect crops and preservation of the environment. This strategy, frequently called "stimulation of natural defenses" arouses more and more interest in the phytosanitary field.

The objectives of this work were 1) to research the natural elicitor molecules of the defence mechanisms in planta at the melon plant (*Cucumis melo* L.), 2) to compare the release of the defence mechanisms at the melon plant in the case of an abiotic elicitation and in the case of a treatment with fruit extracts. Two unripe carob varieties extracts, Lahlou and Tounsi, were used in this study, supposed having simulative properties of natural defences at plants (SND). The studied pathological system was "*Cucumis melo* / prick".

To understand the signaling pathways involved in the defence mechanisms, these works were performed:

- Analysis of the enzymatic activities relating to the stress (the peroxidase (POD) and the catalase).
- Determination of total phenolic and soluble protein.

The main results were expressed by precocity and intensity of enzymatic activities because of the treatment with two carob varieties extracts. Indeed, the induced defence mechanisms would be assimilated to the systemic resistance acquired (SRA) due to the increase of the total phenolic contents and POD. In addition, the phenomenon of priming was generally observed in the site of the second elicitation.

SYNTHETIC SELF-ASSEMBLING PEPTIDES STABLE ADSORPTION ON POLYMER SURFACES AND EFFICIENCY FOR CELLS ADHESION AND GROWTH

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KEYWORDS

Self-assembling peptides, coatings, synthetic culture matrices

ABSTRACT

Currently, cell culture systems intended to therapeutic applications are subjected to constraints aiming at ensuring biosecurity, validation between R&D and industrial scales, use of synthetic and biomimetic culture environments and surface to volume ratios optimization.

In this context, synthetic matrices have defined physico-chemical properties and should thus facilitate scale-up and processes validation. Moreover, self-assembling peptides matrices can mimic extracellular matrix mechanical properties, are cytocompatible and can have bioactive properties.

In this scope, the aim of this work is to assess the stable adsorption and cytoactivity maintenance of synthetic self-assembling bioactive peptides on hydrophilized polystyrene. These peptides, synthesized by Peptisyntha, contain RGD, collagen and fibronectin-derived bioactive sequences that should be recognised by cells and promote their adhesion and spreading.

First of all, peptides coatings stability and coated surfaces topography were studied. The results showed that peptides remain at the polymer surface after coating and washing (XPS, AFM, SEM, ToF-SIMS, HPLC).

Secondly, cells adhesion tests (in serum-free medium) were performed on coated surfaces, using human stem cells. It was shown that peptides coatings provide cell adhesion and growth and have a similar efficiency as animal-derived coatings.

In conclusion, the studied peptides are interesting clues for entirely synthetic culture systems.

IMMUNOMODULATORY ROLE FOR THE PILI OF *LACTOBACILLUS RHAMNOSUS* GG. POSSIBLE APPLICATIONS TOWARDS ASTHMA AND ALLERGIES.

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KEYWORDS

Probiotics, *Lactobacillus rhamnosus* GG, pili, microbe-host interaction, atopic diseases

ABSTRACT

The last decades, the prevalence of atopic diseases has dramatically increased, likely due to a reduced exposure to microorganisms. This led to more interest in the use of probiotics in the prevention and/or treatment of atopic disorders. *Lactobacillus rhamnosus* GG (LGG) is a clinically well studied probiotic with several reported beneficial effects including enhancing certain immune functions and decreasing the development of atopic diseases in susceptible individuals. Nevertheless, outcomes of different clinical trials remain heterogeneous. Thus, health and probiotic organizations highlight the importance of better molecular-based research approaches to unravel the mechanisms underlying the various beneficial effects of probiotics.

The aim of this project is to investigate the interaction and possible immunomodulatory role of the, only recently, discovered pili of LGG with immune cells. Hereto, different immune cells were incubated with LGG wild type and mutant strains to assess the role of the pili in adhesion and their immunomodulatory capacity.

Altogether, our results indicate a key role for pili in the interaction with immune cells, especially for adhesion and uptake. Immunomodulation experiments on the other hand could not attribute a direct role for the pili in the induction of pro- or anti-inflammatory cytokines. Nevertheless, a difference in the interaction capacity with LGG was observed in immune cells from allergic and non-allergic individuals.

ENZYMATIC CONTROL OF MATURE *STAPHYLOCOCCUS EPIDERMIDIS* BIOFILMS

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KEYWORDS

Staphylococcus epidermidis, biofilms, nosocomial infections, enzymatic control

ABSTRACT

Commensal bacterium of human skin and mucosa, *Staphylococcus epidermidis* is now regarded as an opportunistic pathogen. Its virulence, encountered in ca. 30% of cases of nosocomial infections associated to indwelling medical devices, is primarily due to its biofilm-forming ability. Biofilms display very little sensitivity to antibiotics and continuously release planktonic cells, therefore causing acute infections. The development of novel strategies to combat biofilm-borne infections is thus needed, including the potential use of lytic enzymes. This study aims to apprehend the synergistic effects of different enzymes on mature *S. epidermidis* biofilms: dispersin B, lysostaphin, and two phage peptidoglycan hydrolases (Twort and K).

S. epidermidis biofilms were grown in 96-well plates, incubated in presence of the enzyme cocktails for 2h and stained. Absorbance (540nm) indicated the quantity of biofilm left in the wells. The experiments were designed to quantify the impact of each enzyme on the biofilm degradation, as well as the their interactions. Dispersin B and lysostaphin appeared indispensable to clear the wells from any residues whereas the two phage lysins did not significantly participate to the biofilm eradication. Statistical analysis also highlighted the rinsing steps as critical factors affecting reproducibility. Perspectives of enzymatic control of biofilms are numerous, ranging from prevention to curative treatment, notably as potentiating agents for antibiotics.

BACTERIOPHAGES: USEFUL WEAPONS AGAINST EMETIC *BACILLUS CEREUS* ?

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KEYWORDS

Bacillus cereus, emetic toxin, cereulide, food safety, bacteriophages

ABSTRACT

Bacillus cereus is a bacterial foodborne pathogen implicated in more than 1,000 intoxications each year in Europe (EFSA and ECDC, 2014). The ubiquity and the sporulation capacity of this bacterium, as well as the extreme resistance (pH, heat and protease) of its emetic toxin, the cereulide, prevents an efficient sanitation by conventional methods. Therefore, novel means to control the emetic pathotypes of *B. cereus* are necessary. Among these, bacterial viruses, known as bacteriophages (or phages), certainly represent a potential alternative worth to explore. In order to establish a collection of bacteriophages specific to the emetic strains of *B. cereus*, about 300 samples of various origins (e.g. soil, food, animal faeces, wastewater treatment plant) were incubated with a representative panel of emetic strains before isolating individual lytic phages. Results highlight a broad spectrum of sensitivity of the emetic strains to the phages, and, conversely, the absence of a single phage able to lyse all the strains tested. However, 89% of 53 emetic *B. cereus* were lysed by a combination of 7 bacteriophages. These phages originated from Belgian agricultural soils and wastewater treatment plants. The use of bacteriophage cocktails shows multiple advantages as well as a limitation of apparition of resistant bacteria. Further analyses have to be carried out to ensure the safety of these phage cocktails for humans and their efficiencies to control *B. cereus* pathotypes in food matrices.

COMBINED IMPACT OF NUTRITION AND POLLUTION: THE FATTY ACID PROFILE MODULATES THE TOLERANCE TO CADMIUM AND METHYLMERCURY IN RAINBOW TROUT HEPATOCYTES

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KEYWORDS

Metals, Omega-3 fatty acids, Omega-6 fatty acids

ABSTRACT

Aquatic organisms experience stress when an external stressor moves them away from their optimum performance in terms of growth and reproduction. Two relevant examples of stressors for fish are sub-optimal lipid nutrition (e.g. fish oil vs vegetable oil based diet) and heavy metals (HM) exposure (e.g. methylmercury). To date, there is a good knowledge about the biological responses of fish to these two stressors, when considered individually. However, though both dietary lipid quality and HM are known to influence similar biological processes (e.g. redox homeostasis, fatty acids (FA) metabolism), the interactions between them are poorly known. In that context we aimed to further explore the role of lipids in fish sensitivity to HM such as cadmium and mercury, using first an in vitro approach. Practically, seven cell batches, from the rainbow trout liver cell line RTL-W1, differing in their FA profile were developed by enriching the growth medium with specific FA. These modified cells were then challenged with cadmium or mercury. Two ω -3 FA, α -linolenic acid (ALA) and eicosapentaenoic acid (EPA) showed a particularly high protection against cadmium (EC50 3 times higher than in non-enriched cells) and methylmercury (EC50 4-4.5 times higher than in non-enriched cells) but none protected the cells against inorganic mercury. Based on these results, multiple stress scenarios have been selected for further mechanistic investigations (in vitro and in vivo).

