TECHNOLOGY • RESEARCH HIGHLIGHTS • VISIONS • SCIENCE •
342

The 3rd Conference of the International Society for Plant Molecular Farming

June 11-13, 2018: Book of abstracts

Kirsi-Marja Oksman | Anneli Ritala | Heiko Rischer | Suvi Häkkinen | Jussi Joensuu | Päivi Vahala (Eds.)

VTT TECHNOLOGY 342

The 3rd Conference of the International Society for Plant Molecular Farming

June 11-13, 2018: Book of abstracts

Editors:

Kirsi-Marja Oksman

Anneli Ritala

Heiko Rischer

Suvi Häkkinen

Jussi Joensuu

Päivi Vahala

VTT Technical Research Centre of Finland Ltd

ISBN 978-951-38-8674-5 (URL: http://www.vttresearch.com/impact/publications)

VTT Technology 342

ISSN-L 2242-1211
ISSN 2242-122X (Online)
http://urn.fi/URN:ISBN:978-951-38-8674-5

Copyright © VTT 2018

JULKAISIJA – UTGIVARE – PUBLISHER

Teknologian tutkimuskeskus VTT Oy
PL 1000 (Tekniikantie 4 A, Espoo)
02044 VTT
Puh. 020 722 111, faksi 020 722 7001

Teknologiska forskningscentralen VTT Ab
PB 1000 (Teknikvägen 4 A, Esbo)
FI-02044 VTT
Tfn +358 20 722 111, telefax +358 20 722 7001

VTT Technical Research Centre of Finland Ltd
P.O. Box 1000 (Tekniikantie 4 A, Espoo)
FI-02044 VTT, Finland
Tel. +358 20 722 111, fax +358 20 722 7001

Cover image: Maria Teresa Capell

Conference Sponsors

The congress organizers and the International Society of Plant Molecular Farming would like to thank warmly all the sponsors for their valuable contributions.

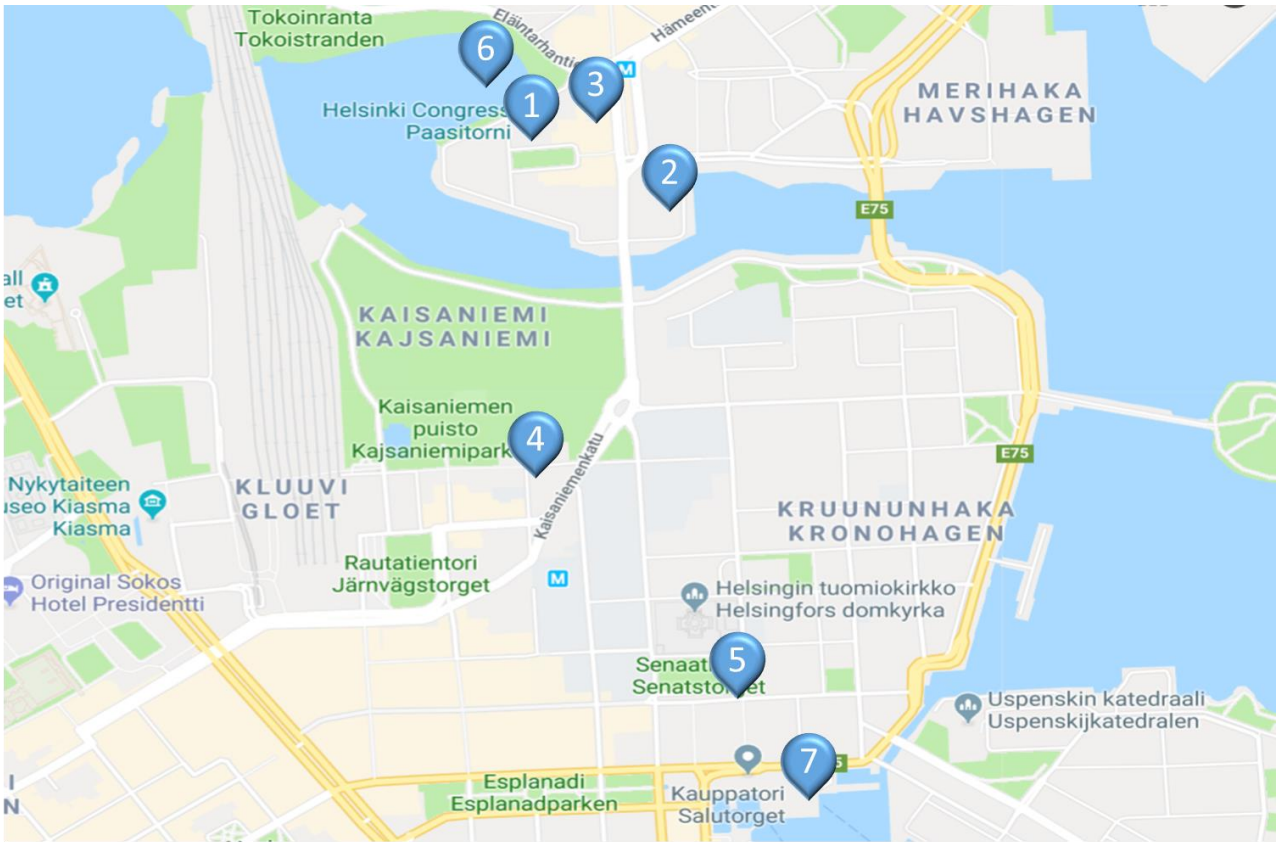
Table of Contents

POSTER PRESENTATIONS

Welcome to Helsinki

Sitting on the edge of the Baltic, the modern, cosmopolitan city of Helsinki was the World Design Capital for 2012. The beauty of the surrounding nature blends seamlessly with high-tech achievements and contemporary trends. Walking tours of the city center show layers of history, while modern architecture and cutting-edge style stake the city's claim to the future. Helsinki during its endless summer days is a refreshing experience.

June is a splendid time of the year to visit Helsinki. The midnight sun allows us to continue discussions outdoors until dawn and over - be ready for an intensive 60 hour package. In the Helsinki city you can find places to listen the sound of silence, for example in the fortress of Suomenlinna - not typical for all capitals in Europe. Helsinki offers a wide variety of attractions to explore: the Market Square with its seagulls, the Esplanade Park with the beautiful Kappeli restaurant building and the option for a lawn picnic, wonderful well-preserved architecture and historic monuments, design, delicious traditional food, and great natural sightseeing opportunities in the Finnish archipelago.



1. Helsinki Congress Paasitorni (Paasivuorenkatu 5A)
   Scandic Hotel Paasi (Paasivuorenkatu 5B)
2. Hilton Helsinki Strand (John Stenbergin ranta 4)
3. Cumulus Hotel Hakaniemi (Siltarsaarenkatu 14)
4. Hotel Arthur (Vuorikatu 19)
5. Old Town Hall (Aleksanterinkatu 20)
6. Restaurant Meripaviljonki
   (Säästöpankinranta 3)
7. Ferries to Suomenlinna Island
   From Paasitorni to Suomenlinna ferry is appr. 25 min walk (1.5 km)

Organizing Committee

The conference is organized by the International Society for Plant Molecular Farming together with VTT Technical Research Centre of Finland Ltd.

Kirsi-Marja Oksman-Caldentey, VTT, Finland (Chair)

Anneli Ritala, VTT, Finland

Heiko Rischer, VTT, Finland

Suvi T Häkkinen, VTT, Finland

Jussi J Joensuu, VTT, Finland

Eva Fredriksson, VTT, Finland

Päivi Vahala, VTT, Finland

George Lomonossoff, John Innes Centre, United Kingdom

Julian Ma, St. George's Hospital Medical School, University of London, United Kingdom

Penny Hundleby, John Innes Centre, United Kingdom

Heribert Warzecha, University of Darmstadt, Germany

Ann Depicker, VIB, Belgium

From the Organizing Committee

It is our great pleasure and honor to welcome you to the 3rd Conference of the International Society of Plant Molecular Farming. The 3rd ISPMF Conference will take place in the amazing city of Helsinki, from the 11th to the 13th of June, 2018. We have selected the Helsinki Congress Paasitorni, established already in 1908, as the meeting venue. Paasitorni is located by the sea in the city center in close vicinity of the Hakaniemi market place and Hakaniemi metro station. The meeting itself takes place in the Congress Hall of Paasitorni, the pearl of Paasitorni with its tall ceilings, splendid chandeliers and impressive balconies.

The topics addressed by the Conference will cover the whole palette of interests for the Plant Molecular Farming community, presenting all possible aspects of recombinant proteins and bioactive small molecules. Besides nine keynote speakers, we have selected over 30 oral presentations and 70 poster presentations. The Conference will provide a delightful forum for 150 attendees to improve their scientific knowledge and explore the latest outcomes and trends in the field. It will offer plenty of networking opportunities, interaction with leading scientists, and stimulating creative exchange among researches and people working in the respective industries.

We hope you will join us for this outstanding scientific event, the 3rd ISPMF, and take a little extra time to enjoy the remarkable and exceptional beauty of Helsinki!

With best wishes,

On behalf of the whole Organizing Committee

Kirsi-Marja Oksman

Anneli Ritala

Keynote Speakers

Dr Jennifer Bromley
Group Leader at British American Tobacco Plant Biotechnology, Cambridge UK.

Jennifer Bromley studied for her Ph.D in Plant Molecular Development at the University of Cambridge. She then held postdoctoral research positions at the University of Cambridge, University of Copenhagen and Lawrence Berkeley National Laboratory in California. She moved to BAT in 2014. She leads a Computational Biology & Gene Discovery group of 7 scientists who have built a number of computational biology resources including the public release of a high-quality tobacco genome sequence.

Professor Paul Christou
ICREA Professor, Universitat de Lleida-Agrotecio Center, Lleida, Spain

Paul Christou has held at least 170 invited lectures; he is named in almost 250 publications in peer-reviewed journals and over 50 chapters in books. He is co-inventor in more than 20 patents and patent applications. His main research interests are in transgene structure and function in genetically engineered crops; metabolic engineering and synthetic biology, production of pharmaceutical macromolecules in crop plants; engineering of multiple novel agronomic traits in crop plants. He is also interested in training and technology transfer for developing country biotechnology, intellectual property issues and regulatory and bio-safety issues of transgenic crops, focusing on developing countries. Furthermore, Paul Christou is also interested in science policy issues and strategic planning covering the interphase between fundamental and applied research.

Prof. Christou holds a bachelor's degree in Organic and Natural Products Chemistry, and a PhD in Plant Biochemistry.

Dr Marc-André D'Aoust
Vice-President Research & Innovation at Medicago Inc., Canada

Dr D'Aoust joined Medicago in July 1999 as Team Leader, Research and Development. He was successively Project Leader, Product Development, and Director, Research and Innovation, prior to his appointment as Vice-President, Research and Innovation. He was closely involved in development of the Medicago technological platform as well as the capacity to produce virus-like particles and biotherapeutics in alfalfa and *Nicotiana benthamiana*. He is co-inventor on more than 330 patents or patent applications related to the technological platform or the company's products.

Dr D'Aoust holds a bachelor's degree in biology and a PhD in plant biochemistry and molecular biology.

Dr Dominik Mojzita
Principal Scientist at VTT Technical Research Centre of Finland Ltd., Finland

Dominik Mojzita received his PhD in Microbiology in 2007 from the Göteborg University in Sweden and his MSc in Cell Biology in 2001 from Charles University in Czech Republic.

Dr Mojzita has a background in molecular biology and genetic engineering of yeast and filamentous fungi. He worked on the identification and characterization of novel genes and metabolic pathways, transcriptional and metabolic regulation, genome-wide and gene-specific expression analysis, and the production of organic compounds in fungal hosts. Currently, his focus is on the development of synthetic expression tools and the establishment of synthetic control circuits in eukaryotic hosts, especially fungi and plants.

Dr Kirsi-Marja Oksman-Caldentey
Research Manager at VTT Technical Research Centre of Finland Ltd., Finland

Kirsi-Marja Oksman received her PhD in pharmacognosy. She has a long experience in plant biotechnology from both academia and pharmaceutical industry. Currently she is a research manager at VTT's Industrial Biotechnology and Food Solutions research area comprising about 130 scientists and research assistants. The focus is to develop technologies using living cells as bioreactors, and plants as raw materials for the production of fuels, chemicals and industrial ingredients. Her own research interest focuses on understanding the complexity of biosynthetic pathways of plant secondary metabolism at systems level. Her newest research path is related to the concept of "Food without fields" evaluating the potential of plant cells as food.

Dr Oksman holds an adjunct professorship at the University of Helsinki, Faculty of Pharmacy since 1994. She is named in over 100 publications in peer-reviewed journals, books chapters and 8 patents or patent applications.

Dr Frank Petersen
Executive Director Natural Product Unit, Novartis Ltd, Switzerland

Frank Petersen studied microbiology at the universities of Hohenheim and Tübingen, Germany. After receiving his PhD at the Institute of Microbiology/Antibiotics with Hans Zähner, he joined the natural products research group at CIBA-Geigy in 1991. As laboratory head in microbiology, he introduced new strategies in the detection of new natural products scaffolds from microorganisms. Since 2001, he heads the natural products research unit at Novartis Pharma. He established microbial sourcing collaborations with various institutes in Africa and Asia.

Dr Pettersen received visiting professorships at the Shanghai Institute of Materia Medica/ Chinese Academy of Sciences and at the SKKU University, Seoul, Korea. Since 2004 he advises governmental committees and scientific organizations on Convention of Biological Diversity and Nagoya Protocol related matters, e.g. at the European Commission, Government of the Federal Republic of Germany, Swiss Government, United Nations, WIPO, or at the NIH.

Professor Kazuki Saito
Dean, Chiba University and RIKEN, Yokohama, Japan

Kazuki Saito obtained his PhD from the University of Tokyo in 1982. He has served as the Dean and a professor of Graduate School of Pharmaceutical Sciences, Chiba University, and a deputy center director and a group director of RIKEN Center for Sustainable Resource Science. He also served as a scientific advisor of several international research activities, such as a European Project 'SmartCell', VIB-Plant Systems Biology, Belgium, and CEPLAS Project, Germany. He was awarded the Prize for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, Japan, JSPP Award 2016 by the Japanese Society of Plant Physiologists, and the Pharmaceutical Society of Japan Award 2017. He was also selected one of Thomson Reuters Highly Cited Researchers in the 'Plant & Animal Science' field, and an ASPB Top Author. Professor Saito's research is focused on metabolomics, functional genomics and biotechnology of plant natural products.

Professor Jochen Schiemann
Director at Julius Kühn Institute, Quedlinburg, Germany

Jochen Schiemann has been director of the Institute for Biosafety in Plant Biotechnology at Julius Kuehn Institute (JKI), Federal Research Centre for Cultivated Plants, until his retirement in September 2016. Since 2006 he is Honorary Professor at University of Lüneburg. He has been coordinating several national and EU-funded cluster projects on biosafety research. From 2003 to 2009 he was member of the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA). From 2002 to 2012 he was member of the Executive Committee of the International Society for Biosafety Research (ISBR), from 2004 to 2008 President of ISBR. From 2004 to 2017 he has been member of the Steering Council of the European Technology Platform "Plants for the Future". In 2016 he has been nominated as plant expert for the Explanatory Note "New techniques in Agricultural Biotechnology" published by the Group of Chief Scientific Advisors. Jochen Schiemann is co-founder of the ISPMF.

Professor Dr Stefan Schillberg
Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany

Stefan Schillberg is member of the management team (provisional) of the Fraunhofer Institute for Molecular Biology and Applied Ecology IME as well as head of the Department Plant Biotechnology in Aachen. He received his PhD in Molecular Biology from the RWTH Aachen University in 1994. Current activities in his laboratory focus on recombinant protein production in various hosts including whole plants and plant suspension cells as well as cell-free expression systems, metabolic pathway engineering, genome editing, strategies to scale-up processes and to improve product levels in plants as well as engineering novel traits in crops.

He holds an honorary professorship at the Justus-Liebig-University in Giessen, Germany.

Social Events

Pre-conference excursion to Suomenlinna

There will be a possibility for an excursion to Suomenlinna (www.suomenlinna.fi) on Monday morning on June 11th, 2018 prior to the conference. This will be on your own costs.

Suomenlinna is a sea fortress that was built on the coast of Helsinki in 1748. Here you can get a real feeling for Finland's position between East and West; Suomenlinna was shaped by the distinct historic eras when helped defend first Sweden, then Russia and ultimately Finland. In 1991 Suomenlinna was listed as a UNESCO World Heritage Site to be preserved for future generations as an example of the European military architecture of its time. Today about 850 people live permanently in Suomenlinna.

You can get to Suomenlinna by a ferryboat leaving from the market place every 20 minutes. The boat drive lasts 15 minutes.

There are several restaurants in Suomenlinna where you can have lunch. The islands themselves are very interesting to explore but you can also visit several small museums and a submarine there.

Get-together party

The City of Helsinki is kindly inviting all the participants of the conference to a reception in the beautiful Empire Hall of the neoclassical style Old Town Hall building, Aleksanterinkatu 20, on Monday 11th June, 2018 at 19 o'clock. The building is located in the heart of Helsinki close to the Senate square, only a 15-20 minutes walk from the conference site. This event is included to the registration fee.

Conference dinner

Conference dinner is included in the registration fee. We invite warmly all the attendees to participate this closing event of our conference. The dinner will take place at the opposite side of our conference venue Paasitorni in the beautiful restaurant Meripaviljonki just over the sea (www.ravintolameripaviljonki.fi) on Wednesday June 13th, 2018 at 19.30.

Useful Information of Helsinki

Here we collected some practical information that maybe useful during your visit.

Finland is a country of forests and lakes. Inland lakes and rivers make up 10% of the country. The large areas of forest cover almost two thirds of the land mass. Only 6% of Finland is arable. It is one of the Nordic countries and bordering Sweden, Norway and Russia. Area wise the country is the fifth largest in Europe (338,424 km²) with only 5.5 million inhabitants. It is the most sparsely populated country in the European Union, with only 16 inhabitants per km². There are four seasons in Finland winters being cold and summers warm.

Finland is a republic and is an independent country since 1917. It became a member of the European Union in 1995 and part of the European Monetary Union in 2002 and the only Nordic country using the Euro as currency. Finland was the third country in the world and the first one in Europe allowing women to vote. This happened as early as 1906. The electronics, machinery, forestry, high-tech and design industries are Finland's most important revenue sources. Finland is the world's biggest producer of mobile phones.

Finnish language is a no*N*-Indo-European language belonging to the Uralic family, along with Estonian and Hungarian. However, language is not a problem. As most Finns take it for granted that you do not speak their language, they are glad to make use of their English or other European languages they master. Finland is in fact a bilingual country, the second official language being Swedish.

Climate and weather

Helsinki's climate combines characteristics of both a maritime and a continental climate. The proximity of the Arctic Ocean and the North Atlantic creates cold weather, while the Gulf Stream brings in warmer air.

Spring arrives in Helsinki usually sometime in April. The days grow rapidly longer and warmer, attracting people to the city while the nature blossoms all around.