SPOILAGE CHARACTERIZATION OF CHICKEN BREAST FILLETS STORED UNDER DIFFERENT PACKAGING AND TEMPERATURE CONDITIONS, USING SIFT-MS

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KEYWORDS

Spoilage, VOCs, volatiles, SIFT-MS, chicken

ABSTRACT

Meat and fish are characterized as very perishable products through spoilage processes due to their intrinsic properties. The majority of these products are packaged under modified atmosphere (MAP). A major disadvantage of this packaging methodology is the fact that quality assessment based on sensory, microbiological or chemical analyses is only possible in an invasive way. The aim of the CheckPack project is the development of an optical sensor, integrated in the packaging material, to be used in a non-destructive way as a tool for fast and accurate determination of the quality status of packaged meat and fish products. This study is therefore going to illustrate the relationship between microbial growth, the production of volatile organic compounds (VOCs), and consumer appreciation and acceptance. The VOCs, which are a result of the microbial metabolism, can be used as biomarkers which will be measured consequently by the sensor. Raw chicken breast fillets were packaged under various modified atmospheres and stored at 4°C and 8°C, respectively. Microbial growth was followed during self-life while VOCs were quantified using selected ion flow tube mass spectrometry (SIFT-MS). The gas composition and the pH were measured respectively as function of time. Sensory evaluation was performed to indicate differences in the spoilage status of the fillets during self-life.

NOVEL ANTIPATHOGENIC ACTIVITIES OF LACTOBACILLI AGAINST NASOPHARYNGEAL PATHOGENS

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KEYWORDS

Lactobacillus, probiotics, upper respiratory tract infections, antimicrobial activity, screenings platform

ABSTRACT

Acute infections of the upper respiratory tract are situated at number one of the most common new diagnoses of Flemish doctors according to figures from the Flemish government with an incidence of 240 new diagnoses per 1000 patients. In too many cases, these infections are treated with antibiotics which have several side effects. Studies indicate that the microbiota in the upper respiratory niche is disturbed during infection. In this research we investigate whether locally administered probiotics can have a positive effect on the infections and can eliminate the microbial disruption. In agreement with the main modes of action of beneficial gut probiotics, lactobacilli could also promote health in the nasopharyngeal niche by (i) having antimicrobial actions against important pathogens; (ii) strengthening the mucosal barrier function; and (iii) modulating host immunity. In this study, different lactobacilli are screened for antipathogenic activity against the 6 most common nasopharyngeal pathogens. Furthermore, the mode of action of this activity is investigated. The early results indicate antipathogenic activity for certain lactobacilli. Investigations for the mode of action are ongoing.

INTERFERENCE WITH BACTERIAL SOCIOBIOLOGY: INHIBITION OF COMMON GOOD PRODUCTION

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KEYWORDS

Cooperation, biofilm, antimicrobial strategy, *Salmonella*

ABSTRACT

Microbes often cooperate by the production of public goods, which are resources that are costly for the individual to produce but provide a benefit to all the individuals in the population. Nevertheless, antibiotics, our main line of defense against micro-organisms, target individual-level traits and thus select for resistance. An interesting alternative lies in targeting mechanisms by which MOs interact with each other, which could weaken the population and increase the susceptibility to antimicrobials and the immune system. Even more interesting is the prediction, based on social evolutionary theory, that these treatments can select against resistance. Indeed, mutants that resist a therapeutic that prevents public good production would not be favored by natural selection, because the sensitive strains would have a small growth advantage as they are not producing the costly public goods.

To deliver a proof of concept we focused on the exopolymeric substances (EPS) of *Salmonella Typhimurium* biofilms. Biofilms are clusters of cells imbedded in the EPS and it is commonly accepted that biofilms are the main form of bacterial growth. The biofilm matrix can be seen as a public good as it can be produced by one cell and offer protection to surrounding cells. We evaluated if non-EPS producers can outcompete EPS producers in short term competition experiments using different types of biofilm assays.

IMPACT OF DIESEL ON THE SYMBIOTIC PARTNERS *MEDICAGO TRUNCATULA*/*RHIZOPHAGUS IRREGULARIS* UNDER IN VITRO CULTURE CONDITIONS

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KEYWORDS

Arbuscular mycorrhizal fungi (AMF), symbiosis, oil pollution, diesel, spore germination, plant growth, in vitro culture

ABSTRACT

The tropical rain forest of Ecuador, Amazon region, in particular the Yasuni reserve, is a hotspot of diversity. It also contains a major reservoir of oil. Since 1970, the oil industry development has become a serious threat to the environment, resulting in pollution of soil. The remediation of polluted soils by crude oil has become a priority for Ecuador.

Currently, there is an increased interest in phytoremediation methods assisted by arbuscular mycorrhizal fungi (AMF). Indeed, these fungi colonize the majority of plant roots and are considered essential for the survival of many plants in polluted environments.

In the present study, we investigated in monoxenic culture conditions the direct impact of oil on the symbiotic partners. The host plant *Medicago truncatula* and the AMF *Rhizophagus irregularis* MUCL 41833 were cultivated in absence or in presence of diesel which was added to the culture medium at the concentrations of 0.05; 0.1; 0.5 and 1%.

The results demonstrated that the fungal spore germination as well as plant growth were inhibited in the presence of the two highest concentrations of diesel. As a consequence, this beneficial association could be hampered under such polluted conditions.

These preliminary results open the door to investigate the mechanisms behind this inhibition. The in vitro system also allows the study of the impact of diesel on one of the major function of the mycorrhizal symbiosis, i.e. the nutrient transport from the fungus to the roots.

ISOLATION OF ACETIC ACID BACTERIA FROM MOROCCAN BIOTOPES AND OPTIMIZATION OF THEIR BIOMASS PRODUCTION USING RESPONSE SURFACE METHODOLOGY

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KEYWORDS

Isolation, vinegar, starter, *Acetobacter*, acetic fermentation, response surface methodology

ABSTRACT

The objective of this work was first the isolation of novel acetic acid bacteria strains from natural Moroccan habitats, and then, the evaluation of their ability to produce microbial starters for vinegar production. The isolation was made from figs, dates, cactus, traditional vinegars and alcohols. Five strains, selected from a total of 63 isolates were confirmed to be belonged to *Acetobacter* species according to biochemical and metabolic studies. Acetic acid fermentation tests, performed on date and apple fermented juices by the selected *Acetobacter* strains, showed high capacity of acidification. The most powerful strain, isolated from traditional apple vinegar, yielded an acidity of about 6 % (w/v) on date juice. A cell growth optimization study was carried out on the most efficient strain using the response surface methodology (RSM). The linear, quadratic and interaction effects of four factors; ethanol, acetic acid, glucose and pH were studied by the application of a central composite design. 30 experiments were designed to predict the maximum concentration of cell biomass. The optimal calculated values of ethanol, acetic acid, glucose and pH allowing the prediction of the maximum biomass production (2,13g/L) were 16 g/L, 8 g/L, 15g/L and 5.1, respectively. Subsequently, further batch fermentation cycles were carried out in a 10L lab-bioreactor to confirm the mathematical model. The results were in line with the predicted values.

M05

**NUMERICAL MODELLING
AND SIMULATION**

MODELLING MICROBIAL COMPETITION IN BIOFILM REACTORS

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KEYWORDS

Biofilm reactors, mathematical modelling, wastewater treatment, nitrogen removal, microbial competition

ABSTRACT

Biological nitrogen removal from wastewater can be considered as a proven technology and has been widely implemented. During nitrification, which is the key reaction in biological nitrogen removal processes, ammonia-oxidizing bacteria (AOB) convert ammonia to nitrite, which is further oxidized to nitrate by nitrite-oxidizing bacteria (NOB). Biofilm reactors display distinct advantages for the cultivation of these slow growing nitrifiers, due to their biomass retention characteristics. Based on molecular techniques, the coexistence of two or more species performing the same function has previously been reported in nitrifying biofilms.

In this contribution, a unique biofilm model including the growth and decay of 60 AOB species and 60 NOB species was developed to investigate the factors influencing microbial competition and coexistence in biofilms. It was found that the microbial composition of the biofilm was clearly different when nitrogen or oxygen was limiting. The maximum growth rate and the affinity for the limiting nutrient, characterizing each species, had a substantial influence on the resulting microbial composition. It was confirmed that species performing the same function were able to coexist if they followed different strategies, i.e. species with a high affinity for the limiting substrate but a low growth rate (K-strategists) were able to live deeper in the biofilm than the species characterized by a high growth rate but a low affinity (r-strategists).

COMBINING IN VIVO AND IN SILICO EXPERIMENTS TO DECRYPT ROOT WATER UPTAKE DYNAMICS

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KEYWORDS

Root system, water uptake dynamics, modelling

ABSTRACT

In our experiments, we monitored in 2D the evolution of soil water content around roots of transpiring maize plants using a light transmission imaging technique on a rhizotron. Subsequently, we digitized the entire root system in order to create an input file for the model RSWMS (HYDRUS-like model). A sensitivity analysis of the modeled experiment highlighted the plant parameters that can be measured thanks to such a procedure. Then fitting the simulated changes of distributed Sink term in Richards equation to experimental data enabled us to depict the local radial and axial conductivities. The use of the RSWMS model in association with experimental data gave us an insight on the water potential distribution in the plant and fluxes by and through individual segments. Now this analysis can be optimized by changing the timing and/or types of measurements included in the protocol in order to maximize the information content. A validation of the results can also take place: the optimized conductivities of the root segments are indeed sensitive to a global conductance measurement. Our new operation pipe clearly shows that the combined use of computer models and experimental data allows a better analysis of the water fluxes in the soil-plant system, can help researchers to decrypt the root water uptake dynamics and will improve the experimental protocol. In the future, this experimental set-up will enable us to compare genotype hydraulic architectures.

MYCOTOXIN PRODUCTION AND PREDICTIVE MODELLING KINETICS ON THE GROWTH OF *ASPERGILLUS FLAVUS* AND *ASPERGILLUS PARASITICUS* IN WHOLE BLACK PEPPERCORNS

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KEYWORDS

Predictive modelling, black pepper, *Aspergillus flavus*, *Aspergillus parasiticus*, growth rate, LC-MS/MS

ABSTRACT

The growth and mycotoxin production of an *Aspergillus parasiticus* and three *A. flavus* isolates were studied in whole black peppercorns (*Piper nigrum* L.) using a full factorial design with seven water activity (aw) (0.826-0.984) levels and three temperatures (22, 30 and 37°C). Diverse secondary models were assessed for their ability to describe the radial growth rate as a function of individual and combined effect of aw and temperature. The bias factors (0.70-1.01), accuracy factors (1.01-1.41) and root mean square error (0.019-0.280) show that the examined models are conservative predictors of the colony growth rate of both fungal species in black peppers. The Rosso square root cardinal model can be recommended to describe the individual aw effect while the extended Gibson model was the best model for describing the combined effect of aw and temperature on the growth rate of both fungal species in peppercorns. Temperature optimum ranged from 29 to 33°C, while aw optimum was 0.93-0.96 as estimated by multi-factorial cardinal model for both species. Following the growth study, production of mycotoxins (aflatoxins B1, B2, G1, G2, sterigmatocystin and O-methyl sterigmatocystin (OMST)) were quantified using LC-MS/MS. Sterigmatocystin (<LOQ-76.7 µg/kg) was the dominant mycotoxin found. High inter- and intra-species variability in mycotoxin production restricted the modelling of mycotoxins production in black pepper.

M06

MOLECULAR TECHNIQUES

A CROSS-DISCIPLINARY STUDY ON CELL WALL BIOSYNTHESIS IN THE GROWING HEMP (*CANNABIS SATIVA* L.) HYPOCOTYL

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KEYWORDS

Hemp, cell wall, lignin, transcriptomic, proteomic, microscopy

ABSTRACT

Hemp is a relevant source of lignocellulosic material for the construction sector. Hemp is also an interesting plant for cell wall studies, as its stem shows the occurrence of tissues with contrasting cell wall composition and features. Despite being a non-woody plant, *C. sativa* undergoes secondary growth: primary and secondary sclerenchymatous fibres are present in the older parts of the stems. By studying hemp hypocotyls aged between 6 to 20 days, we have followed the dynamics of secondary growth and, consequently, secondary cell wall deposition, and showed that hemp hypocotyl can be used as a model to study both fibre formation and secondary growth. Microscopic analysis highlights the presence of cellulosic bast fibres and of lignified core. Secondary growth was clearly associated with different patterns of lignin and cellulose localization. The expression of genes playing a role in cellulose and lignin biosynthesis was analysed by qPCR. A gradual upregulation in the expression of secondary cellulose synthases was accompanied by a parallel increased expression of lignin-related genes, which is the result of the activation of the pathways involved in secondary cell wall deposition. Using a proteomic gel-based approach, a similar change in protein accumulation was observed. While photosynthesis-related proteins were more abundant in young hypocotyls, proteins involved in secondary cell wall formation and defence became more abundant in the proteome profile of older tissues.

UNRAVELING THE EFFECTS OF FUTURE CLIMATE CONDITIONS ON PROLINE METABOLISM IN GRASSLAND SPECIES: AN INTEGRATED BIOCHEMICAL AND MODELING APPROACH

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KEYWORDS

Proline metabolism, drought, elevated temperature, elevated CO₂, grassland species, metabolic control analysis

ABSTRACT

Proline (Pro) is a versatile metabolite that plays a role in the protection of plants against environmental stresses. We aim to gain a deeper understanding of the regulation of Pro metabolism under predicted future climate conditions, including drought stress, elevated temperature and CO₂. Combining measurements in four grassland species (two grasses and two legumes) at multiple organisational levels, i.e. metabolite levels, enzyme activities and gene expression, with a new computational pathway control analysis, allowed to obtain a mechanistic understanding and identify the regulatory changes in Pro metabolism. Drought stress (D) activates Pro biosynthesis and represses its catabolism. Elevated temperature (DT) further increased Pro content, and elevated CO₂ attenuated the DT effect on Pro accumulation. Computational control analysis indicates that the experimentally observed co-regulation of several enzymes is more effective to modulate Pro levels, with pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) playing a central role for grasses (*Lolium perenne*, *Poa pratensis*) and arginase (ARG), ornithine aminotransferase (OAT), and P5CR for legumes (*Medicago lupulina*, *Lotus corniculatus*). Different strategies in the regulation of Pro concentrations under stress conditions were observed. In grasses predominantly the glutamate pathway is activated, and in the legumes the ornithine pathway, possibly related to differences in N-nutritional status.

THE RISK OF SWEET LUPIN AS NEW FOOD ALLERGEN: IGE-MEDIATED CROSS-ALLERGENICITY AMONG ATOPIC PATIENTS' TO LEGUME SEEDS AND NUTS PROTEINS

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KEYWORDS

Conglutins, food allergen, IgE-cross reactivity, legume, *Lupinus angustifolius*, nuts/seeds, seed storage proteins

ABSTRACT

Lupin, a legume with good nutritional value, is used in food production today. Proteins from sweet lupin are increasingly eaten in the form of seeds or additives to wheat flour. The risk of cross-allergenicity with other legumes (lentil, peanut, soybean, chickpea) and/or seeds/nuts (pecan, walnut, pistachio, sunflower) is still poorly known.

The immunological cross-reactivity among major families of proteins from legumes, nuts/seeds, including lupin has been analyzed by in vitro experimental approaches, using SDS-PAGE separations of protein extracts and immunoblots with atopic patients' sera with allergies to different legume and nuts/seeds proteins. The immunoblotting data showed a wide range of IgE-binding responses both displayed towards different extracts and among subjects. Differences were both quantitative and qualitative.

The comparison of the IgE-binding to several legumes and nuts/seeds proteins has allowed us to get an overall broad picture of the immunological cross-reactivities among proteins of widely used different seed species and to hypothesize the role of most conserved specific polypeptides.

As conclusion, the risk of crossed peanut-lupine allergy is high, and moderate to the risk with other legumes. Thus, the use of lupine flour in wheat flour without mandatory labeling makes lupine a hidden allergen, presenting a major risk of cross-reaction in subjects already allergic to other legumes and nuts/seeds contained in food products.

GENETIC STRUCTURE OF MOROCCAN LENTIL LANDRACES FROM DIFFERENT ORIGINS AS REVEALED BY SHORT SEQUENCE REPEAT MOLECULAR MARKERS USING THE SOFTWARE STRUCTURE

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KEYWORDS

Lentil landraces, Morocco, genetic structure, structure software

ABSTRACT

The genetic structure of 252 genotypes of 53 lentil Moroccan landraces from different geographic origins was assessed using 19 microsatellites molecular markers. According to STRUCTURE software based on Bayesian approach, the best genetic structure model displays two gene pools (with the Evanno ad hoc statistic $\Delta K=168.167$) of contrasting sizes under the assignment probability higher than 80%. A small set of landraces, mainly from the dry environment of Abda region, was differentiated from the rest. The largest gene pool includes genotypes from different origins. Although, no clear correlation was found between geographic origin and genetic structure as the two gene pools are represented in all origins. Fifty-three percent of the genotypes from a small location in Abda region, the driest environment where drought and heat stresses are the most important traits targeted by farmers over years for selection of adapted landraces, were assigned to a small discrete gene pool. A fairly high value of ΔK (37.46) compared to other values suggests the possibility of three gene pools displaying the two local cultivars L56 and L24 as major components of the third gene pool with 97.6% as proportion of membership. As for the model with two gene pools, nearly half of the genotypes from the dry Abda region was assigned to a small discrete gene pool. These landraces have a short cycle and high early vegetative growth vigor conferring drought tolerance.

PHENOTYPE SEQUENCING UNCOVERS MUTATIONS IN CANDIDATE GENES THAT MEDIATE TECTIVIRUS-RESISTANCE IN *BACILLUS THURINGIENSIS*

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KEYWORDS

Bacteriophage, tectivirus, *Bacillus cereus*, *Bacillus thuringiensis*, phages receptor, linear plasmid, prophage

ABSTRACT

The Tectiviridae family is a relative rare group that includes tail-less phages having a membrane beneath their icosahedral protein shell. Tectiviruses preying on the *Bacillus cereus* group are represented by phages GIL01/Bam35, GIL16, AP50 and Wip1. GIL01 and relatives are capable to reside as temperate phages that do not integrate into the host genome upon infection and remain as an autonomous linear plasmid in the cell. The aim of this work was to study the primary interaction between these tectiviruses and their host, and the phenotypes of bacterial resistances triggered by the presence of these phages.