Summer in Helsinki is bright. Days are at their longest in the second half of June, when the sun stays above the horizon for 19 hours.

Average temperatures in Helsinki
Entire year: +5.9°C
Warmest month: July +17,8°C
Coldest month: February -4,7°C
For the latest weather forecast, see website (en.ilmatieteenlaitos.fi) by the Finnish Meteorological Institute.

City bikes

The City of Helsinki is attempting to increase levels of cycling in city traffic. The city bike system supports this goal. During 2018 city bike season there will be 1,500 bikes and 150 bike stations in Helsinki.

City sightseeings in Helsinki

Boat tour in Helsinki archipelago. Enjoy a guided sightseeing tour by boat and experience the beautiful archipelago and canals of Helsinki. The tour passes the maritime highlights of the capital.

Guided tours & Hop On Hop Off in Helsinki. Explore the city on a guided sightseeing tour by bus and see all the top sights of Helsinki! Take a tour with Hop On Hop Off or enjoy a city tour with Helsinki Panorama.

Sightseeing by bus and boat in Helsinki: Enjoy Helsinki by bus and boat with our combination tickets. Two guided sightseeing tours that let you discover the main sights in the capital both from land and sea.

More information: www.stromma.fi/en/helsinki/

Currency and payments

The local currency in Finland is Euro (EUR). All major credit cards are widely accepted in Finland. For an ATM machine, look for the sign “OTTO”. These 24-hour cashpoint machines are widely available and accept the following international ATM and credit cards: Visa, Visa Electron, EuroCard, MasterCard, Maestro, Cirrus and EC.
Banks are usually open on weekdays 10am–4:30pm.
In addition to banks, there are several foreign currency exchange points available around the city center. See for example: Forex at Stockmann Department Store or Central Railway Station and Tavex on Fabianinkatu 12

Electricity

Voltage: 220–240 Volts. Electrical sockets (outlets) in Finland are one of the two European standard electrical socket types: ”Type C” Europlug and ”Type E/F” Schuko.

Emergency number

General Emergency number for police, ambulance and fire department is 112.

Postal services

Post offices are open 9am–6pm (some offices even until 8pm) from Monday to Friday. Yellow mailboxes are available for collections on weekdays.
Stamps can be purchased at post offices, bookstores, newsagents, kiosks and hotels.
Helsinki General Post Office is located at the center of Helsinki, Elielinaukio 2 F.

Esplanade Park

Situated in the heart of Helsinki, the Esplanade Park serves as a promenade for visitors and a place to relax for the locals. The park and the Espa Stage host many popular events, including the Marimekko Fashion Show in June and the Jazz-Espa concert series throughout July.

Tax free shopping

Citizens of no*N*-European countries are eligible for tax-free returns upon leaving EU territory. Purchases must be made in shops displaying the Tax Free Shopping sign. The minimum total sum of purchased goods must be €40.

Time zone

The time zone in Helsinki is Eastern European Time (EET), 2 hours ahead of Greenwich Mean Time (GMT+2).

Tourist information

Helsinki Tourist Information (www.myhelsinki.fi) offers free information about the city, sights, events and services including a wide range of brochures and maps all year round.

WiFi

You can connect your laptop or mobile phone to a wireless network in many places throughout the city center e.g. in several cafes, restaurants and libraries.
For free WiFi, connect to “Helsinki City Open Wlan”. There are plenty of hotspots available in the city center and at harbors. In addition, most hotels offer their guests a free internet connection. For the international research and education community, it is also possible to use Eduroam service while in Helsinki.

Scientific Programme

Monday 11.6.2018

09:30 – 14:00	Optional pre-conference excursion to Suomenlinna (trip and lunch)
12:00	Registration opens

Opening session

14.30 – 15:00	*Anneli Ritala-Nurmi (VTT, Espoo, Finland) and George Lomonossoff (John Innes Centre, Norwich, UK)* Welcome and Opening the 3rd ISPMF Congress
15:00 – 15:10	*Kirsi-Marja Oksman-Caldentey (VTT, Espoo, Finland)* Launching a Research Topic article collection for Frontiers in Plant Science
15:10 – 15:30	*Eija Lehmuskallio (NatureGate, Helsinki, Finland)* Video presenting the biodiversity of Finnish nature

Session 1

Chairs

George Lomonossoff and Anneli Ritala

15:30 – 16:10	**Keynote** *Kirsi-Marja Oksman-Caldentey (VTT, Espoo, Finland)* Plant biotechnology beyond genome era in the dawn of synthetic biology
16:10 – 16:40	**Refreshments**

16:40 – 17:00 *Franziska Kellner (Leaf Expression Systems, Norwich, UK)*
Rift Valley Fever Virus – A case study

17:00 – 17:20 *Teresa Capell Capell (University of Lleida, Spain)*
A tríple combination microbiside against HIV-1 expressed in rice

17:20 – 17:40 *Efraim Lewinsohn (The Volkani Centre, Ramat Yishay, Israel)*
Modulation of sesquiterpene biosynthesis in Khat (*Catha edulis*) leaves upon harvesting

17:40 – 18:00 *Lauri Reuter (VTT, Espoo, Finland)*
Future of disruptive technologies and plant molecular farming

19:00 – 21:00 **Get-together in Old Town Hall, Empire Room**
(Addess: Aleksanterinkatu 20, Helsinki)

Tuesday 12.6.2018

Session 2

Chairs

Stefan Schillberg and Suvi Häkkinen

09:00 – 09:40 **Keynote**

Stefan Schillberg (Fraunhofer IME, Aachen, Germany)
A critical view on the business potential of recombinant protein production in plants

09:40 – 10:00 *Andreas Schaaf (Greenovation Biotech GmbH, Freiburg, Germany)*
Pharmacokinetics, pharmacodynamics, and safety of moss-aGalactosidase A in patients with Fabry disease

10:00 – 10:20 *Cornelius Gunter (University of Cape Town, Rodenbosch, South Africa)*
Immunogenicity of plant-produced porcine circovirus-like particles

10:20 – 10:45 Five elevator talks for selected posters

10:45 – 11:15 **Posters with refreshments**

11:15 – 11.55 **Keynote**

Kazuki Saito (Chiba University and RIKEN, Yokohama, Japan)
Phytochemical genomics: from *Arabidopsis* to medicinal plants

11:55 – 12.15 *Hadrien Peyret (John Innes Centre, Norwich, UK)*
Using a synthetic biology approach to develop a novel, easy to use, and open access transient expression system

12:15 – 12:35 *Christina Dickmeis (RWTH Aachen University, Germany)*
Small, smaller, nano: New applications for Potato Virus X in bionanotechnology

12:35 – 12:55 *Katja Härtl (Technical University of Munich, Freising, Germany)*
Tailoring bioactive natural products with glycosyltransferases

13:00 – 14:00 **Lunch**

Session 3

Chairs

Paul Christou and Dominik Mojzita

14:00 – 14:40 **Keynote**

Paul Christou (University of Lleida, Spain)
From genes to products: A plant biotechnology product portfolio for human and animal health and nutrition

14:40 – 15:00 *Heribert Warzecha (Technische Universität Darmstadt, Germany)*
Toward engineering of cannabinoid biosynthesis: *In-planta* characterization of enzymes involved in the synthesis of THCA and derivatives

15:00 – 15:20 *Somen Nandi (University of California, Davis, USA)*
Supporting explorers on Mars: A biomanufacturing solution for producing biologics during deep space exploration

15:20 – 16:00 **Keynote**

Dominik Mojzita (VTT, Espoo, Finland)
On-demand gene expression in plants: New frontiers set by the new synthetic expression system

16:00 – 16:30 **Posters with refreshments**

16:30 – 16:50 *Kaewta Rattanapisit (Chulalongkorn University, Bangkok, Thailand)*
Recombinant human osteopontin expressed in *Nicotiana benthamiana* stimulates osteogenesis related genes in human periodontal ligament cells

16:50 – 17:10 *Ani Barbulova (Arterra Bioscience srl, Naples, Italy)*
Plant somatic embryo cultures are a profitable source of compounds with skin rejuvenating activity

17:10 – 17:30 *Sara Selma García (IBMCP, CSIC-UPV, Valencia, Spain)*
Combinatorial design of modular and programmable transcriptional regulators in plants

17:30 – 17:45 *Julia Jansing (RWTH Aachen University, Germany)*
Molecular Farming 6.0: Hexa-mutant *Nicotiana benthamiana* plants lacking plant-specific *N*-glycans for transient expression

17:45 - 18:15	**Keynote** *Jochen Schiemann (Julius Kühn Institute, Quedlinburg, Germany)* Genome Editing: challenging our views and interpretation of the current regulatory systems
18:15 – 18:45	Introduction of new H2020 plant molecular farming projects *Diego Orzaez*: Newcotiana *Dirk Bosch:* CHIC *Julian Ma:* PharmaFactory
19:00 - 20:00	**ISPMF General assembly (members only)**

Wednesday 13.6.2018

Session 4

Chairs: *Marc-André D'Aoust and Jussi Joensuu*

09:00 – 09:45 **Keynote**

Marc-André D'Aoust (Medicago Inc., Quebec, Canada)
Development of the next generation influenza vaccine

09:45 - 10:05 *Sebastian Mercx (Université catholique de Louvain, Louvain-la-Neuve, Belgium)*
Genome editing of *Nicotiona tabacum* suspension cells by a multiplex CRISPR/Cas9 strategy results in humanized pharmaceutical glycoproteins without plant specific glycans

10:05 – 10:25 *Ingo Appelhagen (John Innes Centre, Norwich, UK)*
Colour bio-factories: Scale-up production of anthocyanins in plant cell culture

10:25 –10:45 *Philippe Varennes-Jutras (University of Oxford, UK)*
pH gradient mitigation in the leaf cell secretory pathway – One goal, several effects

10:45 – 11:20 **Posters with refreshments**

11:20 – 12.00 **Keynote**

Frank Petersen (Novartis Pharma AG, Basel, Switzerland)
Natural products based molecules for target and drug discovery in pharmaceutical research

12:00 – 12:20 *Audrey Teh (St. George´s University of London, UK)*
Prolonging serum half-life of plant-made anti-HIV broadly neutralising antibodies (bNAbs) by improving binding to human neonatal Fc receptor (hFcRn) and reducing binding to human macrophage receptor (hMR)

12:20 – 12:40 *David Ullisch (Phyton Biotech GmbH, Ahrensburg, Germany)*
Green chemistry? Environmental performance evaluation of commercial plant cell culture-derived paclitaxel production

12:40 – 13:00 *Rima Menassa (Agriculture and AgriFood Canada, London, Canada)*
Developing an IgA biobetter antibody against *E. coli* O157:H7 with enhanced stability by rational design of a bovine Fc chain

13:00 – 14:00 **Lunch**

Session 5

Chairs: *Julian Ma and Heiko Rischer*

14:00 – 14:40 **Keynote**

Jennifer Bromley (Bristish American Tobacco, Cambridge, UK)
A multi-omics approach to harness tobacco biochemistry

14:40 – 15:00 *Benjamin Gengenbach (Fraunhofer IME, Aachen, Germany)*
Recombinant production of the toxic anti-cancer lectin viscumin in tobacco plants and microbial cells. A comparative analysis of yield, process costs and toxicity

15:00 – 15:20 *Anatoli Giritch (Nomad Bioscience GmbH, Halle, Germany)*
Plant-made antimicrobial proteins for food safety

15:20 – 15:40 *Mark A Jackson (University of Queensland, Brisbane, Australia)*
Co-expression of a cyclizing asparaginyl endopeptidase enables efficient production of cyclic peptides *in planta*

15:40 – 16:10 **Posters with refreshments**

16:10 – 16:30 *Muriel Bardor (University of Rouen, France)*
Understanding the regulation of the *N*-glycosylation pathway is a prerequisite to optimize microalgae as a cell factory for the production of biopharmaceuticals

16:30 – 16:50 *George Lomonossoff (John Innes Centre, Norwich, UK)*
Global food security – Plant-made Virus-Like Particles as a candidate vaccine against Nervous Necrosis Virus

16:50 – 17:10 *Ann Meyers (University of Cape Town, Rondebosch, South Africa)*
Immunogenicity of a virus-like particle (VLP) vaccine against African horse sickness

17:10 – 17:30 *Luis Matias Hernández (Sequentia Biotech, Barcelona, Spain)*
From *TricoPharming* to *Artennua*: From medicinal trichomes research to business

Closing

17:30 – 18:00 *Kirsi-Marja Oksman-Caldentey (VTT, Espoo, Finland) and George Lomonossoff (John Innes Centre, Norwich, UK)* Closing remarks and announcing **the 4th ISPMF**

19:30 - 22:00 **Conference Dinner at Meripaviljonki, Helsinki**

Video presenting the biodiversity of Finnish nature

Eija Lehmuskallio

NatureGate, Helsinki, Finland

Corresponding author: Eija Lehmuskallio <eija@naturegate.net>

NatureGate (NG) is a comprehensive but easy knowledge website and identification tool. It enables fast species identification and nature observations. As an awareness building nature web service, NatureGate offers solutions for joyful learning of scientific knowledge as well as practical skills.

Current human behavior and ignorance cause dramatic stress to the nature. Climate changes now faster than ever. People's contacts with nature have declined dramatically. We cannot read nature's signs anymore. NG helps create new links between people and the nature. Our solution enables people again to understand nature's signs as well as the relationships between their actions and the environment. Our solution popularizes scientifically accurate natural science, spreading understanding of species as well as the effects of accelerating climate change. We help people read the nature and become conscious of the relationship between our actions and the environment

Global warming alters habitats, species' native ranges and their behavior. A growing number of species will not adapt to the faster-than-ever climate change. When is our time? Experts predict that 25% of Earth's species will be heading for extinction by 2050. Only a fraction of our global population understands the basics of biodiversity and the strong dependency between species. Only people who can read nature are conscious of the relation between their actions and the environment.

NatureGate offers a comprehensive, practical and inspiring way to observe and identify species, and spot the signals of change. It enables you to read nature anywhere on our globe. Learning can replace out-of-date books in nature and species teaching at schools. We provide a field-tested, shared research platform with a vast constantly expanding online database. NatureGate data and information reflect proven changes in our nature. Even small childrens' observations may help scientific research by producing real time nature information. e.g. new species. NatureGate's species identification method helps users find species by sorting the database according to the features, location etc. of the observation. NatureGate is designed to be easy, fast and suitable also for amateurs as well as to professional researchers.

ABSTRACTS FOR

ORAL

PRESENTATIONS

Session 1

Keynote

Plant biotechnology beyond genome era in the dawn of synthetic biology

Kirsi-Marja Oksman-Caldentey
VTT Technical Research Centre of Finland, Ltd., Espoo, Finland

Keynote

Plant biotechnology beyond genome era in the dawn of synthetic biology

Kirsi-Marja Oksman-Caldentey

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Kirsi-Marja Oksman-Caldentey <kirsi-marja.oksman@vtt.fi>

Plant biotechnology is considered to have started from the discovery of the principles of cellular totipotency in the late 19th century, followed by *Agrobacterium*-mediated genetic transformation about 45 years ago. Today, the two main pilars of plant biotechnology are the possibilities to utilize efficiently living plant cells as green factories to produce highly complex small molecules (secondary metabolities), and recombinant proteins. On the other hand, plant biotechnology offers also valuable tools for plant breeding, e.g. introducing novel traits to crop or ornamental plants, nowadays also through new plant breeding techniques with unprecedented efficiency.

The main bottleneck in producing efficiently plant seconday metabolites has been the lack of understanding of how these complex molecules are synthetized and regulated in plant cells. The tissue-specific and subcellular localization adds an extra challenge to this picture. Moreover, only recently the rapid and more economic genome sequencing tools have allowed to obtain genome data, also from non-model plants, thus permitting the modelling of biosynthetic pathways. We are now able to utilize synthetic biology tools to readily map the key genes playing the major roles in pathway engineering, as well as producing entirely new-to-nature molecules with novel acivities through combinatorial biochemistry.

Examples of metabolic engineering of pharmaceutically important plant metabolites in plants and plant cells as well as possibilities to transfer entire pathways to simpler organisms such as yeast will be given. Plant cells can also be used for biotransformation as will be illustrated with the example of a raspberry ketone flavour coumpound. To overcome the additional bottleneck related to the instability of the undifferentiated cell cultures, we have shown the metabolic stability of hairy root cultures for more than 15 years of cultivation.

Recombinant protein production in plants and plant cell cultures has resulted in excellent outcomes, especially for pharmaceutical applications. These discoveries will also be highlighted.

Manufacturing plant based vaccines “Rift Valley Fever Virus – A case study”

Kellner F[1], Holton N[1], Cater P[1], Charlton S[2], Dowall S[2]

[1]Leaf Expression Systems, Norwich, United Kingdom
[2]Public Health England, Porton Down, United Kingdom

Corresponding author: Franziska Kellner <kellnerf@leafexpressionsystems.com>

New and emerging viral diseases pose a growing threat of a global epidemic. Rift Valley Fever (RVF) is an acute viral disease caused by RVF virus (RVFV) transmitted to animals through bites from infected mosquitoes. First reported in cattle in Kenya’s Rift Valley in the early 1910, subsequent outbreaks have been reported throughout sub-Saharan Africa. In livestock, the RVFV causes disease and a high abortion rate, leading to significant societal and economic impacts. Worryingly, the virus can be transmitted to humans through contact with blood and tissues of infected animals. At present no vaccination strategy is approved for human use and veterinary vaccines are not effective or have unwanted side effects.

Within this project we aimed to develop new virus-like particle(VLP) based vaccine against Rift Valley Fever virus (RVFV) using plant transient expression (Sainsbury and Lomonossoff, 2008). In order to produce this vaccine we employed two strategies: (1) Production of a native RVFV VLP, composed of the structural proteins of the virus; (2) Incorporation of RVFV epitopes into the coat protein of Cowpea Mosaic virus to provide a stable vehicle for epitope delivery (Porta el al., 2003). Initial preclinical evaluation indicates the potential effectiveness of this vaccine against RVFV. Further work is however required to develop this product and evaluate its effectiveness in a clinical setting.

References

Porta, C., Spall, V.E., Findlay, K.C., Gergerich, R.C., Farrance, C.E., Lomonossoff, G.P. 2003. Cowpea mosaic virus-based chimaeras: effects of inserted peptides on the phenotype, host range, and transmissibility of the modified viruses. *Virology*, 310*:* 50-63.

Sainsbury, F., Lomonossoff, G.P. 2008. Extremely high-level and rapid transient protein production in plants without the use of viral replication. *Plant Physiology*, 148: 1212-1218.