For this purpose, *Bacillus thuringiensis* strain GBJ002 was subjected to a selective pressure after repetitive propagation with clear plaque mutants of GIL01 and GIL16. Twenty tectivirus-resistant bacterial mutants were isolated. These resistant bacteria showed differences in colony morphology and displayed distinct adaptation features, such as biofilm formation, sporulation rate, swarming motility, exopolysaccharide production and some differences in metabolic profiles. To unravel the genetic changes responsible for the phage-resistant phenotype, a pooled high-throughput genome sequencing analysis of multiple independent mutants was used to identify potential genes causing the tectivirus-resistant phenotype in *B. thuringiensis*. These results will shed new light on phages receptor(s) and on the different strategies used by bacteria to elude tectiviruses infection.

ANTIBIOTIC SUSCEPTIBILITY PATTERNS AND PRESENCE OF GENETIC VIRULENCE DETERMINANTS IN *BACILLUS CEREUS* GROUP ISOLATES FROM A CUBAN POLLUTED RIVER

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KEYWORDS

Almendares River, La Habana, *Bacillus cereus*, antibiotic resistance, virulence genes, polluted fresh water

ABSTRACT

Bacillus species inhabit a wide range of environments including aquatic ecosystems. The objectives of this study was to determine the patterns of antibiotic susceptibility of *Bacillus cereus* group isolates from the polluted freshwater ecosystem of the Almendares River (Havana, Cuba), and to determine the presence of virulence genes potentially involved in food intoxication syndromes. Antibiotic susceptibility patterns of 10 *B. cereus* group isolates from water and rhizo-sediment from the Almendares River were determined for 17 antibiotics. The presence of the genetic determinants of the emetic toxin (em1, ces, cer), the subunit A of the non-haemolytic enterotoxin (nheA), the cereolysin O (clo) and cytotoxin K variants 1 and 2 (cytK1 and cytK2) was also tested by PCR. From the 10 *B. cereus* analyzed, five isolates from water samples showed resistance to penicillin, oxacillin, ampicillin, gentamicin, kanamycin, rifampicin, nalidixic acid, bacitracin and erythromycin. Two of these strains were also resistant to vancomycin. Five rhizo-sediment isolates showed resistance to penicillin and kanamycin. Based to the PCR detection of the toxin genetic determinants, none of the isolates were found as potentially emetic. However all the isolates were positive for cytK2, eight for clo and five for nheA. Further studies will be required to assess the potential toxigenicity of these isolates, as well as their potential contribution to the spread of antibiotic resistance genes.

IS CYTOTOXIN K2 FROM *BACILLUS CEREUS* A BONA FIDE ENTEROTOXIN ?

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KEYWORDS

Bacillus cereus, diarrhoeic syndrome, emetic syndrome, cytotoxin K, enterotoxins, MLST

ABSTRACT

Bacillus cereus is an opportunistic pathogen that can be responsible for severe local or systemic infections and may also cause foodborne diseases. The emetic syndrome is due to the production of the emetic toxin cereulide in food before consumption and causes nausea and vomiting. The diarrhoeal syndrome is caused by one or several enterotoxin(s) that disrupt the integrity of the plasma membrane of the small intestine epithelial cells. The aim of this study was to assess the potential implication of one of the putative enterotoxins, CytK2, in the diarrhoeal syndrome of *B. cereus* pathotypes.

Concerning the occurrence of cytK2, about 47 % of the 190 screened *B. cereus* isolates were positive. Interestingly, this occurrence was lower in isolates from foodborne toxi-infections and clinical strains than in samples coming from the environment or from food. The construction of a phylogenetic dendrogram based on the cytK2 genes from the 70 positive strains showed an important diversity, with the foodborne outbreak isolates widely spread throughout the dendrogram. These observations were confirmed by Multi-Locus Sequence Typing on five different loci of housekeeping genes. Based on these observations, the choice of cytK2 as virulence marker for the diarrhoeal pathotype of *B. cereus* does not seem to be relevant. However, its responsibility in the diarrhoeal syndrome cannot be fully excluded and potential synergistic effects with other putative enterotoxins should be further investigated.

PXO16 FROM *BACILLUS THURINGIENSIS* SEROVAR *ISRAELENSIS*: ALMOST 350 KB OF TERRA INCOGNITA?

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KEYWORDS

Bacillus thuringiensis, *Bacillus cereus*, conjugation, horizontal gene transfer, plasmid

ABSTRACT

The conjugative plasmid pXO16 (350 kb) from *Bacillus thuringiensis* serovar *israelensis* encodes an aggregation-mediated conjugation system and is able of transferring itself and other non-conjugative and non-mobilizable plasmids in a very efficient manner. So far however, the genetic bases for this unique transfer system remain largely unknown. In this study, CDS were predicted from the plasmid sequence and their putative functions were examined with a particular attention to possible conjugative and aggregation functions. pXO16 displayed unexpected characteristics for a conjugative plasmid: its 467 putative CDS (88% of its sequence) are all in the same orientation and more than two third of them showed no homologue in the databases. These features are usually shared by jumbo phages. Concerning conjugative functions, no significant type IV secretion system homologues were found, strongly suggesting that pXO16 encodes an unforeseen conjugative system.

Using double-recombination mutagenesis, knockout of three regions was performed: a 25-kb region including 5 cell-surface-associated CDS, an FtsK-like protein CDS and the gene coding for a bona fide S-layer protein. The 25-kb cell-surface-associated knockout mutant was not able to produce macroscopic aggregation but transfer was still detected, yet at lower frequencies (1,000-fold for conjugative transfer and 100-fold for mobilization). This proves that aggregation is important but not essential in pXO16 transfer.

MICROBIAL DIVERSITY IN AEROBIC GRANULAR SLUDGE

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KEYWORDS

Aerobic granular sludge, aquatic ecosystems, wastewaters, *Candidatus "Accumulibacter"*, DGGE, polyphosphate, polyhydroxyalcanoate

ABSTRACT

The discharge of untreated wastewaters in the environment is responsible of the rapid degradation of aquatic ecosystems. The current leading technology is the activated sludge process, where the dissolved pollution is degraded by free-floating consortia (flocs). The major drawback of these loose aggregates is their low settling velocity. An emerging technology aims to improve this process by selecting consortia that form aerobic granules that settle significantly faster. However, the role of microorganisms on granule formation and pollution removal is still largely unknown. This research aimed to assess the bacterial diversity of granular sludge produced from both urban and industrial wastewaters. Bacterial diversity was assessed by denaturing gradient gel electrophoresis and tag-encoded pyrosequencing.

Successful granulation was achieved with brewery, dairy and urban wastewaters. The bacterial diversity of the initial floccular sludge responded to change in the reactor operating conditions. However, the bacterial composition of granules was different between reactors, which seem to confirm that there is not a single microbial community that leads to granules. It was also shown that bacterial diversity differed between granules from the same reactor. *Candidatus "Accumulibacter"* accumulates intracellular polyphosphate and polyhydroxyalcanoate and it is hypothesized that these inclusions increase the density of the cell, resulting in an improved sedimentation.

M07

**SYSTEMS AND PROCESS
OPTIMISATION**

STUDY OF DURABILITY AND THE PHYSICO-MECHANICAL PROPERTIES OF THERMALLY MODIFIED WOOD

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KEYWORDS

Physical properties, mechanical properties, durability

ABSTRACT

Heat-treated wood is a credible alternative for certain kinds of tropical timber. It is also an alternative for certain polluting wood preservatives. This study aims to determine the changes in physico-mechanical properties and durability of wood that is subjected to heat treatment. For this, we studied five species (oak, ash, natural beech and a variant known as steamed beech, poplar, Douglas-fir). For each species, we had fifteen reference samples and fifteen corresponding treated samples. The results show a decrease in the hygroscopicity and an increase in dimensional stability of heat-treated wood in relation to the degradation of hemicelluloses. The mechanical properties are influence variously, because heat-treated wood is stiffer but also more brittle. The modulus of elasticity increases slightly after treatment. Side hardness and compressive fracture of heat-treated wood may increase up to 30%. On the other hand, the modulus of rupture, the ability to slit and impact may decrease by 50%, 60% and 70%. The durability of heat-treated wood against wood-destroying fungi increases. However, within each species, the durability increase varies greatly. Additional tests conducted to understand this variability, suggests that the heat distribution is not homogeneous within the processing unit. Moreover, our work suggests that durability is related to the fixed carbon content and that this path would be interesting to explore in the future.

MICROAERATION FOR H₂S REMOVAL IN UASB REACTOR

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KEYWORDS

Microaeration, UASB reactor, hydrogen sulfide removal, biogas desulfurization

ABSTRACT

The anaerobic treatment of wastewater with a high sulfate concentration, leads to the production of hydrogen sulfide (H₂S), which ends up into the produced biogas. Since H₂S is toxic to humans and can cause corrosion of concrete and steel, it needs to be removed before using the biogas for energy production. Biogas desulfurization can be achieved by blowing of a small amount of air into the anaerobic reactor, a process which is termed “microaeration”,

This contribution deals with the removal of H₂S from biogas by microaeration in Up-flow Anaerobic Sludge Blanket (UASB) reactors treating synthetic brewery wastewater with sulfur loading rate 0.084 g S L⁻¹ d⁻¹ (COD/S ratio of 95). A fully anaerobic UASB reactor served as a control while air was dosed into a microaerobic UASB reactor (UMSB). The average concentration of H₂S in the produced biogas was 8.9 and 2.2 g m⁻³ for UASB and UMSB, respectively, corresponding with an average H₂S removal efficiency of 73% in the UMSB reactor. Microaeration did not cause any decrease in COD removal or methanogenic activity in the UMSB reactor and the elemental sulfur produced did not accumulate in granular sludge.