A triple combination microbicide against HIV-1 expressed in rice

Capell T[1], Vamvaka E[1], Farré G[1], Molinos-Albert LM[2], Evans A[3], Canela-Xandri A[1], Twyman RM[4], Carrillo J[2], Ordóñez RA[1], Shattock R[3], O'Keefe BR[5], Clotet B[2,6], Blanco J[2,6], Christou P[1,7]

[1]*University of Lleida-Agrotecnio Center, Lleida, Spain*
[2]*IrsiCaixa AIDS Research Institute, Badalona, Barcelona*
[3]*Imperial College London, London, United Kingdom*
[4]*TRM Ltd, York, United Kingdom*
[5]*National Cancer Institute NIH, Frederick, MD, USA*
[6]*Central University of Catalonia, Barcelona, Spain*
[7]*Catalan Institute for Research and Advanced Studies ICREA, Barcelona, Spain*

Corresponding author: Teresa Capell <teresa.capell@pvcf.udl.cat>

The transmission of Human immunodeficiency virus (HIV) can be prevented by the prophylactic application of broadly-neutralizing monoclonal antibodies and lectins. Plants offer an alternative platform for the production of protein microbicides benefiting from inexpensive and highly scalable upstream production and the ability to produce multiple components in a single plant line. The latter is important because multiple components are required to avoid the rapid emergence of HIV-1 strains resistant to single microbicides. Furthermore, crude extracts from plants expressing multiple microbicides can be used directly for prophylaxis to avoid the massive costs associated with downstream processing and purification. Having previously established that rice (*Oryza sativa*) plants can individually express the monoclonal antibody 2G12 and the lectins cyanovir*i*N-N (CV-N) and griffithsin (GRFT) as functional proteins in the endosperm, we wished to investigate the potential of rice to produce all three functional proteins simultaneously. Transgenic plants were generated expressing all three proteins and the crude extracts showed *in vitro* binding to gp120 and HIV-1 neutralization. Therefore, the production of HIV-1 combination microbicides in rice may not only reduce costs compared to traditional platforms but also provide strategies for long term durability by making it difficult for the virus to mutate simultaneously against multiple components in the combination microbicide.

References

Vamvaka, E., Evans, A., Ramessar, K., Krumpe, L. R.H., Shattock, R.J., … Capell, T. 2016. Cyanovirin-N produced in rice endosperm offers effective pre-exposure prophylaxis against HIV-1_{BaL} infection *in vitro*. *Plant Cell Reports* 35: 1309-1319.

Vamvaka, E., Ramessar, K., Evans, A., O'Keefe, B.R., Shattock, R.J., Christou, P., Capell, T. 2016. Rice endosperm is cost-effective for the production of recombinant griffithsin with potent activity against HIV. *Plant Biotechnology Journal* 14: 1427-1437.

Vamvaka, E., Twyman, R. M., Murad, A. M., Melnik, S., Teh, A. Y.H., … Capell, T. 2016. A recombinant HIV-neutralizing antibody produced in rice endosperm accumulates predominantly as an aglycosylated derivative with enhanced neutralizing activity. *Plant Biotechnology Journal* 14: 97-108.

Modulation of sesquiterpene biosynthesis in khat (*Catha edulis*) leaves upon harvesting

Lewinsohn E[1], Bar E[1], Shotland Y[2], Hagel JM[3], Facchini PJ[3], Dudareva N[4], Davidovich-Rikanati R[1]

[1]*Agricultural Research Organization, The Volcani Center, Rishion Le Zion, Israel*
[2]*Shamoon College of Engineering, Beer Sheva, Israel*
[3]*University of Calgary, Calgary, Canada*
[4]*Purdue University, West Lafayette, USA*

Corresponding author: Efraim Lewinsohn <twefraim@volcani.agri.gov.il>

Khat (*Catha edulis*) leaves are common in East Africa and the Middle East and traditionally chewed for their psycho-stimulating amphetamine-like properties. In addition to *S*-cathinone and other pharmacoactive phenylpropylamino alkaloids, leaves also accumulate various terpenoids including volatile mono- and sesquiterpenes and no*N*-volatile quinone methide triterpenes and cathedulin alkaloids exhibiting substantial pharmacological interest. Sesquiterpenes are accumulated in many plant species in response to mechanical injury or biotic challenges.

In this work we characterized the sesquiterpenes present in khat leaves in response to harvesting. Leaf volatiles were sampled using solid-phase microextraction (SPME) coupled to GC-MS. Harvesting the leaves resulted in marked increases in the total sesquiterpene content, and especially in the levels of (*E*)-caryophyllene and α-copaene. (*E*)-Caryophyllene is a modulator of cannabinoid action influencing the therapeutic properties of cannabis by binding to and activating the human CB2 receptor. To test for the potential sesquiterpene biosynthetic capacity of khat leaves, crude protein extracts were incubated with farnesyl diphosphate as a substrate and the sesquiterpenes produced *in vitro* were identified using GC-MS. Increased biosynthetic potential for sesquiterpenes, including (*E*)-caryophyllene, elemol, and α-eudesmol were detected in extracts from harvested leaves as compared to controls. To get a broader view of genes that are up or down regulated in harvested leaves, we performed deep RNA sequencing.

The analysis resulted in the identification of novel putative terpene synthase genes transcriptionally upregulated upon harvesting. Functional expression of three of these genes indicated that they encoded proteins catalyzing the formation of sesquiterpenes from farnesyl diphosphate. CeTps17580 produced mainly α-copaene and α-elemene, CeTps13580 generated mainly elemol and α-eudesmol, while CeTps6019 produced a mixture of α-copaene, α-cubebene, γ-cadinene and δ-cadinene as main components. The identification and characterization of these genes together with the study of their expression levels as a result of harvesting will help us to better understand the mechanism that brings about phytochemical changes in khat leaves upon harvesting.

Future of disruptive technologies and plant molecular farming

Reuter L

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Lauri Reuter <lauri.reuter@vtt.fi>

The speed of technological development accelerates. Changes are coming faster and from ever more unexpected directions. Fast sequencing technologies and cost effective gene editing have already changed the field. That is obvious. What are the developments we are not expecting? What is it that will surprise us all? Where is the next disruption coming?

Unprecedented data processing capabilities of neural networks, machine intelligence, changes massive industries. Image recognition has already transformed radiology and AI keeps newborn children safe in hospitals. Generative design has changed the ways cars and aircrafts will be built. In the digital world, the learning cycle has suddenly shortened. What happens when all the tools are applied to molecular farming? The only way to keep up is to run. Are you running?

Technology has come on and under your skin. Mobile devices and wearable sensors collect data. Social media collects meta-data. Biobanks collect data. Everything collects data and that data will change everything. What are the implications for clinical trials? What happens when n=5 billion, everyone becomes her own control group and cost drops to a fraction? What are the questions you want to ask when all answers exist?

This is a provocation to set the tone for discussions going beyond the obvious.

Session 2

Keynotes

A critical view on the business potential of recombinant protein production in plants

Stefan Schillberg
Fraunhofer IME, Aachen, Germany

Phytochemical genomics: from *Arabidopsis* to medicinal plants

Kazuki Saito
Chiba University, Chiba, Japan
RIKEN, Yokohama, Japan

Keynote

A critical view on the business potential of recombinant protein production in plants

Stefan Schillberg

Fraunhofer IME, Aachen, Germany

Corresponding author: Stefan Schillberg <stefan.schillberg@ime.fraunhofer.de>

The global protein expression market size was estimated at USD 1,654 million in 2017 and is expected to reach USD 2,850 million in 2022. The market is dominated by prokaryotic and mammalian expression systems, whereby prokaryotic platforms provide high production capacities and inexpensive production, and mammalian cells are the favoured system for the production of biopharmaceutical products. Although plants are now gaining widespread acceptance as a general platform for the large-scale production of recombinant proteins, their market share is rather small. This is partly because of low yields, poor and inconsistent product quality and difficulties with downstream processing at larger scale. In addition, examples demonstrating the capacity of plants to provide proteins at lower costs and improved quality are still relatively scarce, and therefore industry is reluctant to switch from their established and approved processes to plant-based production. Nevertheless, plant-produced proteins for application in medicine, research and cosmetics have reached the market demonstrating that production in plants can be competitive and sustainable. The talk will present the strengths of plant expression systems for specific uses, but will also address bottlenecks requiring expedient solutions to expand the implementation of plant systems.

Pharmacokinetics, pharmacodynamics, and safety of Moss-aGalactosidase A in patients with Fabry disease

Schaaf A, Hennermann JB, Arash-Kaps L, Fekete G, Busch A, Frischmuth, T

Greenovation Biotech GmbH, Freiburg, Germany

Corresponding author: Andreas Schaaf <ASchaaf@greenovation.com>

Moss-aGal is a plant-made version of human alpha-galactosidase developed for enzyme replacement therapy (ERT) in Fabry patients. Manufactured in a moss (*Physcomitrella patens*), the enzyme exhibits an extraordinary homogenous *N*-glycosylation profile with more than 90% mannose-terminated glycans. In contrast to mammalian cell produced Fabry ERTs, moss-aGal does not rely on Mannose-6-phosphate receptor (M6PR) mediated endocytosis but targets the mannose receptor (MR) for tissue uptake. Extensive quality testing revealed outstanding and reproducible biochemical product purity and definition.

Between January and October 2017, we conducted a Phase 1 clinical trial evaluating pharmacokinetics, pharmacodynamics, and safety of Moss-aGal in patients with Fabry disease. Recruiting took place in Mainz (Germany) and Budapest (Hungary). In total, 6 patients with confirmed diagnosis of Fabry disease, elevated lyso-Gb3 concentrations and seronegative for antibodies against agalsidase participated in this trial and received a single dose of Moss-aGal with 0.2 mg/kg body weight by intravenous infusion. Following injection, tolerability of the drug, patient's safety and quality of life as well as initial explorative efficacy parameters were assessed during a 28-day clinical schedule. For all six patients, the drug was well tolerated and no safety issues were observed.

The presentation will introduce and discuss the outcome of this first-i*N*-human study 1of the first moss-made enzyme replacement therapy.

Immunogenicity of plant-produced porcine circovirus-like particles

Gunter CJ, Regnard GL, Rybicki EP, Hitzeroth II

University of Cape Town, Rondebosch, South Africa

Corresponding author: Cornelius Gunter <corriegunter@gmail.com>

Porcine circovirus type 2 (PCV-2) is the main causative agent associated with diseases now collectively known as porcine circovirus-associated disease. Costs associated with commercial PCV-2 vaccines currently used to protect pigs have placed a significant economic strain on the global swine industry. Plant-made pharmaceuticals are rapidly becoming a viable alternative to established production systems. Plant-made viral proteins have been demonstrated to self-assemble into virus-like particles (VLPs) eliciting more potent antibody and cellular immune responses. Our objective in this study was the expression of PCV-2 capsid protein (CP) in *Nicotiana benthamiana*, the detection of assembled VLPs, and assessment of their immunogenicity in mice.

The PCV-2 CP was transiently expressed in *N. benthamiana* using the pEAQ-HT *Agrobacterium*-delivered expression vector. Plant-made CP was confirmed using immunoblotting and expression was optimised for the OD_{600} of infiltrated recombinant *A. tumefaciens* and day of harvest. VLPs were isolated using CsCl density purification, visualised by transmission electron microscopy, and used in immunogenicity studies in mice.

The 27-kDa PCV-2 CP was successfully expressing in *N. benthamiana* and the optimum expression conditions were determined to be four days post infiltration at an *A. tumefaciens* optical density of one. The particles of between 17-20 nm diameter were detected that were absent in the control indicating that the CP had assembled into VLPs. The plant-made PCV-2 VLPs elicited an immune response in mice with high antibody titres.

This is the first report describing the assembly of plant-made PCV-2 CP into VLPs and their immunogenicity in mice. These results contribute to our understanding of foreign protein production in plants and may well serve as viable alternative for commercial vaccine development.

Keynote

Phytochemical genomics: from *Arabidopsis* to medicinal plants

Kazuki Saito

Chiba University, Chiba, Japan
RIKEN Center for Sustainable Resource Science, Yokohama, Japan

Corresponding author: Kazuki Saito <ksaito@faculty.chiba-u.jp>

The recent advances of genomics and metabolomics in plants accelerate our understanding about the mechanism, regulation and evolution of biosynthesis and function of plant specialized products. The questions now can be addressed how the metabolomic diversity of plants is originated at the levels of genome and how we should apply this knowledge to medicine and industry. Such study called as 'Phytochemical Genomics' is regarded as the new sector in plant science and natural products chemistry, which elucidates the genomic basis of the biosynthesis and the function of plant metabolites (Saito, 2013; Sumner et al., 2015; Rai et al., 2017; Yamazaki et al., 2018). In this presentation, I will report an overview of our recent studies on the phytochemical genomics in a model plant, *Arabidopsis thaliana*, and medicinal plants. The topics include: 1) Strategy of phytochemical genomics for understanding the origins of the chemical diversity of plants as *A. thaliana* as the model, and 2) A couple of examples for identification of genes involved in the biosynthesis of specialized products in medicinal plants.

References

Rai, A., Saito, K., Yamazaki, M. 2017. Integrated omics analysis of specialized metabolism in medicinal plants. *Plant Journal* 90: 764-787.

Saito, K. 2013. Phytochemical genomics - a new trend. *Current Opinion in Plant Biology* 16: 373–380.

Sumner, L.W., Lei, Z., Basil, N.J., Saito, K. 2015. Modern plant metabolomics: advanced natural product gene discoveries, improved technologies, and future prospects. *Natural Products. Rep*orts 32: 212-229

Yamazaki, M., Rai, A., Yoshimoto, N., Saito. K. 2018. Perspective: functional genomics towards new biotechnology in medicinal plants. *Plant Biotechnology Reports* (in press).

Using a synthetic biology approach to develop a novel, easy to use, and open access transient expression system

Peyret H, Lomonossoff GP

John Innes Centre, Norwich, United Kingdom

Corresponding author: Hadrien Peyret <hadrien.peyret@jic.ac.uk>

Expression systems for the transient overproduction of proteins in plants have come a long way in the past 10-15 years. The development of a range of different expression systems from numerous different research groups and private companies all allow plant biotechnologists to obtain high yields of their proteins of interest. The most successful of these expression systems are deconstructed viral vectors, which include different genetic components from different (usually) viral sources in order to maximise protein yield. We have taken the pre-existing pEAQ-*HT* expression vector (Sainsbury et al., 2009) as a starting point for rationally-designed improvements based on first principles and the current state of knowledge about plant viral gene regulation and recombinant protein expression. In an iterative process, improvements were made to the untranslated regions (UTRs) and to the vector backbone in order to improve ease of use as well as recombinant protein yield. The main result of this is the development of a novel expression system which allows for two-fold higher recombinant protein yield than the original pEAQ-*HT*. Moreover, a suite of novel, synthetic, rationally-designed 5'- and 3'-UTRs have been developed which allow tailoring of expression to suit desired yields. As a side effect of this work, valuable insight has been gained into the relative roles of the pEAQ-*HT* UTRs, and more generally what is required for a transient expression system to be successful. Another aspect of the work has been to focus on the development of free-to-use components that can be used throughout the plant molecular farming community by rethinking the traditional approach to intellectual property and material transfer. Another expression vector has therefore been created which is free of patented components and can be used by anyone in the plant molecular farming community.

Reference

Sainsbury, F., Thuenemann, E.C., Lomonossoff, G.P. 2009. pEAQ: versatile expression vectors for easy and quick transient expression of heterologous proteins in plants. *Plant Biotechnology Journal*, 7: 682-693.

Small, smaller, nano: New applications for Potato Virus X in bionanotechnology

Dickmeis C, Röder J, Commandeur U

RWTH Aachen University, Aachen, Germany

Corresponding author:
Christina Dickmeis <christina.dickmeis@molbiotech.rwth-aachen.de>

Nanotechnology is an emerging interdisciplinary field of interest. Nanostructured materials originate from inorganic or organic compounds such as polymers and peptides. Plant virus nanoparticles became a key instrument for the introduction of tailored functionalities by genetic engineering and conjugation chemistry. The most popular plant viruses include tobacco mosaic virus (TMV) and cowpea mosaic virus (CPMV) and have shown to be applicable for instance in bioimaging, vaccination and electronics. However, little attention was paid to potato virus X (PVX), although exhibiting great potential in diverse application areas in which multi-functional flexible scaffolds with a high aspect ratio are desired. Here we present new applications for PVX and new functionalization methods. By peptide presentation, we could modify PVX for improved cell attachment with the integri*N*-binding domain RGD and improved mineralization with different mineralizatio*N*-inducing peptides. The PVX nanoparticles (NP) support hydroxyapatite nucleation for improved bone tissue replacement in hydrogel applications. By this means, PVX comprises a genetically programmable control of its surface, while showing excellent biocompatibility and biodegradability, which makes it particularly attractive for medical applications.

PVX can further be genetically modified for silicification. We have shown that filamentous, anisotropic, flexible plant viruses offer a versatile platform for the formation of complex hybrid materials including isolated coated virus particles and complex microstructures. These structures formed under mild conditions making them suitable for more delicate viral particles. By silicification the PVX NPs are further suited for chemical modifications for applications as semiconductors, imaging dyes and drug carrier.

Additionally, we developed more diverse fusions strategies for larger protein attachments to the surface of PVX. As an alternative strategy, we utilized a broad variety of 2A sequences for different fusion efficiencies and tested different protei*N*-protein interaction partners. The most promising interacting pair on PVX NPs was the SpyTag-SpyCatcher system from *Staphylococcus pyogenes*. The fusion of the SpyTag to the PVX NP allows a covalent linkage of any desired protein fused to the SpyCatcher expressed separately in its most appropriate expression system.

Tailoring bioactive natural products with glycosyltransferases

Härtl K, Schwab W

Biotechnology of Natural Products, Freising, Germany

Corresponding author: Katja Härtl <katja.haertl@tum.de>

The bioactivity of plant natural products depends on more than just their scaffolds. Tailoring them by addition or removal of functional groups can decisively alter their physicochemical properties. In Nature, the addition of sugar residues to small lipophilic molecules is one of those mechanisms being particularly frequent. In plants, the sugar transfer is mostly conveyed by nucleoside diphosphate (NDP)-sugar dependent glycosyltransferases (GTs). The glucosylated products show increased solubility and stability, and reduced toxicity. Conveniently, the glycosidic bond can later be cleaved on demand by heat or enzymes. As a result, the bioactivity and bioavailability can be manipulated offering numerous opportunities for industrial application.

The interest of the food, pharma and cosmetics industry for new and applicable natural products has increased tremendously. Therefore, we have built a library of plant GTs to glucosylate commercially important small molecules such as the flavour compounds geraniol and vanillin. Furthermore, we employ whole cell biocatalysis to produce the glucosides on large scale and test the purified products in food and cosmetic applications. We have established the first economically viable process for the production of small molecule glucosides.