The results clearly confirm the potential of microaeration for biogas desulphurization in UASB reactors. Moreover, the method does not require the installation of separate reactors for biogas treatment or other expensive equipment or the addition of chemicals.

INFLUENCE OF COVER CROP MANAGEMENT ON SUGAR BEET PRODUCTION

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KEYWORDS

cover crop, soil tillage, crop production, sugar beet

ABSTRACT

Benefits of cover crop before a spring crop are well established and do not to be demonstrated anymore. However, questions remain on the way and the date to destroy it and prepare the next crop. In this poster, we will discuss the impact of cover crop management on sugar beet production. Two field experiments have been settled in the experimental farm of Gembloux Agro-Bio Tech. Rotation in these two fields is the same but delayed by one year. Contrasted soil tillage is applied on field in relation to the burial depth of residue and time of burial, respectively winter ploughing, spring ploughing, shallow tillage and strip tillage. Various observations (such as above ground biomass, root biomass, root notation, weeds observation, disease observation, leaf area index, yield and quality of harvested products) are made on the cover crop and on the following cash crop in order to characterize the overall growth and observe all possible differences between treatments.

So far, our results show that weather is the major influence on crop production. While in 2013 no effect was observed on yield, in 2014 winter ploughing showed a significant higher yield. Weeds seed germination was also impacted by cover crop management and cultural past.

Given the contrasted nature of the results and the large influence of weather conditions, these two trials will be continued in order to identify the impact of cover crop destruction and soil management on crop production with different crops.

LANGMUIR FILM BALANCE- A TECHNIQUE TO STUDY THE INTERACTION OF β -CYCLODEXTRIN WITH CHOLESTEROL IN MILK FAT GLOBULE MEMBRANE MONOLAYERS

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KEYWORDS

Milk Fat Globule Membrane, langmuir film balance, cholesterol, β -cyclodextrin

ABSTRACT

Milk is a natural complex system present as oil in water emulsion. The natural organisation of milk fat globules is stabilized by the presence of a natural membrane, called the milk fat globule membrane (MFGM). It has tripartite structure consisting of a monolayer facing the inner triacylglycerol core and an external bilayer. This MFGM accounts for 2-6% of fat globule. The MFGM is composed of both polar lipids and proteins. Cholesterol is also present in MFGM and accounts for about 2% of MFGM. Langmuir film balance is a technique to study monolayers and is a useful tool to examine the effect of cholesterol removal on surface properties of MFGM. β -cyclodextrin has no surface activity of its own and is thus a useful tool to study the effect of cholesterol desorption on surface properties in membrane. The removal of monolayer cholesterol to the sub-phase is proportional to the area decrease of the monolayer at a constant surface pressure. Amount of cholesterol removed can also be calculated as a function of mean molecular area at a given pressure, time and temperature. Using the monolayer technique, it is possible to study the interactions between β -cyclodextrin and MFGM film as well as effect of cholesterol removal in altering the surface properties MFGM.

UTILITY OF REMOVING CHOLESTEROL FROM AN INDUSTRIAL BY-PRODUCT- BUTTERMILK POWDER

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KEYWORDS

Buttermilk, cholesterol, Milk Fat Globule Membrane

ABSTRACT

Almost 16% of milk produced in European Union is being converted to butter. Buttermilk, is a low cost by-product from butter manufacture and available in large quantities but has been considered invaluable for many years. When cream is churned to butter the stable oil in water emulsion is destabilized and milk fat globule membrane (MFGM) is disrupted which is released in buttermilk. Thus, over the last two decades it has gained considerable attention due to its specific composition (proteins and polar lipids) attributed towards the MFGM. In account to this composition of buttermilk powder, it finds application in food industry as a natural stabilizing and emulsifying agent. It also finds application as a source of total solids and is commercially being added upto 10% in many food products. However, it has limitation in such applications due to the presence of cholesterol (approx. 80mg/100g). Cholesterol is also an integral part of MFGM and is released into buttermilk with MFGM. The most suitable techniques to remove cholesterol from buttermilk powder are using β -cyclodextrin and cholesterol oxidase enzyme. To valorize this abundantly produced industrial by-product of butter industry and increase its application in health food industry, as well as extend its application in pharmaceutical industry and preparation of liposomes, it is important to remove cholesterol from buttermilk.

PHYTOEXTRACTION OF HEAVY METALS WITH *NOCCAEA CAERULESCENS*: FIELD TRIALS ON POLLUTED SOILS IN THE BRUSSELS-CAPITAL REGION

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KEYWORDS

Phytoremediation, soil pollution, trace metals, *Noccaea caerulea*

ABSTRACT

Phytoextraction is a soil remediation method based on the use of plants accumulating heavy metals in harvestable parts. The technique aims at reducing the bioavailable pool of heavy metals and is particularly suitable for moderately polluted soils. One of the most promising candidates for phytoextraction is the Zn/Cd/Ni hyperaccumulating plant *Noccaea caerulea*.

Despite promising results in pot trials the technique has not yet been successfully implemented in the field. One reason is that the best cultural practices are still unknown, resulting in low yields in the few field trials that have already been conducted. Moreover there is a large uncertainty concerning both the long-term behaviour and the influence of soil parameters on extraction efficiency.

The first objective of this project is to optimize the technique of phytoextraction in polluted urban soils. Two original approaches will be developed in situ: the use of non-metallicolous populations of *N. caerulea* known for a high accumulation ability, and the test of different cultural practices. Phytoextraction efficiency will also be estimated by growing vegetable crops sensitive to heavy metals on soils sampled before and after phytoextraction. The second objective aims at improving the phytoextraction predictability. For this purpose, the influence of soil parameters on the phytoextraction ability of each population will be quantified and interannual variations of heavy metal accumulation will be followed.

ETUDE DU TRANSPORT SOLIDE DANS L'OUED EL-HACHEM ET SON IMPACT SUR LA RECHARGE DE LA NAPPE À PARTIR DES LACHERS DU BARRAGE DE BOUKOURDANE (NORD DE L'ALGÉRIE)

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KEYWORDS

Barrage, recharge de la nappe, sédimentation, érosion

ABSTRACT

Dans le Maghreb, les barrages souffrent régulièrement d'un envasement excessif lié aux problèmes d'érosion présent dans les impluviums situés en amont des barrages. Les origines de cette érosion sont multiples : aménagement du territoire, déforestation, affectation des terres,...

Une gestion adéquates des lâchers, couplée à une meilleure gestion de l'érosion en amont du barrage permettra d'allonger la durée de vie des barrages Algériens. Plus particulièrement pour le barrage de Boukourdane, une amélioration de la gestion des lâchers est primordiale pour améliorer la recharge de la nappe, lutter contre l'intrusion des eaux marines, et prévenir l'accumulation des particules fines qui réduisent la perméabilité du sol.

IDENTIFICATION OF CHARRED BOTANICAL REMAINS PROVIDES MORE ACCURATE INFORMATION ON PAST HISTORY IN CENTRAL AFRICA

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KEYWORDS

Central Africa, taxonomical identification, radiocarbon dating, calibration, chronology, archaeology

ABSTRACT

In palaeoenvironmental studies, charred botanical remains have rarely been identified to the species level before being sent to radiocarbon dating. Moreover, the age of most tropical spp. and thereby the age of the carbon sequestered during plant growth is not known. Dating unidentified charred wood in the tropics should be thus treated with caution because the accuracy of the dates is not guaranteed. Here we present 71 dates obtained on charred endocarps and wood charcoals sampled in soil pits in Cameroon and in the Rep. of the Congo. We taxonomically identified 43 samples then selected both identified and unidentified individual fragments for radiocarbon dating. We performed summed probability distributions of the dates calibrated in BP for the 43 identified and the 28 unidentified samples separately then for the whole dates. Results showed that the dates obtained on unidentified samples better fit the established chronology for Central Africa but that they also presented less precise standard deviations than the dates obtained on identified short-lived material, and that the dates on identified samples provide more detailed trends about the phases of human occupation in Central Africa after 2,500 BP. We can assume that dating unidentified material may introduce some blur into chronologies and that the selection of identified charred botanical remains should be systematically applied for palaeoenvironmental reconstructions in tropical contexts to refine the chronologies.

FACTORS AFFECTING THE ADOPTION OF PASSION FRUIT IN PRODUCTION SYSTEMS IN MATONGO

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KEYWORDS

Agriculture, innovation*, adoption

ABSTRACT

The drop and instability of coffee price have prompted some producers of Matongo to adopt passion fruit as an alternative to meet the need of food security of their households. The adoption of passion fruit has been a spontaneous innovation initiated by farmers. In this context, identifying the explanatory variables of its adoption has become necessary. This study aimed to identify factors determining the adoption of passion fruit by farmers in Matongo in a context of extreme poverty and food insecurity and to suggest solutions for its sustainable production in this district and other regions of the country. Using the socio-economic approach will be used because we are in a dynamic and spontaneous non-diffusionist. The results show that age influenced the adoption but against culture was adopted by most illiterate people who have a high level of study. This is contrary to the diffusion approach in which the level of education influences the adoption.

MOLECULAR ANALYSIS OF VIABLE BUT NON CULTURABLE CELLS FORMED DURING GLUCONIC ACID FERMENTATION

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KEYWORDS

Fermentation, viable but non culturable cell, gluconic acid, acetobacter, 2D-DiGE, catalase

ABSTRACT

Despite progress towards gluconic acid production, fewer researches are available regarding its bacterial production at high temperature. In our previous study, Na-gluconate was successfully produced in batch and semi-continuous process at high temperature (38-40 °C). In this study, the reasons of VBNC (viable but non culturable cells) formation were investigated by using 2D-DiGE. Four cycles of semi-continuous fermentation process were performed in a 15 L bioreactor using a thermotolerant acetic acid bacterium, *A. senegalensis*. Samples were taken from exponential and stationary phase of each cycle. The total proteome of cells was extracted and analyzed by 2D-DiGE. A major challenge associated with semi-continuous process was inability of cells in glucose consumption during the stationary phase and next fermentation cycles. But, a culture medium supplemented with ethanol and acetic acid could resuscitate a great percentage of the VBNC cells. Analysis by 2D-DiGE exhibited that ethanol and acetic acid caused stress conditions for VBNC cells. Thereby, some proteins such as Heat shock proteins (Hsp) and some enzymes such as catalase and superoxide dismutase over expressed after exposure to ethanol and acetic acid. In conclusion it seems that stressors such as ethanol and acetic acid may increase the expression of some proteins involved in repair of the injured cells (VBNC cells).

M08

**FIELD/FULL-SCALE
MODELLING**

SITE-SPECIFIC HEIGHT-DIAMETER ALLOMETRY OF CENTRAL AFRICAN MOIST FORESTS

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KEYWORDS

Allometry, biomass estimation, carbon stock, forest deciduousness, tropical forests

ABSTRACT

In this study we aimed to identify the variation in height-diameter allometry between forest types and among species in Central African moist forests. We also examined the consequences on biomass estimation. Two forest types (evergreen and semi-deciduous) in southern Cameroon with contrasting levels of deciduousness. Height and diameter were measured for a total of 521 trees belonging to 12 timber species over a large range of diameter. Commercial forest inventory data were gathered for the two sites for estimation of aboveground biomass. A total of nine allometric models (including polynomial, asymptotic and non-asymptotic models) were fitted to the height-diameter data at species ($n=12$) and site ($n=2$) level. Biomass estimates were computed based on forest inventory data and general allometric models using both site-specific and published height-diameter equations. The height measurements performed over a wide range of diameters, 10-240 cm, tended to support an asymptotic shape for the height-diameter allometry either at species and site level. We identified a significant difference in height-diameter allometry between the two study sites. For a given diameter, trees tended to be taller in the more semi-deciduous than in the more evergreen. These specific height-diameter allometric models are developed to further improve the estimation of biomass and carbon stock contained in tropical forests.

THE GROWTH ZONE OF MAIZE LEAVES IS AN IDEAL MODEL SYSTEM FOR SYSTEMS BIOLOGY APPROACHES TO INVESTIGATE THE EFFECTS OF DROUGHT STRESS ON ORGAN GROWTH REGULATION

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KEYWORDS

Maize, leaf growth, transcriptomics, drought stress

ABSTRACT

Drought is the most important yield-limiting factor under natural and agricultural conditions and therefore detailed knowledge of its impact on plant growth regulation is crucial. The maize leaf represents an attractive system for growth studies, because of its spatial gradient, allowing sampling of dividing, expanding and mature cells at the same time, and its big size, providing enough material for molecular analyses: a big advantage over the model plant *Arabidopsis thaliana*.

By means of a kinematic analysis we showed that drought inhibits leaf growth by reducing cell division rates in the meristem and cell expansion rates in the elongation zone. A transcriptome analysis provided a molecular basis for the observed inhibition of cell division rates, but also pointed at distinct effects on the development of the photosynthetic machinery, and antioxidant and redox systems. The effects on these pathways were subsequently confirmed by detailed biochemical analysis of the activity of key enzymes and quantification of metabolites.

We demonstrated the functional significance of the identified transcriptional and physiological changes, showing that 1. Increasing the antioxidant capacity in the growth zone, by overexpression of iron superoxide dismutase, increases leaf growth under control and drought conditions. 2. Increased expression of photosynthesis genes under stress facilitates faster growth upon re-watering compared to unstressed controls.

PATTERNS OF CARBON DIOXIDE AND METHANE EMISSION OF GRAZING COWS ON PASTURE

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KEYWORDS

CH4 emission, cow, pasture

ABSTRACT

Mitigating CH₄ emissions from cattle is a major challenge facing the livestock industry. Implementation of efficient strategies for grazing cattle requires an accurate understanding of the daily CH₄ emission patterns on pasture. Current methods use SF₆ as tracer gas or punctual measurement during concentrate feeding or milking to estimate daily production but the extent of this variation in emission intensity over the day is not documented. A novel original method is being developed to assess instantaneous CH₄ emission of grazing cattle and link emission intensities to the animal behavior and pasture attributes. Cows are equipped with 2 IR CH₄ and CO₂ sensors analyzing in real-time (4 Hz) the composition gas exhaled and eructated. Animals are also equipped with inertial measurement units to provide information on their behavior using a prediction algorithm. First results show that CH₄ and CO₂ peaks are more intense during rumination than grazing or resting (P<0.01). Further studies will validate, through comparison with metabolic chamber measurements, if the calculation of the ratio between both gases allows to quantify daily CH₄ production.

INVESTIGATING THE EFFECT OF PLANT ROOT EXUDATES ON PAHS BIOAVAILABILITY TO SOIL MICROORGANISMS IN BROWNFIELD CONTAMINATED SOILS: RESEARCH METHODOLOGY

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KEYWORDS

PAH, organic pollutant, brownfield, microorganism, plant, phytoremediation, soil, exudates, bioavailability

ABSTRACT

In Wallonia, there are 6,000 estimated brownfields (rising to over 3.5 million in Europe) that require remediation. Polycyclic Aromatic Hydrocarbons (PAHs) represent 17% of all encountered pollutants.

The project focusses on improving less aggressive PAHs remediation techniques in brownfields, as an alternative to current expensive and sometimes not environmentally friendly strategies. More specifically, it is built on several authors' observation that PAHs soil content decreases in the presence of plants and is articulated around three research axes.

The first axis focusses on plant exudates and how they may improve PAHs bioavailability to soil microorganisms, enhancing further degradation. This will be investigated by (i) characterizing several brownfield contaminated soils (agronomic parameters) and determining their PAHs content and bioavailability, (ii) selecting a plant model and collecting root exudates, and (iii) evaluating the effects of exudates on PAHs bioavailability.

The second axis objective is to evaluate the effect of plant exudates on PAHs degrading microorganisms by (i) comparing PAHs biodegradation in the presence/absence of exudates and (ii) assessing the toxic effects of exudate compounds on the microbial community.

The third axis aim is to study pollutants-plant interactions by (i) establishing the plant tolerance to several contamination levels and (ii) following PAHs bioavailability when facing real exudation rates, on the field.

HEAT RECOVERY FROM A WWTP TREATING HIGHLY CONCENTRATED WASTEWATER

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KEYWORDS

Biological nitrogen removal, energy balance, experimental work, heat recovery, modelling, simulation

ABSTRACT

This research assesses the heat recovery potential from a biological wastewater treatment plant (WWTP) treating the liquid fraction of manure, which is characterized by a high chemical oxygen demand and nitrogen content. A heat balance was set up to calculate the temperature profile of the aeration basin enabling the calculation of the heat recovery potential over a one-year period. Changes in weather conditions and other input parameters were accounted for on a half-hour basis, allowing a dynamic basin temperature prediction. Evaporation and atmospheric radiation were responsible for most of the heat loss to the environment. Due to the high load of the evaluated wastewater, the heat during the biological reactions in the system was significant. The heat recovery potential was defined as all energy that increases the basin temperature above 20°C, which is the minimal temperature to ensure sufficient biological activity. It was concluded that the heat recovery potential from this WWTP could reach 780MWh/year. The treatment of heavy-loaded wastewater is thus also attractive in view of renewable energy production.

M09

**PHYSIOLOGICAL
OBSERVATIONS**

EXPLOITING IONOMIC VARIATION TO CLONE GENES REGULATING MAGNESIUM TISSUE CONCENTRATION IN PLANTS

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KEYWORDS

Arabidopsis, mineral element profile, natural variation

ABSTRACT

Magnesium (Mg) is the fourth-most common cation in the human body and half of its dietary intake is from plant origin. Worldwide, nearly two-thirds of the population do not consume the recommended amount of Mg in their diet. Therefore, understanding how plants take up Mg from soil and regulate their internal content could have significant implications for offering humans improved Mg sources. Our primary interest is to gain better knowledge on the mechanisms governing Mg homeostasis in the model species *Arabidopsis thaliana*, a domain that is relatively unexplored. To achieve that goal, our experimental outline is to exploit changes in tissue mineral concentrations of natural populations and to identify genes and alleles controlling Mg homeostasis. Since those populations grow in contrasted soil conditions, genetic variation is expected to be found in traits linked to mineral uptake and tissue concentration. We observed a variation of up to 50% in Mg concentration inside large diversity sets of accessions. Physiological characterization of most contrasting accessions in response to Mg depletion is undertaken. Our aim is to identify loci controlling the elemental concentration in plants through linkage and genome-wide association mapping strategies. The identification of loci regulating Mg content could help drawing Mg biofortification strategies in crops.