Session 3

Keynotes:

From genes to products: A plant biotechnology product portfolio for human and animal health and nutrition

Paul Christou
University of Lleida, Spain

On-demand gene expression in plants: New frontiers set by the new synthetic expression system

Dominik Mojzita
VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Genome editing: challenging our views and interpretation of the current regulatory systems

Joachim Schiemann
Julius Kühn Institute, Quedlinburg, Germany

Keynote

From genes to products: A plant biotechnology product portfolio for human and animal health and nutrition

Christou P[1,2], Capell T[1], Zhu C[1]

[1]*University of Lleida-Agrotecnio Center, Lleida, Spain*
[2]*Catalan Institute for Research and Advanced Studies (ICREA), Barcelona, Spain*

Corresponding author: Paul Christou <christou@pvcf.udl.cat>

We recreated the carotenoid and ketocarotenoid pathways in elite South African white maize inbred lines. One of the resulting high carotenoid transgenic lines was registered in Spain under the name of Carolight®. We describe the process used to generate Carolight® and also a breeding program and experimental field trials to assess the performance of elite transgenic hybrids using locally adapted commercial inbred lines. We present data on the interactions of Carolight® with pests and diseases in the field. The use of Carolight® in poultry and swine production in a commercial setting will be discussed. Experiments demonstrating the beneficial effects of a high carotenoid corn diet specifically delivered through Carolight® in human health, using an experimental animal model will be described. The performance of a high ketocarotenoid line in fish production will be used as a case study to illustrate transition from the laboratory to a commercial setting. IP and FTO analysis as well as regulatory aspects will also be discussed to address remaining barriers to commercialization.

References

Eritja, N., Arjó, G., Santacana, M., Gatius, S., Ramírez-Núñez, O., Arcal, L., ... Christou, P. 2016. Oral intake of genetically engineered high-carotenoid corn ameliorates hepatomegaly and hepatic steatosis in PTEN haploinsufficient mice. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1862: 526-535

Farré, G., Perez-Fons, L., Decourcelle, M., Breitenbach, J., Hem, S., … Sandman, G. 2016. Metabolic engineering of astaxanthin biosynthesis in maize endosperm and characterization of a prototype high oil hybrid. *Transgenic Research* 25: 477–489.

Henriquez-Rodriguez, E., Pena, R. N., Seradj, A. R., Christou, M., Tor, M., Estany, J. 2017. Carotenoid intake and SCD genotype exert complementary effects over fat content and fatty acid composition in Duroc pigs. *Journal of Animal Science* 95: 2547–2557.

Moreno, J. A., Díaz-Gómez, J., Nogareda, C., Angulo, E., Sandmann, G., Portero-Otin, M., ... Christou, P. 2016. The distribution of carotenoids in hens fed on biofortified maize is influenced by feed composition, absorption, resource allocation and storage. *Scientific Reports* 6: 35346.

Nogareda, C., Moreno, J. A., Angulo, E., Sandmann, G., Portero, M., Capell, T., ... Christou, P. 2016. Carotenoid-enriched transgenic corn delivers bioavailable carotenoids to poultry and protects them against coccidiosis. *Plant Biotechnology Journal* 14: 160-168.

Zanga, D., Capell, T., Slafer, G. A., Christou, P., Savin, R. 2016 A carotenogenic mini-pathway introduced into white corn does not affect development or agronomic performance. *Scientific Reports* 6: 38288.

Zanga, D., Capell, T., Zhu, C., Christou, P., Thangaraj, H. 2016. Freedom-to-operate analysis of a transgenic multivitamin corn variety. *Plant Biotechnology Journal* 14: 1225-1240.

Zhu, C., Zhu, C., Naqvi, S., Breitenbach, J., Sandmann, G., Christou, P., Capell, T. 2008. Reconstruction and extension of the carotenoid biosynthetic pathway in maize through combinatorial nuclear genetic transformation. *Proceedings of National Academy of Sciences USA*, 105: 18232-18237.

Toward engineering of cannabinoid biosynthesis: *In-planta* characterization of enzymes involved in the synthesis of THCA and derivatives

Warzecha H[1], Geissler M[1], Volk J[1], Stehle F[2], Kayser O[2]

[1]*Technische Universität Darmstadt, Darmstadt, Germany*
[2] *TU Dortmund University, Dortmund, Germany*

Corresponding author: Heribert Warzecha <warzecha@bio.tu-darmstadt.de>

Cannabis sativa L. or hemp has been a crop plant for centuries, providing fibers and oil but is most prominent for its content of pharmacologically highly active compounds, with THC being the main psychotropic ingredient and the reason for the plants treatment as an illicit drug in many countries. However, numerous of the cannabinoids have been shown to be effective in the treatment of various diseases, making them interesting drug targets or lead compounds for the development of semisynthetic derivatives. To uncouple targeted cannabinoid production from a THC-producing plant, a biotechnological production system with defined products would be advantageous (Schachtsiek et al., 2018).

Here we describe the engineering of late biosynthetic genes into *Nicotiana benthamiana* to evaluate biosynthetic capacity in a transient expression system. With THCA synthase, we were able to show that active enzyme can be obtained after ER/apoplast-targeting (Geissler et al., 2018). Moreover, THCAS seems to be glycosylated in *N. benthamiana,* suggesting that this modification has an influence on the stability of the protein. Activity assays with cannabigerolic acid as substrate showed that the recombinant enzyme not only produces THCA (123 ± 12 fkat g^{-1}_{FW} activity towards THCA production) but also cannabichromenic acid (CBCA; 31 ± 2.6 fkat g^{-1}_{FW} activity towards CBCA production). This preliminary work shows that *N. benthamiana* could be a suitable host for cannabinoid production, but towards whole pathway integration careful analysis of subcellular localization is necessary.

References

Geissler, M., Volk, J., Stehle, F., Kayser, O., Warzecha, H. 2018. Subcellular localization defines modification and production of Δ^9-tetrahydrocannabinolic acid synthase in transiently transformed *Nicotiana benthamiana. Biotechnology Letters* (in press).

Schachtsiek, J., Warzecha, H., Kayser, O., Stehle, F. 2018. Current perspectives on biotechnological cannabinoid production in plants. *Planta Medica* (in press).

Supporting explorers on Mars: A biomanufacturing solution for producing biologics during deep space exploration

Nandi S, Karuppanan K, McNulty M, McDonald KA

University of California, Davis CA, USA

Corresponding author: Somen Nandi <snandi@ucdavis.edu>

The Center for the Utilization of Biological Engineering in Space (CUBES), one of two inaugural National Aeronautics and Space Administration (NASA) Space Technology Research Institutes (STRIs), recently formed to pioneer research in biomanufacturing to produce fuel, materials, pharmaceuticals, and food. These biotic solutions aspire to realize inherent advantages of *in situ* resource utilization in space biotechnology over traditional abiotic approaches.

Deep space exploration mission environments pose a variety of obstacles for sustained human life support systems. Continuous exposure to lower gravity reduces bone mass, muscle mass, and alters cardiac performance. Increased levels of cosmic radiation outside of Earth's protective atmosphere result in high risks of cancer and accelerated degradation of many human organs. Physical isolation from earth-reliant supply chains introduce no*N*-negligible complications in nutritional provisions and access to therapeutics. The primary tasks of our team are to develop platform technologies that could be practiced to provide necessary nutritional and medical support to overcome these human life support system challenges.

Plants are well-studied as a food source for astronauts. More than a dozen plant cultivation experiments have been performed on space missions since the 1970's. Plants harness the abundance of solar energy and close the human waste loop with complementary metabolism. Our overarching research goal is to demonstrate the multi-functional utility of plants not only as a source of food but also for human pharmaceuticals that address unmet needs in deep space exploration. Our primary aims are (1) to design codo*N*-optimized synthetic genes of relevant pharmaceutical molecules, (2) to optimize production and delivery modalities tailored to the specific needs, and (3) to screen and select transient and transgenic plant host lines for sustained production of therapeutic biologics in a Mars-like environment.

Our initial therapeutic target is the gravity-associated bone anabolism of astronauts. We are developing a plant-based platform for expression of Parathyroid Hormone (*N*-terminal fragment) fused to the Fragment Crystallizable (Fc) antibody region. We are also in the process of developing a novel biologically-derived purification platform to reduce the complexity of biopharmaceutical production in the limited resource environment of deep space. The integrated strategic plan and initial experimental research will be presented, along with future research aimed at safeguarding the health of deep space explorers.

Keynote

On-demand gene expression in plants: New frontiers set by the new synthetic expression system

Dominik Mojzita

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Mojzita Dominik <Dominik.Mojzita@vtt.fi>

We have developed a novel orthogonal expression system (SES) for plants that offers broad range and highly constitutive expression levels of target genes. The expression system is based on a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The sTF expression is driven by a universal core promoter, which was obtained by a specifically designed screening assay. The sTF is employed in the SES system as a potent transcription activator for the target gene. The sTF-dependent promoter regulating the expression of the target gene also contains a similar type of universal core promoter, making the whole expression system independent of the host's native regulation and therefore functional in diverse species. The varying number of the sTF-binding sites in combination with a choice of core promoters, and trans-activation domains in the sTF, enable adjustment of the target-gene expression levels over a wide range, from very low to very high. On the high end, the expression is substantially exceeding the levels achievable by the established double-enhancer CaMV35S promoter system. This expression system provides robust expression platform in a broad spectrum of host organisms with numerous applications in metabolic engineering and protein/enzyme production.

Recombinant human osteopontin expressed in *Nicotiana benthamiana* stimulates osteogenesis related genes in human periodontal ligament cells

Rattanapisit, K[1], Abdulheem S[1], Chaikeawkaew D[1], Kubera A[2], Mason HS[3], Ma JKC[4], Pavasant P[1], Phoolcharoen W[1]

[1]*Chulalongkorn University, Bangkok, Thailand*
[2]*Kasetsart University, Bangkok, Thailand*
[3]*Arizona State University, Tempe, USA*
[4]*St. George's, University of London, London,* United Kingdom

Corresponding author: Kaewta Rattanapisit <som_kaewza@hotmail.com>

Tissue engineering aims to utilise biologic mediators to facilitate tissue regeneration. Several recombinant proteins have potential to mediate induction of bone production, however, the high production cost of mammalian cell expression impedes patient access to such treatments. The aim of this study is to produce recombinant human osteopontin (hOPN) in plants for inducing dental bone regeneration. The expression host was *Nicotiana benthamiana* using a geminiviral vector for transient expression. OPN expression was confirmed by Western blot and ELISA, and OPN was purified using Ni affinity chromatography. Structural analysis indicated that plant-produced hOPN had a structure similar to commercial HEK cell-produced hOPN. Biological function of the plant-produced hOPN was also examined. Human periodontal ligament stem cells were seeded on an OP*N*-coated surface. The results indicated that cells could grow normally on plant-produced hOPN as compared to commercial HEK cell-produced hOPN determined by MTT assay. Interestingly, increased expression of osteogenic differentiatio*N*-related genes, including *OSX*, *DMP1*, and *Wnt3a*, was observed by realtime PCR. These results show the potential of plant-produced OPN to induce osteogenic differentiation of stem cells from periodontal ligament *in vitro*, and suggest a therapeutic strategy for bone regeneration in the future.

Plant somatic embryo cultures are a profitable source of compounds with skin rejuvenating activity

Barbulova A, Sena LM, Apone F, Colucci MG

Arterra Bioscience srl, Naples, Italy

Corresponding author: Ani Barbulova <ani@arterrabio.it>

The use of plant tissue cultures as a source of safe, sustainable and effective skin care ingredients became not only a trend but also a valid alternative to the traditional plant extract preparations. Currently, a number of plant cell suspension culture derived ingredients with proven efficacy have been already available on the cosmetic market. Among the advantages of the plant cell suspension cultures as source of bioactive compounds is their versatility. This means that it is possible to induce the overproduction of specific compounds by simply modifying the growth conditions or inducing metabolic pathways by elicitor treatments. Moreover, by modulating growth regulator ratios or applying physical stresses, plant cell cultures can be further stimulated to produce differentiated tissues, which are more specialized and therefore able to synthesise compounds that are not produced in cell cultures at detectable levels (Barbuloba et al., 2014).

One example is represented by the differentiation of the somatic embryos, bipolar structures, morphologically and physiologically identical to the zygotic embryos contained in the seeds. The somatic embryos pass through the same developmental stages as zygotic embryos, but without employing any gamete fecundation. In these terms, somatic embryo cultures, and the process of somatic embryogenesis itself, represent the maximum expression of plant totipotency. It has been demonstrated that the somatic embryos express differentiation regulating genes and normally specific changes in the synthesis and mobilization of proteins, carbohydrates and lipids occur during their development (Horstman et al., 2017). Moreover, the somatic embryos are newly formed plant structures in constant mitotic activity, which makes them an interesting source of bioactive compounds promoting cell growth and differentiation in the skin.

By employing molecular and analytical approaches, we investigated on the different composition of somatic embryo and cell culture derived ingredients, and demonstrated that extract preparations, obtained from somatic embryo-enriched cultures, were capable of attenuating senescence associated phenotypes in skin fibroblasts, by inducing important components of the extra cellular matrix and activating cell rejuvenation factors.

References

Barbulova, A., Apone, F., Colucci, G. 2014. Plant cell cultures as source of cosmetic active ingredients. *Cosmetics* 1: 94-104.

Horstman, A., Bemer, M., & Boutilier, K. 2017. A transcriptional view on somatic embryogenesis. *Regeneration* 4: 201-2014.

Combinatorial design of modular and programmable transcriptional regulators in plants

Selma García S, Bernabé Orts J, Ajenjo Bauzá M, Diego Martin B, Vazquez Vilar M, Granell A, Orzaez D

Institute for Plant Molecular and Cellular Biology (CSIC-UPV), Valencia, Spain

Corresponding author: Sara Selma García <saselgar@doctor.upv.es>

The RNA-guided DNA binding activity of CRISPR/Cas9 offers unprecedented possibilities in the fields of synthetic biology, molecular farming and metabolic engineering, among others. Modifications of the Cas9 protein or its guide RNA (gRNA) enable the expansion of the range of Cas9 activities, from its original nuclease activity to other DNA binding-related activities, like transcriptional activation/repression. This opens the possibility of modulating the expression of selected genes or sets of genes in plants through CRISPR/Cas9-based programmable transcription factors, thus directing metabolic fluxes towards the accumulation of certain products.

The objective of this work is to design modular gRNA modifications that allow the attachment of regulatory domains to the CRISPR/Cas9 complex via its gRNA for plants. To achieve this, the CRISPR/gRNA-directed synergistic activation mediator or SAM strategy was adapted via Goldenbraid cloning system. SAM strategy consists of the adding of an RNA aptamer that binds the Ms2 phage coat protein, to free positions of the gRNA. This creates a structure comprising the chimeric RNA-guide and Cas9, which binds to Ms2 fused to a domain or protein of interest. In order to optimize the process, two chimeric RNA-guides were designed containing two copies of the Ms2 aptamer in two different positions. One of them adds the aptamer to the 3´end of the RNA-guide, whereas the second one incorporates it in a free loop of the RNA-guide hairpin. These structures were used for the attachment of several combinations of transcriptional activators to the gRNA/Cas9 complex fused to the Ms2 protein. Taking advantage of the modular Goldenbraid cloning system, the construction of a combinatorial array of programmable transcriptional regulation with other proteins that bind aptamer sequences, like PP7 and COM, and several regulatory domains was facilitated.

After test different strategies, the conclusion was that SAM/Ms2 strategy with the aptamer in the 3' of the scaffold was the most efficient in *N. benthamiana*. Furthermore, the efficiency of using SAM/Ms2 strategy was compared with others where the activator domain was fused directly to the dCas9 testing different promoters, like Nopaline synthase promoter (pNos), Metallothionei*N*-like protein type 2B promoter (pMTB) or dihydroflavonol 4-reductase promoter (pDFR), in transient and stable expression in *N. benthamiana*. The results obtained show the great potential of the Cas9/SAM system to modulate metabolic pathways and optimize the production of metabolites or proteins of interest.

References

Nowak, C. M., Lawson, S., Zerez, M., Bleris, L. 2016. Guide RNA engineering for versatile Cas9 functionality. *Nucleic Acids Research*, 44: 9555-9564.

Vazquez-Vilar, M., Bernabé-Orts, J. M., Fernandez-del-Carmen, A., Ziarsolo, P., Blanca, J., Granell, A., Orzaez, D. 2016. A modular toolbox for gRNA–Cas9 genome engineering in plants based on the GoldenBraid standard. *Plant Methods*, 12:1, 10.

Molecular Farming 6.0: Hexa-mutant *Nicotiana benthamiana* plants lacking plant plant-specific *N*-glycans for transient expression

Jansing J[1], Sack M[1], Augustine SM[1], Fischer R[1], Bortesi L[1]

[1]*RWTH Aachen University, Aachen, Germany*
[2]*Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Aachen, Germany*

Corresponding author: Julia Jansing <julia.zischewski@molbiotech.rwth-aachen.de>

The field of genome editing has been revolutionized by the recent emergence of the CRISPR/Cas9 technology, which makes the engineering of genomes simpler and cheaper than before. The RNA-guided nuclease Cas9 can easily be programmed to introduce a double strand break (DSB) in one or multiple sequences of choice simultaneously. In plants, DSBs are preferentially repaired by no*N*-homologous end-joining, which can result in frameshift mutations and thus the knock-out (KO) of targeted genes.

Plants are a fast and flexible alternative for the production of pharmaceutical proteins, but differences between plant and mammalian *N*-glycosylation can affect the activity and immunogenic potential of a plant-produced protein. Thus it is desirable to modify the endogenous *N*-glycosylation machinery of *Nicotiana benthamiana* – by far the species of choice for protein production via transient expression – to make mammalia*N*-like *N*-glycan structures. Previous efforts to eliminate the plant-specific α-1,3-fucose and β-1,2-xylose from *N*-glycans by RNAi (Strasser et al.), classical mutagenesis (Weterins & van Eldik) and TALEN (Li et al.) have only been partially successful, as the presence of α-1,3-fucose could be reduced but never eliminated. Here, we employed multiplexed CRISPR/Cas9 genome editing to generate plant lines completely deficient in α-1,3-fucosyltransferase and β-1,2-xylosyltransferase activity. We generated constructs targeting either the xylosyl- (X) or the fucosyl-transferases (F) separately, and both sets of genes simultaneously. In the T2 generation, F-KO and X-KO lines were identified and the functional gene knock-out was confirmed by mass spectrometric analysis of their *N*-glycans. FX-KO lines were then generated by crossing, and - thanks to the favorable appearance of new germinal mutations by ongoing Cas9 activity - homozygous knock-out lines were quickly identified.

References

Li, J., Stoddard, T. J., Demorest, Z. L., Lavoie, P. O., Luo, S., ... Yabandith, A. 2016. Multiplexed, targeted gene editing in *Nicotiana benthamiana* for glycol-engineering and monoclonal antibody production. *Plant Biotechnology Journal* 14: 533-542.

Strasser, R., Stadlmann, J., Schähs, M., Stiegler, G., Quendler, H., ... Steinkellner, H. 2008. Generation of glyco- engineered *Nicotiana benthamiana* for the production of monoclonal antibodies with a homogeneous human- like *N*-glycan structure. *Plant Biotechnology Journal*, 6: 392-402.

Weterings, K., Van Eldik, G. 2015. *Nicotiana benthamiana* plants deficient in fucosyltransferase activity. *U.S. Patent Application No. 14/347,752*.

Keynote

Genome editing: challenging our views and interpretation of the current regulatory systems

Joachim Schiemann

Julius Kühn Institute, Quedlinburg, Germany

Corresponding author: Schiemann, Joachim <joachim.schiemann@julius-kuehn.de>

Genome editing is a transformative technology with general applicability providing a very wide range of potential uses to tackle societal challenges. Worldwide, several genome-edited plants and products thereof are already approved as no*N*-regulated articles and are reaching the market. In January 2017 the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) have published four documents related to the pre-market regulatory oversight of a variety of biology-based agricultural tools, including genetically engineered plants and plants and animals derived from certain newer precision breeding techniques, such as genome editing. EASAC, the European Academies Science Advisory Council, has published a Report on Genome Editing: scientific opportunities, public interests and policy options in the EU in March 2017. In this report, EASAC takes a broad perspective on the research advances, applications, policy implications and priorities for EU strategy for promoting innovation and managing regulation. EPSO, the European Plant Science Organization, has highlighted that in the implementation of the EU biotechnology regulatory framework there is a disproportionate focus on the genetic improvement technique used. This has led to the misinterpretation that GMOs are merely defined by the use of certain techniques. This is incorrect. Whether or not the resulting organism is a GMO depends on the fact if a novel combination of genetic material has been produced beyond the natural barriers of mating and recombination. This is not the case for several new plant traits obtained by genome editing. Based on the documents mentioned above future perspectives on biotechnology legislation will be discussed.

In a first reaction on the Advocate General's Opinion regarding mutagenesis and the Genetically Modified Organisms Directive the European Plant Science Organisation welcomes the Advocate General's opinion in case C-528/16 published on 18 Jan 2018 as an important step in clarifying the European Directive 2001/18/EC concerning organisms modified by recent mutagenesis techniques that appeared after 2001. The opinion provides a general scheme to follow now by the European Court of Justice.

Looking over the edge of the European plate (Sprink et al., 2016), regulatory triggers, the regulatory status of genome edited crops, and biotechnology oversight efforts in Argentina, Australia, Canada, China, Japan, and the USA will also be discussed.

Reference

Sprink, T., Eriksson, D., Schiemann, J., Hartung, F. 2016. Regulatory hurdles for genome editing: process- vs. product-based approaches in different regulatory contexts. *Plant Cell Reports* 35: 1493-506.

Session 4

Keynotes

Development of the next generation influenza vaccine

Marc-André ’Aoust
Medicago Inc., Québec, Canada

Natural products based molecules for target and drug discovery in pharmaceutical research

Frank Petersen
Novartis Pharma AG, Basel, Switzerland

Keynote

Development of the next generation influenza vaccine

Marc-André D'Aoust

Medicago Inc., Québec, Canada

Corresponding author: Marc-André D'Aoust <daoustma@medicago.com>

In the 2017-2018 influenza season, vaccination only provided a limited protection against the virus. It has even been calculated that, next year's vaccine could provide only 20% protection against the dominant subtype H3N2 (Bonomo & Deem, 2018). Such suboptimal efficacy of the flu vaccines is the results of mutations resulting from egg-based vaccine production process, stressing the need for a new generation of influenza vaccines. Medicago has developed a recombinant seasonal influenza vaccine in the form of virus-like particles (VLPs). The plant-based production technology used allows for the production of vaccines that perfectly match the circulating strains and the VLP display of antigens results in strong humoral and cell mediated immune responses that have the potential to provide higher efficacy than the current vaccines. Today, our seasonal influenza vaccine is being evaluated in a human efficacy study involving 10 000 subjects. The presentation will detail the development of the next generation influenza vaccine, emphasising on the platform, the process and the analytical developments needed to bring this product to PIII clinical stage along with the most significant clinical results obtained to date.

Reference

Bonomo, M. E., Deem, M. W. 2018. Predicting influenza H3N2 vaccine efficacy from evolution of the dominant epitope. *Clinical Infectious Diseases* (in press).

Genome editing of *Nicotiana tabacum* suspension cells by a multiplex CRISPR/Cas9 strategy results in humanized pharmaceutical glycoproteins without plant specific glycans

Mercx S[1], Smargiasso N[2], Chaumont F[1], De Pauw E[2], Boutry M[1], Navarre C[1]

[1]Université catholique de Louvain, LouvaiN-la-Neuve, Belgium
[2]de Liège, Liège, Belgium

Corresponding author: Sébastien Mercx <sebastien.mercx@uclouvain.be>

Plants or plant cells can be used to produce pharmacological glycoproteins such as antibodies or vaccines. However these proteins carry *N*-glycans with plant-typical residues (β(1,2)-xylose and core α(1,3)-fucose), which can greatly impact the immunogenicity, allergenicity, or activity of the protein. Two enzymes are responsible for the addition of plant-specific glycans: β(1,2)-xylosyltransferase (XylT) and α(1,3)-fucosyltransferase (FucT). Our aim consisted of knocking-out two *XylT* genes and four *FucT* genes (12 alleles altogether) in *Nicotiana tabacum* BY-2 suspension cells using CRISPR/Cas9. Three *XylT* and six *FucT* sgRNAs were designed to target conserved regions. After transformation of *N. tabacum* BY-2 cells, three lines showed a strong reduction of β(1,2)-xylose and α(1,3)-fucose, while two lines were completely devoid of them, indicating complete gene inactivation. The absence of these carbohydrates was confirmed by mass spectrometry analysis of the extracellular proteins. The KO lines did not show any particular morphology and grew as the wild-type. One KO line was transformed with genes encoding a human IgG2 antibody. The IgG2 expression level was as high as in a control transformant which had not been glycoengineered. The IgG glycosylation profile determined by mass spectrometry confirmed that no β(1,2)-xylose or α(1,3)-fucose were present on the glycosylation moiety and that the dominant glycoform was the GnGn structure. These data represent an important step towards humanizing the glycosylation of pharmacological proteins expressed in *N. tabacum* BY-2 cells.

Colour bio-factories: scale-up production of anthocyanins in plant cell cultures

Appelhagen I[1], Wulff-Vester AK[2], Hvoslef-Eide AK[2], Russell J[1], Oertel A[4,5], Martens S[3,4], Mock H-P[5], Martin C[1], Matros A[5]

[1]John Innes Centre, Norwich, United Kingdom
[2]Norwegian University of Life Sciences, Ås, Norway
[3]Edmund Mach Foundation, San Michele all'Adige (TN), Italy
[4]TransMIT GmbH, Giessen, Germany
[5]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK-Gatersleben), Stadt Seeland OT Gatersleben, Germany

Corresponding author: Ingo Appelhagen <ingo.appelhagen@jic.ac.uk>

Anthocyanins are widely distributed, glycosylated, water-soluble plant pigments, which give many fruits and flowers red, purple or blue colouration. Their beneficial effects in a dietary context have encouraged increasing use of anthocyanins as natural colourants in the food and cosmetic industries. However, the limited commercial availability and diversity of anthocyanins have initiated searches for alternative sources of these natural colourants. In plants, high-level production of secondary metabolites, such as anthocyanins, can be achieved by engineering of regulatory genes as well as genes encoding biosynthetic enzymes. We have used tobacco lines which constitutively produce high levels of cyanidin 3-*O*-rutinoside, delphinidin 3-*O*-rutinoside or a novel anthocyanin, acylated cyanidin 3-*O*-(coumaroyl) rutinoside to generate cell suspension cultures. The cell lines are stable in their production rates and superior to conventional plant cell cultures. Scale-up of anthocyanin production in bioreactors has been demonstrated. The cell cultures also have proven to be a valuable production system for ^{13}C-labelled anthocyanins. Our method for anthocyanin production is transferable to other plant species, such as *Arabidopsis thaliana*, demonstrating the potential of this approach for making a wide range of highly-decorated anthocyanins. The tobacco cell cultures represent a customisable and sustainable alternative to conventional anthocyanin production platforms and have considerable potential for use in industrial and medical applications of anthocyanins.

pH gradient mitigation in the leaf cell secretory pathway – One goal, several effects

Jutras PV[1], Goulet M-C[1], Lavoie P-O[2], Tardif R[2], Hamel L-P[2], D'Aoust M-A[2], Sainsbury F[3], Michaud D[1]

[1]*Université Laval, Québec, Canada*
[2]*Medicago Inc., Québec, Canada*
[3]*The University of Queensland, Brisbane, Australia*

Corresponding author: Philippe V. Jutras <philippe.varennes-jutras@plants.ox.ac.uk>

Partial neutralization of the Golgi lumen pH by ectopic expression of Influenza virus M2 proton channel is a useful way to stabilize acid-labile recombinant proteins and peptides in the leaf cell secretory pathway (Jutras et al., 2015). Here we assessed the 'off-target' effects of M2 channel expression in agroinfiltrated *Nicotiana benthamiana* leaves. Transient co-expression assays with fusion protein hybrids differentially susceptible to proteolytic breakdown first allowed to detect a significant alteration of host protease activities and recombinant protein processing in leaves upon M2 channel expression. An iTRAQ proteomics analysis then revealed a cell-wide impact for this protein on the leaf proteome. As expected given previously described effects of *A. tumefaciens* on the proteome (Goulet et al., 2012; Goulet et al., 2015), infiltrated leaves showed an altered soluble protein content compared to no*N*-infiltrated control leaves, associated with a strong decrease of photosynthesis-associated proteins (including Rubisco) and a concomitant increase of stress-related proteins. M2 co-expression partly compromised these effects on the proteome to restore original soluble protein and Rubisco contents in leaf tissue, associated with higher levels of translatio*N*-associated (ribosomal) proteins and reduced levels of stress-related proteins. Proteome alterations in M2-expressing leaves were determined both transcriptionally and post-transcriptionally, to alter the steady-state levels of proteins not only in the secretory pathway but also in other cellular compartments. These data suggest the potential of M2 channel-mediated proton export to modulate the stability of protease-susceptible secreted proteins *in planta* via an indirect, pH-related effect on host resident proteases. They also reveal cell-wide effects for M2 on the leaf proteome, underlining the relevance of carefully considering the eventual 'off-target' effects of accessory proteins used to modulate specific cellular or metabolic functions in plant protein biofactories.

References

Goulet, C., Khalf, M., Sainsbury, F., D'Aoust, M. A., Michaud, D. 2012. A protease activity–depleted environment for heterologous proteins migrating towards the leaf cell apoplast. *Plant Biotechnology Journal*, 10: 83-94.

Jutras, P. V., D'Aoust, M. A., Couture, M. M. J., Vézina, L. P., Goulet, M. C., Michaud, D., Sainsbury, F. 2015. Modulating secretory pathway pH by proton channel co-expression can increase recombinant protein stability in plants. *Biotechnology Journal*, 10: 1478-1486.

Robert, S., Goulet, M. C., D'Aoust, M. A., Sainsbury, F., Michaud, D. 2015. Leaf proteome rebalancing in *Nicotiana benthamiana* for upstream enrichment of a transiently expressed recombinant protein. *Plant Biotechnology Journal*, 13: 1169-1179.

Keynote

Natural products based molecules for target and drug discovery in pharmaceutical research

Frank Petersen

Novartis Pharma AG, Basel, Switzerland

Corresponding author: Frank Petersen <frank.petersen@novartis.com>

Natural products with their specific structural features deliver chemical starting points in drug discovery to develop innovative therapies for diseases for which no or only unsatisfactory treatments exist. The evolved function of natural products in regulating a plethora of diverse biological pathways in nature makes them to a biologically biased and complementary source of chemical probes to identify novel mechanisms of molecular interactions. As there is a high need for specific modulators of new targets, the identification of novel natural product chemotypes and the elucidation of their mechanism of action are gaining an increasing attraction in today's drug research. Selected examples will illustrate how the investigation of new chemotypes from natural products and natural products-inspired synthetics in phenotypic assays can be shuttles to a new biological space of therapeutic relevance.

The technological driving forces of Synthetic Biology, genome sequencing and DNA-synthesis, are currently changing the face of natural products research. Whole genome data or the identification of cryptic natural products pathways *iN-silico* shed light on so far u*N*-accessed genetically encoded small molecules and helping us to print and refactor active pathways of scientific interest.

The human gut microbiome became a new research field in modern natural product discovery where big data analyses open the door to the discovery of beneficial commensals with essential roles in the homeostasis of the human body. The fragmentation of natural products is a further scientifically exciting example how the broad chemical diversity of natural products can be leveraged in the science of drug discovery.

References

Bürstner, N., Roggo, S., Ostermann, N., Blank, J., Delmas, C., ... Mihalic, M. 2015. Gift from nature: cyclomarin A kills mycobacteria and malaria parasites by distinct modes of action. *ChemBioChem*, 16: 2433-2436.

Ganesan, A. 2008. The impact of natural products upon modern drug discovery. *Current Opinion in Chemical Biology* 12: 306-317.

Hoepfner, D., McNamara, C. W., Lim, C. S., Studer, C., Riedl, R., ... Plikat, U. 2012. Selective and specific inhibition of the Plasmodium falciparum lysyl-tRNA synthetase by the fungal secondary metabolite cladosporin. *Cell Host & Microbe* 11: 654-663.

Koch, M. A., Schuffenhauer, A., Scheck, M., Wetzel, S., Casaulta, M., ... Waldmann, H. 2005. Charting biologically relevant chemical space: a structural classification of natural products (SCONP). *Proceedings of the National Academy of Sciences of the United States of America*, 102: 17272-17277.

Krastel, P., Roggo, S., Schirle, M., Ross, N. T., Perruccio, F., ... Mapa, F. 2015. Nannocystin A: an elongation factor 1 inhibitor from myxobacteria with differential anti- cancer properties. *Angewandte Chemie International Edition* 54: 10149-10154.

Kuhn, T.H., Wang, Y. 2008 Artemisin – an innovative cornerstone for anti-malaria therapy. In: Natural Compounds as Drugs Vol II, Progress in Drug Research 65. (Eds F. Petersen & R. Amstutz), Birkhäuser Verlag.

Prescher, H., Koch, G., Schuhmann, T., Ertl, P., Bussenault, A., ... Lizos, D. E. 2017. Construction of a 3D-shaped, natural product like fragment library by fragmentation and diversification of natural products. *Bioorganic & Medicinal Chemistry* 25: 921-925.

Rottmann, M., McNamara, C., Yeung, B. K., Lee, M. C., Zou, B.,... Cohen, S. B. 2010. Spiroindolones, a potent compound class for the treatment of malaria. *Science* 329: 1175-1180.

Schmitt, E. K., Hoepfner, D., Krastel, P. 2016. Natural products as probes in pharmaceutical research. *Journal of Industrial Microbiology & Biotechnology* 43: 249-260.

Schmitt, E. K., Moore, C. M., Krastel, P., Petersen, F. 2011. Natural products as catalysts for innovation: a pharmaceutical industry perspective. *Current Opinion in Chemical Biology* 15: 497-504.

Spillman, N. J., Allen, R. J., McNamara, C. W., Yeung, B. K., Winzeler, E. A., Diagana, T. T., Kirk, K. 2013. Na+ regulation in the malaria parasite *Plasmodium falciparum* involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. *Cell Host & Microbe* 13: 227-237.

Wlodarska, M., Luo, C., Kolde, R., d'Hennezel, E., Annand, J. W., ... Garner, A. L. 2017. Indoleacrylic acid produced by commensal Peptostreptococcus species suppresses inflammation. *Cell Host & Microbe* 22: 25-37.

Prolonging serum half-life of plant-made anti-HIV broadly neutralising antibodies (bNAbs) by improving binding to human neonatal Fc receptor (hFcRn) and reducing binding to human macrophage receptor (hMR)

Teh AY-H., Stelter S, van Dolleweerd C, Ma JKC

St. George's University of London, London, United Kingdom

Corresponding author: Julian Ma <jma@sgul.ac.uk>

The new generation of anti-HIV broadly neutralising antibodies (bNAbs) have great potential to complement existing antiretroviral therapy (ARV) to control viremia and for use as pre-exposure prophylaxis. The recent phase I clinical trial of VRC01[1] showed that although it can control viremia for up to two weeks longer than conventional ARV, it is not clinically significant. Apart from using combinations of bNAbs as an alternative method, we believe improving their serum half-life can also contribute to the efficacy of the bNAbs. To that end, we have generated different variants of the anti-HIV bNAbs VRC01 and 3BNC117 with either the YTE[2] or LS[3] mutation in their Fc region in ΔXF *N. benthamiana* plants. This was done using our i*N*-house Modular Idempotent DNA Assembly System (MIDAS). We found that the mutations in the Fc region improved biding to hFcRn, the receptor responsible for IgG recovery, while not compromising gp120 biding and HIV-1 neutralisation capabilities. Further experiments investigating the serum half-life of these bNAbs will be performed using transgenic mice expressing hFcRn. In lieu of recent research[4], we also hypothesized that removal of the exposed light chain glycan could decrease binding to the hMR which clears proteins containing specific glycan from the bloodstream. However, we showed that VRC01 with aglycosylated light chain produced in wild-type and ΔXF *N. benthamiana* did not bind to hMR *in vitro*. The removal of light chain glycan also affected HIV-1 neutralisation in 3BNC117.

References

Bar, K. J., Sneller, M. C., Harrison, L. J., Justement, J. S., Overton, E. T., ... Learn, G. H. 2016. Effect of HIV antibody VRC01 on viral rebound after treatment interruption. *New England Journal of Medicine* 375: 2037-2050.

Dall'Acqua, W. F., Kiener, P. A., Wu, H. 2006. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). *Journal of Biological Chemistry* 281: 23514-23524.

Zalevsky, J., Chamberlain, A. K., Horton, H. M., Karki, S., Leung, I. W., ..., Desjarlais, J. R. 2010. Enhanced antibody half-life improves in vivo activity. *Nature Biotechnology*, 28: 157.

Production of secondary metabolites with plant cell cultures from an eco-sustainable view

Ullish DA, Sankar-Thomas YD, Selge T, Ellert C, Pump M, Leibold T, Schütte K, Gorr G[1]

Phyton Biotech GmbH, Ahrensburg, Germany

Corresponding author: David Ullisch <David.Ullisch@phytonbiotech.com>

Plants have been considered as a source of natural substances for ages. Recently secondary metabolites gain interest as cosmetical ingredients and in the field of nutraceuticals, but most famous they are for medical applications. However, supply of compounds from natural harvest can be limited by numerous factors i.e. batch to batch variations, endangered species and low product content. In addition, CBD issues and the Nagoya protocol have to be considered. Often the complexity of secondary metabolites precludes chemical synthesis at least on a reasonable commercial basis. Extraction processes of intact plant material often require large amounts of hazardous and toxic solvents. In terms of sustainability the use of organic solvents should be minimized.

With the successful production of the taxane paclitaxel at 75,000 L scale complying with GMP standards, Phyton demonstrates the power of plant cell fermentation (PCF®) as a tool for the production of complex secondary metabolites from plants. Reaching titers of 60 g/kg dry weight implies long history of process optimization.

In addition also ingenanes produced by Euphorbiaceae are used in medical applications, i.e., in Picato® which is approved against actinic keratosis. In intact plants, however, the ingenanes are predominantly present in the milky latex at very low concentrations. The generation of cell lines expressing significant amounts of different ingenanes is another example underlining the strength of plant cell culture.

A process optimization strategy for paclitaxel applying multivariate data analysis (MVDA) and the critical evaluation of a green chemistry survey are presented. Focus is on differences between natural harvest and Phyton's PCF® route.