INFLUENCE OF NITROGEN ON THE ROOT MORPHOLOGY OF *BRASSICA NAPUS* (OILSEED RAPE) CULTIVARS GROWN IN BELGIUM

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KEYWORDS

Lateral root development, root system architecture, nitrogen use efficiency, nitrate leaching

ABSTRACT

Crop productivity relies quantitatively on nitrogen fertilization, which in excess is detrimental to the environment. Ameliorating the Nitrogen Use Efficiency (NUE) is an important approach for breeding future in order to reduce nitrate leaching and denitrification. We study *Brassica napus* (oilseed rape) which has small recovery of applied N in the seed. Our goal is to ameliorate NUE by redesigning a more branched root system that explores a larger soil volume for better N capture. For validation of our concept, we screened 57 cultivars from the Belgian Catalogue of Agricultural Plant Varieties upon in vitro culture at two N supplies (0.01 vs. 10 mM nitrate). Organ biomass and root morphology were measured after one week of treatment. Low N supply stimulated lateral root growth (on average 3-fold longer) and increased root biomass (~1.5-fold higher), while high N favoured shoot biomass production (1.1 to 1.2-fold increase). Our results also showed that there was considerable variation among the cultivars. We identified some with a poorly or highly ramified root system regardless of N supply and others with a high root foraging capacity towards nitrate. Interestingly, a positive correlation was found between lateral root length and aerial biomass production. Our concept proved to be pertinent to laboratory set-ups, suggesting that high root branching can be a positive NUE indicator.

MUTAGENIZED-INDUCED VARIATION OF THE ROOT MORPHOLOGICAL RESPONSE OF *ARABIDOPSIS THALIANA* IN RESPONSE TO THE NITRATE SUPPLY

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KEYWORDS

Arabidopsis thaliana, root architecture, nitrate, forward genetic, nitrogen use efficiency

ABSTRACT

Crop production heavily relies on nitrogen (N) fertilizers. However a considerable fraction of mineral fertilizer, mainly in form of nitrate, is lost with harmful environmental impacts. A sustainable solution is to maximize N uptake and utilization in plants. This can be achieved by breeding crops with a more branched and deeper root system. Our goal is to identify key mechanisms regulating root morphology of the model species *Arabidopsis thaliana* in response to nitrate. During a forward genetic screen with a population treated with ethylmethane sulfonate, hn74 mutant was isolated for the presence of numerous lateral roots upon high nitrate supply — a restrictive condition for the wild type. A positional cloning of the mutation, which was carried with an experimental population segregating for the root morphological trait and with polymorphic markers, identified a 2,128 kb zone of interest on the upper extremity of the lower arm of chromosome 4. Further in vitro phenotypic characterization revealed that hn74 mutant did not undergo the negative repression on lateral root elongation exerted by high salt and osmotic conditions. Finally, higher nitrate content was found in the mutant tissues compared to wild type (1.5-fold increase) suggesting dysfunction of N metabolism. Those results place hn74 as a material of choice to study the mechanisms of lateral root growth repression exerted by high nitrate supply and the signaling pathways overlapping with other nutrient stresses.

GENETIC ANALYSIS OF CD TOLERANCE IN *A. HALLERI*: THE INVOLVEMENT OF CAX1 TRANSPORTER IN LIMITING OXIDATIVE BURST BY MAINTAINING CA HOMEOSTASIS UPON CD STRESS

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KEYWORDS

Arabidopsis halleri, cadmium, calcium, CAX1, heavy metal tolerance, oxidative stress, ROS.

ABSTRACT

Arabidopsis halleri is a model species for studies on adaptation to extreme metallic conditions. In this species, Cd-tolerance seems to be constitutive but the mechanisms underlying the trait are still poorly understood. A previous QTL analysis pinpointed the metal-pump HMA4 as the major genetic determinant for Cd tolerance. However, although necessary, HMA4 alone was proved to be insufficient for determining this trait. After fine-mapping, we identify the Ca²⁺/H⁺ antiporter CAX1 as candidate gene for the second major QTL for Cd tolerance. We showed that CAX1 expression in *A. halleri* was 3 times higher than in the non-tolerant species *A. lyrata* at low external Ca levels, and a second QTL analysis suggested a Ca-dependent involvement of this gene to Cd tolerance in *A. halleri*. Moreover, the loss of function of CAX1 in *A. thaliana* led to higher Cd sensitivity and stronger accumulation of ROS species upon Cd treatment. Together, the results here presented suggest an involvement of CAX1 in the maintenance of Ca homeostasis during Cd stress limiting ROS accumulation and oxidative burst. The high expression of CAX1 in *A. halleri* may then have a contribution to its capability of tolerate metal stress.

FIELD EVALUATION OF A MISCANTHUS COLLECTION REVEALS A BROAD RANGE OF COLD STRESS TOLERANCE AND OPPORTUNITIES FOR BREEDING

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KEYWORDS

Miscanthus, abiotic stress, early vigor

ABSTRACT

The C4 grass miscanthus can produce high biomass yields for low inputs of pesticides, fertilizers and labor. Breeding of cold tolerant varieties of miscanthus would allow the expansion of the possible area of cultivation as well as expanding the possible growing season in areas where it can already grow. Early vigor was measured on plants of 120 *Miscanthus sinensis*, *M. sacchariflorus* and hybrid genotypes on a field trial in Melle, Belgium. Further data were supplied by the partners of the OPTIMISC project involved in a multilocation field trial (11 genotypes out of the 120, six locations across Europe). Base temperature was estimated by maximizing the fit of a linear regression of shoot length in function of thermal time.

A large variation in early vigor was revealed, with base temperatures ranging between 2 and 10°C in the collection of 120 genotypes evaluated in Melle. Several miscanthus genotypes that had lower base temperature for early growth than *M. x giganteus* were identified. Base temperatures calculated using the data of 11 genotypes in the OPTIMISC multilocation trial were similar in the trials in Belgium, Wales and Germany, but were higher for the Ukrainian trial, revealing an effect of the environment on the base temperature. The findings show that large variation in cold tolerance and early growth characteristics is available in the tested collection.

NATURAL BELGIAN POPULATIONS OF *ARABIDOPSIS THALIANA* DIFFER IN ROOT MORPHOLOGY UPON IN VITRO CULTURE

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KEYWORDS

Root architecture, *Arabidopsis thaliana*, natural variation, Belgian population

ABSTRACT

Arabidopsis has a broad geographical distribution and consequently is subject to varying environments which makes it a useful model for studying adaptation and selection. The existence of over a thousand accessions that grow on a wide range of soil conditions could provide a rich source of genetic diversity to explore potentially adaptive differences in root architecture in response to nitrate availability. Here, our approach was to collect populations growing on sandy areas in Belgium. The idea originated from the observation of the lack of lateral roots of Pyl-1 from the Pyla area (Baie d'Arcachon, France) known for having the tallest sand dune in Europe. We link the phenotype as a possible adaptation to sandy soil conditions, where the primary root grows downwards to lower strata to take up water, without elongating lateral roots. Preliminary results suggest that accessions of *Arabidopsis* growing on Belgian coastal areas possess less lateral roots (upon in vitro culture). Nitrate content in the soil of interest ranged from 0.26 to 1.88 ug NO₃- g⁻¹ DW. Including an ecological context to classical molecular studies could help to understand adaptive traits in natural populations.

A NEW PROGRAMMED CELL DEATH PATHWAY IN *ESCHERICHIA COLI*

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KEYWORDS

Programmed cell death, apoptosis, bacteria, RecA, TA modules

ABSTRACT

Programmed cell death (PCD) in bacteria has long been controversial due to the belief that only multicellular organisms would benefit from this kind of altruistic behavior. However, over the past decade, new compelling experimental evidence established a consensus on the existence of such pathways in bacteria. Recently, we discovered a new bacterial PCD pathway. This pathway is triggered by expression of a mutated form of an essential GTPase in *Escherichia coli* and carries many of the markers of eukaryotic apoptosis, including chromosome condensation, DNA fragmentation, exposure of phosphatidylserine on the cell surface and loss of membrane potential. Importantly, this cell death pathway is fundamentally different from all other described bacterial PCD pathways. Previous reports on this type of cell death attributed essential roles to either RecA and its function in the SOS response, or to the toxic protein encoded by toxin-antitoxin modules, especially MazF. However, our results indicate that cell death is not influenced by deletion of the *recA* gene, *mazF* or several other chromosomally encoded TA-modules. Research into the genetic and molecular basis of this new bacterial PCD mechanism may prove useful in unraveling the function and mechanisms of genetically encoded cell death pathways in bacteria and might shed light on eukaryotic PCD as well, since the GTPase under investigation is conserved in eukaryotic organisms.