Developing an IgA biobetter antibody against *E. coli* O157:H7 with enhanced stability by rational design of a bovine Fc chain

Chin-Fatt A, Menassa R

London Research and Development Centre and Western University, Ontario, Canada

Corresponding author: Rima Menassa <rima.menassa@AGR.GC.CA>

Immunoglobulin A (IgA) antibodies are useful therapeutics but production can be complicated by downstream product heterogeneity, often associated with degradation and aggregation of end products due to structural instability. Given the cost of producing these molecules, even small increases in stability, and resultant yield and product homogeneity, can translate to significant savings. An IgA-nanobody fusion is a modular type of molecule that comprises two highly ordered constant domains, collectively called the fragment crystallisable (Fc), fused to an antigen binding partner known as the variable heavy chain fragment (VHH) derived from camelids. Considering that the Fc is a key stability determinant when fused to a VHH partner, we hypothesized that improving the stability of the Fc could improve accumulation of the VHH-Fc fusion. Therefore, rational design of a bovine Fc was performed by bioinformatic analysis and molecular modeling to predict key amino acid substitutions that may induce either supercharging or disulfide bond formation to improve the stability of the fused molecule. The native bovine Fc was mutagenized to construct these candidates which were then transiently expressed in leaves of *Nicotiana benthamiana* and screened for protein accumulation, thermostability and solubility. We have identified and characterised five supercharging and one disulfide mutant that, in comparison to the native Fc, all improve accumulation and enhance thermostability. We also have found that pyramiding of these mutations results in a complementary increase of accumulation. Binding and neutralisation assays are currently being performed to verify that these mutations do not affect binding efficacy. The goal of this project is to demonstrate as a proof of concept that a bovine Fc chain can be rationally designed for improved stability and be a viable strategy for improving accumulation of the therapeutic antibody without sacrificing efficacy.

Session 5

Keynote

A multi-omics approach to harness tobacco biochemistry

Jennifer Bromley
Bristish American Tobacco, Cambridge, United Kingdom

Keynote

A multi-omics approach to harness tobacco biochemistry

Jennifer Bromley

Bristish American Tobacco, Cambridge, United Kingdom

Corresponding author: Jennifer Bromley <jennifer_bromley@bat.com>

From its biochemistry and the underlying genetic architecture that regulates it, the tobacco plant is highly complex and poorly understood. Traditional incremental improvements have in the past provided successes to adapting tobacco but these techniques alone may no longer be sufficient to sustain the crop for future applications and to meet regulatory requirements.

Technological developments in areas such as sequencing and mass spectrometry, as well as data storage and analytical capabilities, have led to an era in which 'big data' plays a major role in biological research. This is pertinent to tobacco, where increasing amounts of genomic and transcriptomic data are becoming publicly available, including assemblies of the tobacco genome. This data is providing fresh insight into the workings of the tobacco plant, and the tens of thousands of genes that its genome contains.

We have recently published (and continue to improve) an assembly of the tobacco genome (Edwards et al., 2017), which has facilitated much of our recent progress into understanding the plant's complexities. The genome serves as a scaffold from which we are able to hang various 'omics data sets. Because the tobacco genome is annotated to contain 69,500 genes, generating and positioning this data is only the starting point, decoding it is where the real challenge lies.

Using a combination of data and hypothesis-driven approaches, we have worked to mine and integrate transcriptomic and metabolomic datasets to lead us to long-lists of candidate genes. From these lists, we are able to apply association techniques, inserting these genes into co-expression networks. These networks have allowed us to short-list and prioritise candidate genes based on pockets of pre-existing knowledge and to also hypothesise their function.

Reference

Edwards, K. D., Fernandez-Pozo, N., Drake-Stowe, K., Humphry, M., Evans, A. D., ... Bromley, J. R. 2017. A reference genome for *Nicotiana tabacum* enables map-based cloning of homeologous loci implicated in nitrogen utilization efficiency. *BMC Genomics* 18 448.

Recombinant production of the toxic anti-cancer lectin viscumin in tobacco plants and microbial cells. A comparative analysis of yield, process costs and toxicity

Gengenbach BB[1], Keil LL[2], Opdensteinen P[1], Müschen CR[2], Buyel JF[1,2]

[1]Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Aachen, Germany
[2]RWTH Aachen University, Aachen, Germany

Corresponding author:
Benjamin Gengenbach <benjamin.gengenbach@ime.fraunhofer.de>

Cancer is the leading cause of death in industrialized countries. Cancer therapy often involves monoclonal antibodies or small-molecule drugs, but carbohydrate-binding lectins such as mistletoe (*Viscum album*) viscumin offer a potential alternative treatment strategy (Zwierzina et al., 2011). Viscumin is toxic in mammalian cells, ruling them out as an efficient production system, and it forms inclusion bodies in *Escherichia coli* such that purification requires complex and lengthy refolding steps (Eiberle and Jungbauer, 2010). We therefore investigated the transient expression of viscumin in intact *Nicotiana benthamiana* plants and *N. tabacum* plant-cell packs (PCPs) (Rademacher et al., 2018), comparing a full-length viscumin gene construct to separate constructs for the A and B chains. The maximum yield of heterodimeric viscumin in PCPs was 5.0±1.2 mg kg^{-1} fresh biomass (arithmetic mean ± standard deviation, n=11) with the full-length construct, 10-fold higher than the separate A and B chains. In intact plants, the maximum yield was 3.0±1.3 mg kg^{-1} fresh biomass (arithmetic mean ± standard deviation, n=9) again with the full-length construct, which was 1.5 fold lower than the yield of the equivalent product in PCPs. Using a lactosyl-Sepharose affinity resin, we increased the purity of viscumin by 150-fold compared to the clarified plant extract, to ~7%. The absence of refolding steps resulted in estimated cost savings of more than 35% when transient expression in tobacco was compared to *E. coli*. Toxicity of the plant derived viscumin product was increased more than 10-fold compared to the microbial counterpart. We conclude that plants offer a suitable alternative for the production of complex biopharmaceutical proteins that are toxic to mammalian cells, require authentic post translational modifications and that form inclusion bodies in bacteria.

References

Eiberle, M.K., Jungbauer, A. 2010. Technical refolding of proteins: Do we have freedom to operate? Biotechnology Journal 5: 547-559.

Zwierzina, H., Bergmann, L., Fiebig, H., Aamdal, S., Schöffski, P., Witthohn, K., Lentzen, H. 2011. The preclinical and clinical activity of aviscumine: a potential anticancer drug. *European Journal of Cancer* 47: 1450-1457.

Rademacher, T., Sack, M., Blessing, D., Fischer, R., Holland, T., Buyel, J.F. 2018. Plant cell packs as a versatile and scalable platform for biotechnology applications. *Plant Biotechnology Journal* (in preparation).

Plant-made antimicrobial proteins for food safety

Giritch A[1], Hahn S[1], Stephan A[1], Schulz S[1], Schneider T[1], Kazanavičiūtė V[2], Razanskiene A[2], Shaversky A[1], Gleba Y[1]

[1]*Nomad Bioscience GmbH, Halle, Germany*
[2]*Nomads UAB, Vilnius, Lithuania*

Corresponding author: Anatoli Giritch <gyrych@nomadbioscience.com>

Foodborne diseases are a growing public health problem worldwide. They are the result of ingestion of foodstuffs contaminated with microorganisms or chemicals. The contamination of food may occur at any stage in the process from food production to consumption. Pathogenic *Escherichia coli*, *Salmonella*, *Listeria*, *Clostridium* and *Campylobacter* are most common foodborne bacterial infections. The prevention of microbial contamination mostly relies on such generic approaches as continuous cold chains, cooking, pasteurization, treatment with organic acids and oxidizing agents and proper animal farm and food processing plant hygiene. Use of traditional antibiotics for the treatment of food is inappropriate, particularly because of increased antibiotic resistance found in almost all foodborne pathogenic bacteria.

Using antimicrobial proteins (bacteriocins and phage endolysins) as food additives or food processing aids is a promising new approach. It is especially attractive because of the magnitude of current food safety issues and because these product candidates can be approved relatively quickly using the so-called GRAS (Generally Recognized As Safe) regulatory approval path in USA.

We showed that most bacteriocins and endolysins active against *E. coli*, *Salmonella*, *Listeria* can be manufactured efficiently in green plants using plant-virus-based production systems. Most antimicrobial proteins are expressed at high yields and are expected to command low commercially viable manufacturing costs. Nomad colicin (bacteriocins from *E. coli*) cocktails show very high activity against all major enterohemorrhagic *E. coli* serotypes defined by USDA/FDA. Salmocins (bacteriocins from *Salmonella*) are highly active against pathogenic *Salmonella* strains. Two salmocins, SalE1a and SalE1b, possess highest potency and broad antimicrobial activity against all 99 major *Salmonella* pathovars. Treatments with low (less than 10 mg bacteriocins per kg of treated food product) reduce the bacterial load of different pathogenic strains by >6 logs. In spike experiments using meats and vegetables and fruits spiked with pathogenic *E. coli* or *Salmonella enterica*, proposed cocktail-based antibacterial food additives and food processing aids efficiently reduce the titer of pathogenic bacteria.

Nomad already has FDA's GRAS status registration for its two first filings (GRN 593 and GRN676). We are preparing new GRAS applications which will deal with plant-made antimicrobial proteins for control of *Salmonella* and *Clostridium perfringens*.

Co-expression of a cyclizing asparaginyl endopeptidase enables efficient production of cyclic peptides *in planta*

Jackson MA[1], Gilding EK[1], Shafee T[2], Harris KS[2], Kaas Q[1], Poon S[2], Yap K[1], Jia H[1], Guarino R[2], Chan LY[1], Durek T[1], Anderson MA[2], Craik DJ[1]

[1]*The University of Queensland, Brisbane, Australia*
[2]*La Trobe University, Melbourne, Australia*

Corresponding author: Mark Jackson <m.jackson1@uq.edu.au>

Peptides as therapeutic agents have advantages over small molecule drugs in both specificity and potency, resulting in reduced side effects and improved user compliance. However, one disadvantage is that peptides are particularly prone to degradation, resulting in short *in vivo* half-lives and the requirement for injectable dosing. Thus, strategies to enhance the stability of peptide therapeutics are of prime importance. In certain plants, peptides termed cyclotides are known to accumulate as ultra-stable plant defense molecules. This stability is largely attributed to their cyclic backbone and unique disulfide bond arrangement. Together these structural features make cyclotides ideal scaffolds for peptide engineering where bioactive epitopes are inserted to stabilize bioactivity. One limitation to the commercial deployment of cyclotide-based peptide drugs is the lack of a cost-effective scalable production system. A plant-based approach is an appealing option, however early attempts at expressing cyclic peptides in biofactory host plants resulted in poor peptide cyclisation and low yields. Here we show that by co-expressing plant asparaginyl endopeptidases (AEPs) sourced from cyclotide producing plant species that cyclic peptide yields *in planta* can be dramatically improved, opening up opportunities for large scale production in plant hosts. Furthermore, we use molecular modelling, protein sequence space analysis and functional *in planta* testing of AEP variants to identify the structural features that differentiate AEPs that preference peptide cyclization over proteolysis. These structural features represent hot spots for further rational engineering of ligase type AEPs to broaden substrate specificity and kinetics.

Understanding the regulation of the *N*-glycosylation pathway is a prerequisite to optimize microalgae as a cell factory for the production of biopharmaceuticals

Lucas P-L[1], Dumontier R[1], Loutelier-Bourhis C[1], Plasson C[1], Burel C[1], Mareck A[1], Afonso C[1], Lerouge P[1], Kiefer-Meyer M-C[1], Mati-Baouche N[1], Bardor M[1, 2]

[1]*University of Rouen, Normandy, UNIROUEN, Rouen, France*
[2]*Institut Universitaire de France (IUF), Paris, France*

Corresponding author: Muriel Bardor <muriel.bardor@univ-rouen.fr>

Few microalgae species, such as the green microalgae, *Chlamydomonas reinhardtii* and the diatom, Phaeodactylum tricornutum have been investigated as potential biofactories for the production of biopharmaceuticals including monoclonal antibodies (mAbs). Indeed in this context, microalgae are cheap, classified as Generally Recognized As Safe (GRAS) organisms and can be grown easily. However, problems remain to be solved before any industrial production of microalgae-made biopharmaceuticals. Among them, post-translational modifications of the proteins need to be fully understand and control. Especially, *N*-glycosylation acquired by the secreted recombinant proteins is of major concern since most of the biopharmaceuticals are *N*-glycosylated and it is well recognized that glycosylation represent one of their critical quality attribute. Therefore, the evaluation of microalgae as alternative cell factory for biopharmaceutical productions thus requires to investigate their *N*-glycosylation capability in order to determine to what extend it differs from their human counterpart and to determine appropriate strategies for remodelling the microalgae glycosylation into huma*N*-compatible oligosaccharides. In this presentation, we will present the work done recently to characterize the *N*-glycosylation pathways of *C. reinhardtii* and *P. tricornutum* and comment the consequences on the glycan engineering strategies that may be necessary to render the future microalgae-made biopharmaceuticals compatible with human therapy.

Global food security – Plant-made Virus-Like Particles as a candidate vaccine against Nervous Necrosis Virus

Lomonossoff GP[1], Marsian J[1], Paley R[2], Hurdiss DL[3], Ranson NA[3]

[1]*John Innes Centre, Norwich, United Kingdom*
[2]*Cefas, Weymouth, United Kingdom*
[3]*University of Leeds, Leeds, United Kingdom*

Corresponding author: George Lomonossoff (JIC) <george.lomonossoff@jic.ac.uk>

As the global population reaches nine billion, aquaculture - the fastest growing food production sector, rather than stagnating capture fisheries, will play a key role in future food security. However, infectious diseases still represent one of the most significant threats to this food production (FAO 2016). Nervous Necrosis Virus (NNV) is a nodavirus that infects over 40 marine fish species and is a significant threat for the fish industry as it causes mass mortality in many economically important fish species worldwide. It is a small, spherical, no*N*-enveloped virus carrying a bipartite positive-sense RNA genome. RNA-2 (1.4 kb) encodes the coat protein precursor alpha (43 kDa) which rapidly self-assembles into T = 3 provirions when expressed in a number of different systems. Provirions mature by spontaneous autocatalytic cleavage of the coat protein alpha post assembly; producing proteins beta (38 kDa) and gamma (5 kDa) which remain part of the mature virion.

We have used transient expression of the full-length coat protein of Atlantic cod NNV (ACNNV) to produce virus-like particles (VLPs) *in N. benthamiana* as a candidate vaccine for use in aquaculture. The expressed coat protein assembled efficiently into VLPs which could be readily purified. In these particles, the majority of the coat protein is unprocessed, suggesting that they are essentially provirions. Cryo-electron microscopy of the plant-produced VLPs yielded a 3.7 Å resolution structure that was characteristic of a nodavirus. Administration of the purified VLPs to sea bass showed that they were able to confer a degree of protection (up to 86.5 relative percent survival) against subsequent challenge with NNV.

Immunogenicity of a Virus-like Particle (VLP) vaccine against African horse sickness

Meyers AE, Dennis SJ, Hitzeroth II, Rybicki EP

University of Cape Town, Rondebosch, South Africa

Corresponding author: Ann Meyers <ann.meyers@uct.ac.za>

African horse sickness is a devastating, infectious, but no*N*-contagious disease that causes great suffering and many fatalities amongst the horse population in sub-Saharan Africa. It is caused by African horse sickness virus (AHSV), an orbivirus of the family *Reoviridae,* and is spread by midges. The disease has significant economic consequences for the equine industry both in southern Africa, and increasingly further afield as the geographic distribution of the midge vector broadens with global warming. Live attenuated vaccines have been in use with relative success for more than five decades, but there is a risk of reversion to virulence as well as re-assortment of the segmented genome between outbreak and vaccine strains. Furthermore, the vaccines lack DIVA capacity, the ability to distinguish between vaccine-induced immunity and that induced by natural infection. Several studies have demonstrated the potential for the use of plant expression systems for the production of VLPs, which are excellent vaccine candidates as they do not contain the virus genetic material, there is no risk of reversion to virulence or re-assortment with wild virus strains and they are DIVA compliant.

In this study we report on the immunogenicity of *N. benthamiana*-produced AHSV serotype 5 VLPs in horses: this serotype is not included in the attenuated live vaccine mix because of safety problems. We previously showed that co-infiltration of constructs encoding the four AHSV structural proteins VP2, VP3, VP5 and VP7 resulted in the assembly of complete VLPs. Production of these VLPs was scaled up using density gradient ultracentrifugation, and immunogenicity and serum neutralisation was tested in guinea pigs. Serum from the guinea pigs was shown to be reactogenic with all four structural proteins in western blots, and able to neutralise live virus after one prime and a single boost inoculation. The ability of plant-produced VLPs to stimulate immunogenicity in horses - the main target animals - was subsequently tested. Serum from horses immunised with 2 doses of plant-produced VLPs was shown to be highly reactive with VLPs. The serum is currently being tested in neutralisation assays. These results show that there is great potential for the production of a novel and effective AHSV serotype 5 vaccine in plants.

From *TricoPharming* to *Artennua*: From medicinal trichomes research to business

Sanseverino C, Acosta A, Murray H, Cigliano RA, Sanseverino W, Matías-Hernández L

Sequentia Biotech, Edificio Eureka, Campus UAB, Barcelona, Spain
Sequentia Biotech, ICIA headquarters, Valle Guerra, Tenerife, Spain

Corresponding author: Luis Matias <lmatias@sequentiabiotech.com>

***TricoPharming**,* a plant molecular farming research line, was created nearly four years ago by *Sequentia Biotech*, a spi*N*-off which goal is to create the know-how and the technologies for a sustainable development for the improvement of agriculture and human health. Trichomes' peculiarity is that they are able to synthesize, store and secrete large amounts of specialized metabolites which could have significant commercial value as pharmaceuticals. *TricoPharming* use trichomes as natural bio-factories to produce high-quality medicines at low cost. Moreover, the isolated compartmentalization in trichomes allows the production of potentially detrimental compounds without affecting plant development. Therefore, *TricoPharming* represent an unprecedented opportunity with high social impact to manufacture affordable modern medicinal compounds and make these available at a global scale.

Our first product, ***Artennua***, is an optimized version of the *Artemisia annua* plant achieved using different plant molecular farming strategies in order to produce the valuable product *artemisinin*; the most efficient molecule to treat malaria as recommended by the WHO. However, over the past decade diverse clinical trials have been carried out to elucidate the enormous therapeutic potential of artemisinin to treat not just malaria and related-diseases, but many other very different diseases that include cancer, autoimmune & inflammatory diseases, bacterial, viral & parasitic diseases, dermatological disorders and even mental diseases. Due to its high market potential, *Artennua* extracts will be produce in a variety of formats in order to encompass market segments such as nutraceuticals, pharma, dermatology, natural cosmetics, herbal and animal health.

Based on these successful research results, in 2017 we started the pilot production in the field of our optimized plant in the Canary Islands; while large-scale production of *Artennua* will begin in 2019. However in the meantime, diverse struggling situations have happened through to the development of the final products. *This is the true story behind the step from "peaceful" research to the challenging business development world.*

ABSTRACTS FOR

POSTER

PRESENTATIONS

1. The development of a chimaeric Rift Valley Fever Virus-like Particle vaccine candidate made in *Nicotiana benthamiana*

Mbewana S, Meyers A, Rybicki E

University of Cape Town, Rondebosch, South Africa

Corresponding author: Sandiswa Mbewana <sandiswa.mbewana@uct.ac.za>

Rift Valley Fever (RVF) is a zoonotic disease endemic in parts of Africa and the Arabian Peninsula but is described as an emerging virus due to the wide range of mosquitoes that could spread the disease into no*N*-endemic areas. RVF is characterised by high rates of abortion in ruminants and haemorrhagic fever, encephalitis, or blindness in humans. RVF is caused by Rift Valley fever virus (RVFV) which is an enveloped negative-stranded RNA virus and belongs to the genus *Phlebovirus* in the family *Bunyaviridae*. The virus major structural proteins Gn and Gc are the main immunogenic determinants of the virus. The disease can be prevented by vaccination, but there is currently no commercially available vaccine that can be used outside of endemic areas. Virus-like Particles (VLPs) are more immunogenic than simple subunit vaccines because they resemble the virus in shape and morphology, and display multiple repeating epitopes. In the case of RVFV, chimaeric VLPs presenting the immunogenic antigens could be a safe and effective vaccine candidate.

We describe the generation of chimaeric RVFV VLPs consisting of a truncated RVFV Gn envelope glycoprotein fused to the H5N1 influenza HA transmembrane domain and cytosolic tail (TMD/CT) to make budding VLPs. The RVFV GnGc polyprotein gene was initially plant codon optimised, synthesised and cloned into a plant expression vector. It was then modified by truncation and fusion to the HA TMD/CT-encoding sequence, and protein expression was achieved by transient expression in *Nicotiana benthamiana* via Agrobacterium tumefaciens-mediated gene transfer. The modified Gn polyprotein (Gne-HA) was successfully expressed in *N. benthamiana* seven days post infiltration. Protein expression analysis was carried out by western blotting. Recombinant plant-produced Gne-HA was purified by differential centrifugation on a 20–50 % iodixanol step gradient and fractions were analysed by polyacrylamide gel electrophoresis. Purified protein yields were calculated to be ~57 mg/kg fresh weight. Transmission electron microscopy (TEM) of gradient fractions was used to confirm particle formation. TEM showed Gne-HA particles of ~49–60 nm in the 30% iodixanol fraction which were no*N*-uniform in shape. The immunogenicity of purified Gne-HA particles was tested in BALB/c mice. The presence of anti-Gn specific antibodies was analysed by indirect ELISA using plant-produced Gn protein as coating antigen. Preliminary immunogenicity studies of chimaeric RVFV Gne-HA VLPs illustrated that the vaccine candidate was immunogenic in mice.

2. Tobacco seeds as sustainable production platform of the natural biopolymer cyanophycin as co-product to oil and protein

Nausch H, Broer I

University of Rostock, Rostock, Germany

Corresponding author: Henrik Nausch <henrik.nausch@uni-rostock.de>

Cyanophycin (CGP) is a cyanobacterial polypeptide which can be used both as valuable *N*-rich ingredient for the feed and as biopolymer substituting chemicals. We introduced CGP-synthesis into leaf plastids of the commercial tobacco cultivars Burley and Virgin, yielding up to 9.4% CGP per dry weight (dw) when using the constitutive 35s CaMV promotor (Nausch et al., 2016). Since CGP is only soluble at pH < 2 and insoluble at neutral pH, it can be easily purified from leaf tissue.

Here we investigated CGP expression in seeds of the high producing events and analyzed a potential correlation to the CGP levels in leaves. The protocol, used for the extraction of CGP from tobacco leaves, was optimized for the CGP-isolation from seeds. Now, a field trail with the elite events in Argentina, done by the company Bioceres, will be prepared. In order to optimize the expression in seeds, we will use tissue-specific promotors and a cultivar specifically bred for high seed yield by the company Sunchem/Idroedil for the next generation of CGP-producing transgenic plants. We are also combining the CGP-purification method with that for oil and protein of Sunchem for the large scale isolation of all three components at the same time. Tobacco oil is used as biofuel, while the protein serves as feed in livestock industry.

Combined with the development of new CGP-derived products, an economic analysis and socioeconomic studies, CGP might be one of the first transgene-encoded biomaterials that reach the market.

The international consortium is funded by the ERA CoBioTech Call 1 as part of the European Union’s Horizon 2020 research and innovation program. The partners of this project are:

Bioceres SA, Argentina
Idroedil SRL, Italy
University of Rostock, Germany
Wageningen Research Centre, Netherlands
Wageningen University, Netherlands
Leuphana University Lüneburg, Germany

3. Feed supplementation with β-Asp-Arg dipeptides via stable co-expression cyanophycin and cyanophycinase in plants

Nausch H[1], Zeyner A[2], Broer I[1]

[1]*University of Rostock, Rostock, Germany*
[2]*MartiN-Luther-University Halle-Wittenberg, Halle (Saale), Germany*

Corresponding author: Henrik Nausch henrik.nausch@uni-rostock.de

Livestock diets can be supplemented with dipeptides in order to promote optimal growth and wellbeing. Due to the dual role of arginine as building block for proteins and regulator of physiological functions, pronounced effects were observed after addition of β-aspartate-arginine (β-Asp-Arg) dipeptides to feed. Currently, β-Asp-Arg is generated in vitro from the cyanobacterial storage polymer cyanophycin (CGP) via incubation with the cyanophycinase enzyme (CGPase) which are both produced in *E. coli*. Because of the high costs and limited scalability, bioreactor-based production is commonly used for the synthesis of high-value but not for cost-sensitive products such as supplements for animal diets.

Alternatively, recombinant low-value products can be produced in plants in an economic manner using existing agricultural infrastructure and farming practices. We already established the production of CGP in plastids of tobacco and potato, yielding up to 9.4% of the dry weight in stably transformed plants. We also demonstrated that CGPases can be transiently co-expressed in the cytosol of CGP-producing tobacco via the MagnICON system without affecting the CGP accumulation in intact chloroplasts. Amongst different CGPases, CphE showed the highest yield and was able to degrade CGP in homogenized leaf tissue when the spatial separation of cytosol and plastid stroma was destroyed. Oral administration of feed pellets which contained purified CGP and CGPase to mice showed that the released dipeptides were absorbed into the blood.

Now we could demonstrate that CGP degradation is not only possible after transient expression of CphE in the cytosol of CGP-producing tobacco, but also when the CGPase is introduced into these plants via stable transformation. Constitutive expression of CphE in parallel to CGP synthesis does not affect the accumulation of CGP in leaf chloroplasts and degrades CGP completely after decomposition of cells. The transfer of this system to feed plants would therefore provide an easy and cheap system to directly release the β-Asp-Arg dipeptides in the intestine of animals in the digestive process.

4. Potyvirus vectors to produce proteins and metabolites in biofactory plants

Martí M, Daròs JA

IBMCP, CSIC-Universistat Politècnica de València, Valencia, Spain

Corresponding author: Mari Carmen Martí Botella <mcmarti@ibmcp.upv.es>, José-Antonio Daròs <jadaros@ibmcp.upv.es>

The small genomes of plant viruses can be easily engineered to transform these pathogens into powerful biotechnological tools able to induce the production of large amounts of recombinant proteins in the host plant, which is converted in a biofactory. Plant virus biotechnology approaches have traditionally focused on viral species that induce the production of huge amounts of some of their proteins, such us Tobacco mosaic virus (TMV) or Cowpea mosaic virus (CPMV). However, the amazing wealth of genome architectures and expression strategies found in plant viruses open many other possibilities. One of them is the use of potyviruses as viral vectors. Potyviruses (genus Potyvirus) make one of the largest groups of plant viruses with around 200 different species known to date. Their genome is composed of an about 10,000-nt long plus-strand RNA molecule that is bound at the 5' end to a genome-linked viral (VPg) protein and is polyadenylated at the 3' end. This genomic RNA is encapsidated in elongated and flexuous virions. What makes potyviruses special is their gene expression strategy. Basically, potyviruses express a single polyprotein that is processed by three viral proteases. The highly specific NIa protease (NIaPro) performs most cleavages in the viral polyprotein based on a seven amino acid recognition motif. We set up a potyvirus-derived vector system in which the viral cistron encoding the RNA-dependent RNA polymerase (NIb) is replaced by a cassette to produce several proteins. The cDNAs encoding for these proteins are flanked with sequences corresponding to the NIaPro cleavage site. This way, the viral vector produces equimolar amounts of several proteins in plants that co-express the viral NIb. Insertion of the recombinant cDNA at the most amino terminal end of the viral polyprotein has allowed us to target heterologous proteins to specific organelles, such as chloroplasts and mitochondria. Using these potyvirus vectors we have produced antibodies, transcription factors and metabolic enzymes.

5. Production of a protein subunit candidate vaccine for *Mannheimia haemolytica* in lettuce and tobacco chloroplasts

Martin C, Kaldis A, Kolotilin I, Menassa R

The University of Western Ontario, London, Canada
Agriculture and Agri-Food Canada, London, Canada

Corresbonding author: Coby Martin <cmart245@uwo.ca>

The cattle industry worldwide is ravaged by bovine respiratory disease (BRD), caused mainly by the bacterium *Mannheimia haemolytica*. We have designed a chimeric protein subunit vaccine against *M. haemolytica* based on recent evidence demonstrating the protective potential of antigens against a virulence factor, leukotoxin, in addition to a surface lipoprotein. Plant-based production of this protein vaccine provides a safe and inexpensive alternative to traditional production methods. Plant-base production also supports the use of an edible vaccine that will deliver antigens to the pharyngeal mucosa to provide local immunization against *M. haemolytica* to prior to its progression into the lungs. Chloroplasts can produce and accumulate bacterial proteins, such as those comprising the vaccine we have designed, due to their prokaryotic translational machinery and lack of mechanisms of glycosylation and gene-silencing. Transplastomic production of our vaccine is therefore an attractive option. Transient expression by *A. tumefaciens*-mediated transformation offers another avenue for production. Transient expression allows large amounts of foreign protein to be produced within a short time following agroinfiltration. In this project, we conducted chloroplast transformation procedures on lettuce and tobacco, and conducted agroinfiltration of *Nicotiana benthamiana* to produce a *M. haemolytica* vaccine. This endeavor necessitated the development and optimization of lettuce chloroplast transformation and regeneration techniques. While lettuce transformants have not been generated thus far, this candidate vaccine was successfully produced transplastomically in N. tabacum and through agroinfiltration of *N. benthamiana*.

6. SES: Synthetic expression system for plants

Rantasalo A, Joensuu J, Rischer H, Mojzita H

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Heiko Risher <heiko.rischer@vtt.fi>

We have developed a novel orthogonal expression system (SES) that functions in a wide spectrum of eukaryotic organisms, including fungi and plants. The expression system is based on a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The sTF expression is driven by a universal core promoter, which was obtained by a specifically designed screening assay. The universal core promoter provides highly constitutive expression level of the sTF, which is employed in the SES system as a potent transcription activator for the target gene. The sTF-dependent promoter regulating the expression of the target gene also contains a similar type of universal core promoter, making the whole expression system independent of the host's native regulation and therefore functional in diverse species. The varying number of the sTF-binding sites in combination with a choice of core promoters enable adjustment of the target gene expression levels over a wide range, from very low to very high, which is particularly difficult in plant hosts with current genetic tools. This expression system provides robust and stable expression levels of target genes in a broad spectrum of host organisms with numerous applications in metabolic engineering and protein/enzyme production.

The method for selecting the universal core promoters, construction of the expression system, and demonstrating its performance in comparison with the established CMV promoter system will be presented. In addition, the utility of the expression system will be demonstrated for the production of diverse recombinant proteins in tobacco leaves.

7. Production in plant of a chimeric vaccine against Infectious Bursal Disease Virus

Rage E[1], Marusic C[1], Lico C[1], Scaloni A[2], Salzano AM[2], Baschieri S[1], Donini M[1]

[1]*ENEA Research Center, Rome, Italy*
[2]*ISPAAM, National Research Council, Napoli, Italy*

Corresponding author: Emile Rage <emile.rage@gmail.com>

Infectious Bursal Disease virus (IBDV) is the cause of an economically important highly contagious disease of poultry. The capsid constituent VP2 is considered as the major host-protective antigen. Vaccines are regarded as the most beneficial interventions to prevent this infection but are still based on whole pathogens and do not permit a differentiation of infected from vaccinated animals (DIVA).

The aim of this study is to use plants as biofactories for the production of a low cost innovative recombinant vaccine against IBDV allowing DIVA. The “vaccine” is composed of an immunogenic VP2 domain fused to the constant domain of an IgY antibody. The fusion may result in the enhancement of the vaccine efficacy through the interaction with receptors on antigen presenting cells in chicken. Furthermore, the Fc portion may work as stabilizing partner and may result in the simplification of the extraction and purification procedures.

The secretory VP2-FcY gene construct was cloned into a plant expression vector. Transformed Agrobacterium tumefaciens, was used to infiltrate *Nicotiana benthamiana* plants and leaf tissues were assayed for recombinant protein expression by Western Blot (WB) analysis showing maximum accumulation of a product corresponding to the assembled VP2-FcY dimer at 5 days post infiltration. The secretion of the protein in the leaf intercellular fluids was also demonstrated, while the correct conformation of the VP2 domain was verified by ELISA. To optimize the production in plants, an extraction protocol was set-up that allowed to minimize contamination by unwanted compounds/proteins. Purification was performed using a chromatography column that specifically binds to the highly hydrophobic FcY portion. The characterization of the purified product by SDS-PAGE indicated also the presence of high molecular weight multimers. Additionally, Mass Spectrometry analysis revealed an Fc glycosylation pattern very similar to that of chicken serum IgY with the formation of high mannose sugar chains.

In conclusion, the VP2-FcY fusion protein was successfully produced in plants, where it correctly assembles in a dimeric form with the Fc portion showing an IgY-like glycosylation profile. Overall these results indicate that the fusion protein may bind to Fc receptors involved in immune response activation in chickens. The developed fast and convenient extraction and purification protocol will allow to verify this hypothesis through in vivo functional studies.

8. Plant-made Bet V 1 for molecular diagnosis

Santoni M[1], Avesani L[1], Mari A[2], Ciardiello MA[3], Zampieri R[1]

[1]*University of Verona, Verona, Italy*
[2]*Associated Centre for Molecular Allergology, Rome, Italy*
[3]*Institute of Bioscience and BioResources, Naples, Italy*

Corresponding author: Mattia Santoni <mattia.santoni@univr.it>

The traditional diagnosis of allergies is based on a skin test system (called also “Prick test”), which has different drawbacks, mainly its difficult standardization. As a consequence, allergy diagnosis is moving towards a molecular approach, which, in turns, currently lacks of a cost-effective diagnostic method tailored on the patient’s clinical history and a flexible technique that allows a multiplexed analysis (Giangrieco et al., 2012).

The Enzyme-Linked Immunosorbent Assay (ELISA), is one of the most popular technique for the detection and quantification of specific biomarker proteins and, as a consequence it is widely used for diagnostic kits set-up.

Given the need of costs saving for the production of a diagnostic system, the antigen production should be cost-effective. Plant Molecular Farming (PMF) may help in this, being a production platform able to produce complex recombinant proteins in short time-frames and at a low-price (Ma et al., 2003).

We was exploited the production of Bet v 1, a major allergen associated with birch pollen allergy. The production of the protein was measured using two different transient expression systems in *N. benthamiana* plants, one based on a binary vector (Karimi et al., 2002) and one using a vector based on the Potato Virus X genome (Porta, & Lomonossoff, 1996) their purification from leaf material and their suitability in a new generation allergy diagnosis system, the Friendly Allergen nano-Bead Array (FABER) which allows, the simultaneous analysis of different allergens and their choice based on the patient’s clinical history is described.

Giangrieco, I., Rafaiani, C., Liso, M., Palazzo, P., Pomponi, D., ... Ciardiello, M. A. 2012. Allergens in allergy diagnosis: a glimpse at emerging new concepts and methodologies. *Translational Medicine@ UniSa* 4: 27.

Karimi, M., Inzé, D., Depicker, A. 2002. GATEWAY™ vectors for Agrobacterium-mediated plant transformation. *Trends in Plant Science* 7: 193-195.

Ma, J. K., Drake, P. M., Christou, P. 2003. Genetic modification: the production of recombinant pharmaceutical proteins in plants. *Nature Reviews Genetics* 4: 794.

Porta, C., Lomonossoff, G. P. 1996. Use of viral replicons for the expression of genes in plants. *Molecular Biotechnology* 5: 209.

Sino Biological Inc. ELISA encyclopedia. 2018. http://www.elisa-antibody.com/ELISA-Introduction/ELISA-types

9. Production of human blood clotting factor IX in moss bioreactors

Top O, Reski R, Decker EL

University of Freiburg, Freiburg, Germany

Corresponding author: Oguz Top <oguz.top@sgbm.uni-freiburg.de>

Hemophilia B is a congenital bleeding disorder caused by a malfunction or deficiency of coagulation factor IX (FIX), a vitamin K-dependent (VKD) serine protease. Current treatment is restricted to a protei*N*-replacement therapy. Approximately 1.6 billion units of FIX were consumed in 2016 (World Federation of Hemophilia). However, still about 70-80% of the patients, mainly in developing countries, receive inadequate or no treatment because of unavailable and/or unaffordable FIX concentrates. Our aim is to produce high levels of recombinant human FIX in Physcomitrella patens bioreactors. This moss is an important model organism for evolutionary and functional genomics approaches and also a promising biopharmaceutical production platform for complex recombinant glycoproteins, with the first product, moss-aGal, successfully completed phase I clinical trials (www.greenovation.com). The ease of gene targeting was employed for precise genome engineering resulting in the elimination of plant-specific protein *N*-glycan residues. Transgenic lines were generated for the stable production of FIX and its amount reached up to 0.32% of total soluble protein. Additionally, the moss-produced FIX amino acid sequence was confirmed by mass spectrometry. For the production of bioactive FIX, additional genes encoding gamma-glutamyl carboxylase (GGCX) and paired basic amino acid cleaving enzyme (PACE), which are responsible for the processing of FIX, will be introduced into the moss genome as both enzymatic functions do not exist in plants. Recombinant bioactive FIX-producing moss lines will be generated and characterized for gamma-carboxyglutamic acid content and *N*-glycosylation via mass spectrometry. Furthermore, the activity of the moss-produced FIX will be evaluated by activated partial thromboplastin time assays.