MYCORRHIZAL STATUS OF THREE *LEUCAENA* SPECIES INTRODUCED IN SOUTHERN MADAGASCAR

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KEYWORDS

Leucaena., endomycorrhizae., consortia., characterization.

ABSTRACT

Mycorrhizal fungi contribute to nutrition of plant species and successful introduction of alien forage species depends not only on the soil and the climate but also on the ability of a species to develop an appropriate symbiotic microbial community at root level to sustain growth requirements. Three *Leucaena* species (*L. leucocephala*, *L. pallida*, *L. diversifolia*) were introduced in Manombo area in the South of Madagascar. Foliage production was assessed and soil samples were characterized on 6 individual per species after 18 months of growth by (1) counting spores, (2) evaluation the rate of mycorrhization with trap cultures and (3) by an indirect assessment of infectious propagules.

Results showed that *L. diversifolia*, whose biomass yield is significantly higher than the two other species, had a specific consortium made of more than 50 % light brown fungi spores with a size between 80 and 150 µm probably of the *Gigasporineae* family. This species of *Leucaena* had a great amount of infectious propagules. However, the rate of mycorrhization was not significant until 6 months of trap culture growth. Further works are being done to characterize the consortium using pyrosequencing and fiels experiment being run to test the dependence of *Leuceana* growth performances on specific consortium and fungi species.

EFFECT OF FLOODING ON FOUR CITRUS ROOTSTOCKS (TWO SOUR ORANGES (*CITRUS AURANTIUM* L.) AND TWO CITRANGES [*CITRUS SINENSIS* (L.) OSB. X *PONCIRUS TRIFOLIATA* (L.) RAF])

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KEYWORDS

Stress ecophysiology, flooding stress, citrus rootstocks, tolerance

ABSTRACT

The effects of flooded soil on chlorophyll content and vegetative growth were studied in four potted six months-old citrus rootstock seedlings: *Citrus aurantium* L. var gou tou (GT), Moroccan sour orange (*C. aurantium* L.) (SO), Troyer (CT) and Carrizo (CC) citrange (*C. sinensis* (L.) Osb.xPoncirus trifoliata L.). Each rootstock seedling was grown in a black plastic bag in a greenhouse at El Menzeh, INRA Morocco. Plants were submitted outdoors to four treatments: T0, not flooded (control); and three flood treatments by submerging pots in 10L containers filled with tap water; during 3 (T1), 6 (T2) and 60 (T3) days. For each rootstock, stem diameter, plant height and number of leaves were measured in the beginning and at the end of the experiment. Root and shoot dry weight and leaf chlorophyll content were measured at the end of the experiment. After 60 days, relative growth, number of leaves, plant height, fresh total biomass, dry total biomass and leaf chlorophyll content had significantly decreased by flooding (respectively by 50%, 30%, 50%, 47%, 42% and 58%). Our study shows that the CC and CT rootstocks were the most-tolerant to flooding. The latter seedlings grew better and accumulated more chlorophyll and dry biomass, and had a higher number of leaves than the other seedlings in different flood stress treatments. Results indicate that Carrizo and Troyer citrange are more appropriate rootstocks than Gou-Tou and Moroccan SO to tolerate occasional soil flooding situations.

M10

COMPUTATIONAL BIOLOGY

THE USE OF BULK SEGREGANT ANALYSIS TO IDENTIFY SINGLE NUCLEOTIDE POLYMORPHISM RELATED TO LATERAL ROOT MORPHOLOGICAL TRAITS

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KEYWORDS

Bulk segregant analysis, single nucleotide polymorphism, root morphology, lateral root, SHOREmap

ABSTRACT

The root morphology is highly responsive to nutrient availability in soil. This is particularly true for nitrate, which acts at multiple stages of lateral root development. An F2 population was generated from a cross between two contrasting natural accessions of *Arabidopsis thaliana* to identify the genetic variation related with root morphology traits. Upon in vitro culture at 10 mM NO₃⁻, Pyl-1 (Pyla, France) has almost no visible lateral root (LR), while Bor-1 (Borky, Czech Republic) about a dozen of LRs that grow long. Around 450 F2 individuals were phenotyped and two pools of 40 plants with extreme LR length were identified. Bulked DNAs were sequenced by Illumina NextSeq platform. Around 200 million reads were generated, filtered and then mapped to Col-0 genome followed by consensus-calling programs. The SHOREmap pipeline (Nature Methods 8:550-551) was used for estimating allele frequencies throughout the bulk of recombinant genomes. Interestingly, we observed more bulk-specific SNPs for the high LR length population, thereby indicating more genetic variations among LR-rich accessions. Top ranking SNPs fell into genes with a higher representation into functional categories of nucleotide binding, ion binding and kinase activity. Further studies of those genes might deepen our understanding of the influence of nitrate on lateral root morphology.

INFLORESCENCE ARCHITECTURE IN TOMATO: GENE FUNCTIONS WITHIN A ZIGZAG MODEL

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KEYWORDS

Solanum lycopersicum, flowering, morphogenesis, sympodial growth, biological model, AGL24

ABSTRACT

Tomato is a major crop plant and several mutants have been selected for breeding but also for isolating important genes that regulate flowering and sympodial growth. Besides, current research in developmental biology aims at revealing mechanisms that account for diversity in inflorescence architectures. We developed a kinetic model of the tomato inflorescence development where each meristem was represented by its “vegetativeness” (V), reflecting its maturation state toward flower initiation. The model followed simple rules: maturation proceeded continuously at the same rate in every meristem (dV); floral transition and floral commitment occurred at threshold levels of V ; lateral meristems were initiated with a gain of V (DV) relative to the V level of the meristem from which they derived. This last rule created a link between successive meristems and gave to the model its zigzag shape. We next exploited the model to explore the diversity of morphotypes that could be generated by varying dV and DV and matched them with existing mutant phenotypes. This approach, focused on the development of the primary inflorescence, allowed us to elaborate on the genetic regulation of the kinetic model of inflorescence development. We propose that the lateral inflorescence meristem fate in tomato is more similar to an immature flower meristem than to the inflorescence meristem of *Arabidopsis*.

INTERACTIONS OF NATURAL RHAMNOLIPIDS PRODUCED BY *PSEUDOMONAS AERUGINOSA* WITH PLANT MODEL MEMBRANES

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KEYWORDS

Rhamnolipid, *Pseudomonas aeruginosa*, elicitor, plant plasma membrane, biomimetic membrane, biopesticide

ABSTRACT

It is well known that chemical pesticides have harmful effects on human health and environment. In this context, the interest for alternative products such as biopesticides is increasing. Among them, elicitors act on the plants by inducing systemic resistance against diseases caused by fungal, viral, bacterial agents and insects. Rhamnolipids are surface active molecules produced mainly by various strains of the bacterium *Pseudomonas aeruginosa*. These secondary metabolites are composed of one to three fatty acids with various chain lengths linked through a glycosidic bond to one or two rhamnose moieties. The fatty acids are linked together through an ester bond. These molecules have shown several biological activities including plant defense stimulation. It has been suggested that this elicitor activity could be related to an interaction of rhamnolipids with the lipid bilayer of the plant plasma membrane (PPM) and lead to its destabilization, which can activate the plant defense signaling pathways. In this context, interactions of two rhamnolipids (Rha-C10-C10 and Rha-Rha-C10-C10) with biomimetic membranes of PPM such as Langmuir monolayers and multilayers were investigated using biophysical and in silico approaches.

RESPONSE TO DROUGHT STRESS OF 'SIDI AISSA' CLEMANTINE (*CITRUS RETICULATA* SWINGLE) GRAFTED ON FIVE CITRUS ROOTSTOCKS

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KEYWORDS

Stress ecophysiology, drought, citrus rootstocks

ABSTRACT

In Morocco, most citrus orchards are planted in the semi-arid area where water is scarce. Furthermore, Moroccan citrus orchards face increasing combinations of biotic (*Citrus tristeza virus* (CTV) / *Phytophthora* spp.) and abiotic stress (salinity, alkalinity and drought). Drought in citrus affects plant morphology and physiology, and ultimately leads to yield reduction. Growth, morphological and physiological parameters were monitored, to assess the effect of drought stress on young citrus plants of 'Sidi Aissa' Clementine variety grafted on five citrus rootstocks (*Citrus macrophylla*, *C. volkameriana*, Mand. Cleo. X CC 30577, Carrizo and Troyer citrange). The experiment was performed under controlled drought conditions on two-year old trees. Two treatments were applied for each rootstock: a control treatment, irrigated at 100% Hcc (field capacity), and a stress-treatment, irrigated at 50%Hcc. Drought stress affected all parameters under consideration. Under stress conditions, 'Sidi Aissa' grafted on citrange Carrizo, *C. macrophylla* and *C. volkameriana* produced larger canopy sizes than trees grafted on other rootstocks, whereas Cleo. X CC 30577 had the lowest tree canopy volume. This study showed that *Carrizo citrange*, *C. macrophylla* and *C. volkameriana* are the better rootstocks for 'Sidi Aissa' Clementine grafting under drought conditions.