10. Production of biologically active recombinant factor H in glyco-optimized *Physcomitrella patens*

Bohlender LL[1], Parsons J[1], Hoernstein SNW[1], Michelfelder S[1], Niederkrüger H[2], Busch A[2], Krieghoff N[2], Koch J[3], Fode B[2], Schaaf A[2], Frischmuth T[2], Poh lN[1], Zipfel PF[3], Häffner K[1], Decker EL[1], Reski R[1]

[1]*University of Freiburg, Freiburg, Germany*
[2]*Greenovation Biotech GmbH, Freiburg, Germany*
[3]*Friedrich Schiller University, Jena, Germany*

Corresponding author: Lennard Bohlender <lennard.bohlender@biologie.uni-freiburg.de>

Complement Factor H (FH) is the major regulator of the alternative pathway of complement activation. The complement system is a part of the innate immunity and defends the human body against pathogen infections. FH protects host cells from complement attack by controlling the activation of the system. Deficiency or impaired FH-activity can lead to severe kidney diseases (aHUS, C3G) or to serious visual impairment (AMD). To date, therapeutic options to treat FH-related diseases are limited. Attempts to produce biologically active recombinant FH in mammalian, insect or yeast production systems have failed to demonstrate therapeutic value. We showed earlier that the moss Physcomitrella patens is able to produce this complex and highly glycosylated protein (Büttner-Mainik et al., 2011). Here, the production of an improved moss-derived recombinant human factor H (FHmoss) devoid of potentially immunogenic plant-specific sugar residues on protein *N*-glycans will be reported. We found a homogeneous and predominantly di-antennary complex-type GnGn glycan pattern on FHmoss. Further, FHmoss displayed full in vitro activity concerning its binding affinity to cell surfaces, which is important for the host cell protective function of FH, as well as inhibition of specific downstream processes of the complement activation. In addition, in vivo studies with FH-knockout mice (FH-/-) showed that a treatment with FHmoss is effective in reducing pathologic complement intermediates in the kidney, which makes FHmoss a promising biopharmaceutical for the treatment of complement dysregulations (Michelfelder et al., 2016).

Büttner‐Mainik, A., Parsons, J., Jérôme, H., Hartmann, A., Lamer, S., ... Decker, E. L. 2011. Production of biologically active recombinant human factor H in *Physcomitrella*. *Plant Biotechnology Journal* 9: 373-383.

Michelfelder, S., Parsons, J., Bohlender, L. L., Hoernstein, S. N., Niederkrüger, H., ... Frischmuth, T. 2016. Moss-produced, glycosylatio*N*-optimized human factor H for therapeutic application in complement disorders. *Journal of the American Society of Nephrology* ASN-2015070745.

11. Not shown

12. Using colicins to broaden coverage of pathogenic, antibiotic resistant *E. coli*

Szeto T[1], Gochera T[1], Stephan A[2], Giritch A[2], Gleba Y[2], Planche, T[1] Ma JKC[1]

[1]*St George's University of London, London, United Kingdom*
[2]*Nomad Bioscience GMBH, München, Germany*

Corresponding author: Tim Szeto <tszeto@sgul.ac.uk>

Decades of careless utilisation and abuse of antibiotics have engendered a global public health crisis of antibiotic resistance. While multidrug and extensively drug-resistant pathogens are becoming more prevalent and a burden to our health systems worldwide, the development of new classes of antibiotics to combat this threat has not kept pace.

In the continuous struggle for survival advantage, bacteria have developed an arsenal of molecular weaponry directed against competing bacteria. Bacteriocins are antimicrobial proteins produced by bacteria to kill closely related competing strains. Colicins are bacteriocins produced by Esherichia coli whose modes of action include nuclease activity, cell depolarisation by forming pores in the target membrane, or inhibiting peptidoglycan wall synthesis. Therefore, they have potential biotechnology applications as food additives to prevent spoilage and safeguard consumer health, and in the prevention or control of infectious diseases.

Our collection of colicins has been engineered for high expression in the tobacco Nicotiana benthamiana. We have transiently expressed twelve colicins in tobacco plants with high yields (up to approximately 40% total soluble protein). Screening colici*N*-containing plant extracts against a panel of 70 multidrug-resistant (MDR) and extensively drug-resistant (XDR) *E. coli* clinical isolates, revealed that these colicins exhibit varying bactericidal activity with the most potent in the picomolar range. Crucially, none of the bacterial isolates exhibited resistance to all colicins. We have therefore combined colicins based on potency, molecular and biochemical characteristics, resistance profile, and the mechanism of actions to enhance coverage. We have tested the efficacy of these colicin cocktails compared to the individual colicins using standardised in vitro kill assays against pathogenic *E. coli*. As predicted colicin cocktails significantly reduce the incidence of resistant bacteria. Concomitantly, a larger percent of the pathogens were susceptible to lower doses of the cocktails compared to constituent colicins. Our observations indicated that for the most part the potency of the cocktail reflects the effect of the most active component in that cocktail. However, we also observed synergy leading to enhanced potency against some isolates. The mechanism(s) for this synergy are currently unknown.

Colicins are effective, evolutionarily honed antimicrobials. Rational combinations of colicins offers a way of subverting resistance. We believe the use of colicins and colicin cocktails in combination with antibiotics, could circumvent nosocomial infections, and stave development of resistance, respectively.

13. African horse sickness virus VP7 crystalline-particles: A plant-produced candidate vaccine

Fearon SH, Hitzeroth II, Rybicki EP, Meyers AE

University of Cape Town, Cape Town, South Africa

Corresponding author: Shelley Fearon <SMTSHE005@myuct.ac.za>

African horse sickness (AHS) is a debilitating viral disease affecting equines and has resulted in many disastrous epizootics. To date, no successful therapeutic treatment exists for AHS and the commercially used live-attenuated vaccines (LAVs) have various side effects. Insoluble particulates have been shown to increase immunogenicity when compared to soluble subunit vaccines and previous studies demonstrated protection of BALB/c mice immunised with AHSV VP7 against a lethal challenge of AHSV-7. This study investigates a safer alternative based on plant-produced crystalline-particles of the serogroup-specific AHSV structural protein, VP7. Crystalline-particles were produced in *N. benthamiana* by means of Agrobacterium-mediated infiltration, using a recombinant A. tumefaciens strain containing the expression vector pRIC3.0 encoding AHSV-5 VP7. Crystalline-particles were purified using a discontinuous iodixanol density gradient and ultracentrifugation. Subsequent analysis by SDS PAGE, Coomassie staining and western blotting with anti-AHSV guinea-pig serum confirmed satisfactory levels of purified recombinant VP7 protein. Crystalline-particle assembly was confirmed by transmission electron microscopy and concentrations of approximately 163ug/ml were quantified by gel densitometry. In guinea-pig immunogenicity studies the experimental (n=5) group was inoculated with prime- and boost-inoculations of between 10 and 50ug of purified AHSV VP7, and the control (n=5) group inoculated with plant sap lacking the VP7 candidate. Analysis of the humoral and cellular immune response is presently underway: Guinea pig serum will be assayed for the presence of anti-VP7 antibodies by western blotting and ELISA and its live virus neutralisation capability will be assessed by serum virus neutralisation assays. In addition, RNA-seq transcriptome profiling of guinea-pig spleen derived RNA will be used to investigate the cell-mediated immune response to AHSV VP7 crystals. To the best of our knowledge, this is the first time AHSV VP7 crystalline-particles have been produced in plants and, furthermore, the first time that their effects on the cellular immune response have been investigated. If AHSV VP7 crystals demonstrate higher levels of immunogenicity when compared to commercially used LAVs this may highlight a safer, easy to produce, inexpensive vaccine alternative.

14. Manufacturing of antimicrobial and flavor modifier food additives using inducible transgenic *N. benthamiana* plants as production hosts

Hahn-Löbmann S, Stephan A, Schulz S, Schneider T, Giritch A, Gleba Y

Nomad Bioscience GmbH, Halle (Saale), Germany

Corresponding author: Simone Hahn <hahn@nomadbioscience.com>

Bacteriocins are natural, no*N*-antibiotic bacterial proteins with potent antimicrobial activity against bacteria of (mostly) related species. Colicins, bacteriocins from Escherichia coli, and salmocins, colici*N*-like bacteriocins from Salmonella enterica, have been proposed for control of Shiga toxi*N*-producing *E. coli* and S. enterica ssp. enterica, respectively, on foods (Schulz, et al., 2015; Schneider et al., 2018) both pathogens being leading causes of food poisoning worldwide. In USA, colicins produced in edible plants such as spinach and leafy beets have already been accepted by the U. S. Food and Drug Administration (FDA) and U. S. Department of Agriculture (USDA) as food-processing antibacterials through the GRAS (generally recognized as safe) regulatory review process (NOMAD's GRN 593 and GRN 674), and GRAS application for *N. benthamiana* as a production host has also been submitted. Thaumatin proteins are natural sweet-tasting proteins originating from the Katemfe fruit of the African bush Thaumatococcus daniellii. Thaumatin extracted from its natural plant source as a mixture predominantly consisting of related proteins thaumati*N*-I and thaumati*N*-II, has been used as low-calorie sweetener and flavor modifier, is classified as GRAS and is permitted for general use in food (Codex Alimentarius 2016). Recently, NOMAD received GRAS approval for plant-made recombinant thaumatins as sweeteners (GRN 738). We describe here generation, selection and analysis of stable transgenic *N. benthamiana* plant lines for ethanol-inducible expression of colicins, salmocins and thaumatins. Employment of this simple and inexpensive production and purification methods based on N. bethamiana as the production host, provides scalable and economically viable process for colicins, salmocins and thaumatins as multiple use food additives (Stephan et al., 2018).

Schneider, T., Hahn-Löbmann, S., Stephan, A., Schulz, S., Giritch, A., ... Gleba, Y. 2018. Plant-made *Salmonella bacteriocins* salmocins for control of *Salmonella pathovars*. *Scientific Reports* 8: 4078.

Schulz, S., Stephan, A., Hahn, S., Bortesi, L., Jarczowski, F., ... Gleba, Y. 2015. Broad and efficient control of major foodborne pathogenic strains of *Escherichia coli* by mixtures of plant-produced colicins. *Proceedings of the National Academy of Sciences USA* 112: E5454-E5460.

Stephan, A., Hahn-Löbmann, S., Rosche, F., Buchholz, M., Giritch, A., Gleba, Y. 2017. Simple purification of *Nicotiana benthamiana*-produced recombinant colicins: high-yield recovery of purified proteins with minimum alkaloid content supports the suitability of the host for manufacturing food additives. *International Journal of Molecular Sciences* 19:1, 95.

15. Plant-expressed pyocins as weapons to combat pathogenic bacteria

Paškevičius Š[1,3], Starkevič U[1,3], Misiūnas A[1], Dapkutė V[1,3], Vitkauskienė A[4], Gleba Y[2], Ražanskienė A[1]

[1]*Nomad UAB, Vilnius, Lithuania*
[2]*Nomad Bioscience GmbH, Halle (Saale), Germany*
[3]*Vilnius University, Vilnius, Lithuania*
[4]*Lithuanian University of Health Sciences, Kaunas, Lithuania*

Corresponding author: Sarunas Paskevicius <paskevicius.sarunas@gmail.com>

Pseudomonas aeruginosa is an ubiquitous gram-negative bacterium belonging to Gamma Proteobacteria class, which persists in the environment as well as inside human body. *P. aeruginosa* is one of the six pathogens causing hospital ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) infections, which readily develop resistance to antibiotics. Recently, the WHO published a list of bacteria for which new antibiotics are urgently needed, and carbapenem-resistant Pseudomonas aeruginosa was declared a problem of critical importance in such context, a new generation of antimicrobial substances is urgently needed.

Bacteriocins are instruments of microbial warfare in competition for ecological niches. *P. aeruginosa* strains rival with each other by secreting various activity spectrum antibacterial proteins called pyocins: deoxyribonucleases, ribonucleases, pore-forming proteins, peptidoglycan synthesis-blocking proteins, lecti*N*-like proteins, and bacteriophage tail-like protein complexes. We attempted to express pyocins of all known types in highly efficient protein synthesis platform – a plant transient gene expression system. Pyocins involved in cell wall synthesis, pore-forming pyocins and lecti*N*-like pyocins were expressed most efficiently reaching 10-50% of total soluble leaf protein. Pyocins S5, PaeM, three lecti*N*-like pyocins (L1, L2 and L3) and one new pyocin, PaeM4, were purified to homogeneity and their antibacterial activity was tested in several assays. Since pyocins tend to be strain specific, we evaluated the spectrum of their activity with a collection of one hundred clinical strains. By using only three pyocins (S5, PaeM and PaeM4), we were able to target as much as 68% of all tested strains, including multidrug resistant isolates. Among the three tested pyocins, PaeM4 targeted the largest number (53%) of clinical isolates.

Plant-produced pyocins reduced *P. aeruginosa* CFU counts in liquid culture assays by several orders of magnitude and efficiently reduced biofilm growth. We further demonstrated the ability of plant-produced pyocins to protect *Galleria melonella* larvae against lethal *P. aeruginosa* infection. Also, mutation rates by fluctuation analysis were determined for pyocins. We propose that plant-produced pyocins should be considered as a viable alternative to antibiotics for the control of pathogenic *P. aeruginosa*.

16. Plant-made putative rodent-specific contraceptive small ZP3-peptide

Ghasemian K, Broer I, Huckauf J

University of Rostock, Rostock, Germany

Khadijeh Ghasemian <khadijeh.ghasemian@uni-rostock.de>

Rodents are the most important group of mammals in terms of the problems they create in agriculture, horticulture, forestry and public health. Fertility is a key factor that determines the population density. Zona pellucida (ZP) glycoproteins, that are located on the surface of the oocyte and mediate the gamete recognition, have been proposed as candidate immunogens for development of a contraceptive vaccine. Oral application of the ZP antigens could be a promising alternative to control rodent pests populations. Sterilization of no*N*-target species might be prevented by limiting the vaccine to small, species specific peptides. We tried to establish the transient expression of putative mice-specific contraceptive small mZP3-peptide in Nicotiana benthamiana, via viral MagniCON expression system, as a rapid and high-level transient expression system in plants. Nevertheless, the expression of small peptides can be a challenge. We have examined the production of immunocontraceptive antigen that comprised of a ‘promiscuous’ T cell epitope of tetanus toxoid (TT: 15 amino acid (aa)) followed by putative contraceptive mZP3-peptide (15 aa), a (6x)His-tag and an ER-retention signal SEKDEL. Although no band representing a protein with the expected size of the mZP3 peptide was detected by Western blot of the crude extract, fusion of antigen to GFP protein revealed a dominant protein band of the expected size. Successful production of mZP3 antigen and improved recombinant protein stability in plant were also achieved by tripling of the antigenic mZP3 epitope that can increase antige*N*-antigenicity as well. The fusion protein of Oleosin and triple-mZP3 was also successfully produced in N. benthamiana. With regards to already reported features of the oleosin fusion technology, this step could lead to the desired specific immunocontraceptive for oral application.

17. Fc engineering of anti-virus monoclonal antibody to alter binding to the neonatal Fc receptor in expression system

Park S[1], Song I[1], Han D[2], Lee HK[2], An HJ[3], Ko K[1]

[1]*Chung-Ang University, Seoul, Korea*
[2]*Korea Centers for Disease Control and Prevention, Osong, Korea*
[3]*Chungnam National University, Daejeon, Korea*

Corresponding author: 고기/성 <ksko@cau.ac.kr>

Plant expression systems has several advantages such a large-scale production of mAb and absence of pathogenic animal contaminants. However, oligomannose (OM) type glycans structure of mAbpK SO57 in ER have showed a faster clearance compared to antibodies produced in animal cells. The neonatal Fc receptor (FcRn) regulates the persistence of IgG by the FcR*N*-mediated recycling pathway, which salvages IgG from lysosomal degradation within cells. In this study, Fc-engineering of plant-derived mAb SO57 with an ER-retention motif (KDEL) (mAbpK SO57) was conducted to enhance its binding activity to human FcRn (hFcRn), consequently improve its serum half-life. ELISA and SPR assay showed altered binding affinity between the Fc region of three different mAbpK SO57 mutants [M281Y/S283T/T285E (MST), M457L/N463S (MN), H462K/N463F (HN)] and hFcRn compared to wild type of mAbpK SO57. *N*-glycan structure was also confirmed that all of mAbpK SO57 mutants had OM type glycans structure similar to the parental mAbpK SO57. In addition, after engineering of mAbpK SO57, the three variants were effective as mAbpK SO57 in neutralizing the activity of the rabies virus CVS-11.

This research was supported by a grant (Code# PJ0134372018) from the Korean Rural Development Administration, National Research Foundation of Korea Grant funded by the Korean Government (NRF-2017R1A2A2A0569788).

18. Expression of a human prostatic acid phosphatase (PAP)-IgM Fc fusion protein in transgenic plants using in vitro tissue subculture

Kang JK, Ko K

Chung-Ang University, Seoul, Korea

Correspondind: 고기성 <ksko@cau.ac.kr>

In this study, prostatic acid phosphatase (PAP), which is overexpressed in human prostate cancer cells, was cloned to be fused to the IgM constant fragment (Fc) for enhancing immunogenicity and expressed in transgenic tobacco plants. Then, the transgenic plants were propagated by in vitro tissue subculture. Gene insertion and expression of the recombinant PAP-IgM Fc fusion protein were confirmed in each tested the first, second, and third subculture generations (SG1, SG2, and SG3, respectively). Transcription levels were constantly maintained in the SG1, SG2 and SG3 leaf section [top (T), middle (M), and base (BA)]. The presence of the PAP-IgM Fc gene was also confirmed in each leaf section in all tested subculture generations. RNA expression was confirmed in all subculture generations using real-time PCR and quantitative real-time PCR. PAP-IgM Fc protein expression was confirmed in all leaves of the SG1, SG2, and SG3 recombinant transgenic plants by using quantitative western blotting and chemiluminescence immunoassays. These results demonstrate that the recombinant protein was stably expressed for several generations of in vitro subculture. Therefore, transgenic plants can be propagated using in vitro tissue subculture for the production of recombinant proteins.

This research was supported by a grant (Code# PJ0134372018) from the Korean Rural Development Administration, National Research Foundation of Korea Grant funded by the Korean Government (NRF-2017R1A2A2A0569788).

19. A plant-derived antigen–antibody complex induces anticancer immune responses by forming a large quaternary structure

Kim, DS[1], Lee, KJ[1], Qiao, L[1], Ko, K[2], Myeung, SC[1], Ko, K[1]

[1]*Chung-Ang University, Seoul, Korea*
[2]*Konkuk University, Seoul, Korea*

Corresponding author: 고기/성 <ksko@cau.ac.kr>

The effectiveness of antigen–antibody complex (AAC)-mediated immunomodulation has spurred the development of diverse quaternary protein structures for vaccination. The technique of producing AAC vaccines with large quaternary structures in plants was developed to enhance immune responses and to generate anticancer IgGs which recognize the corresponding antigens. Both antigen and antibody proteins were expressed in a F1 plant obtained by crossing the plants expressing each protein. In the F1 plant, both the antigen and antibody assembled to form a large (~ 30 nm) quaternary circular structure. These large quaternary protein structures induced immune responses to generate anticancer immunoglobulin G (IgGs), which inhibited the growth of human colorectal cancer cells in the xenograft nude mouse model. Thus, antigens and antibodies can be assembled to form large, complex quaternary protein structures in plants. Plant crossing represents an alternative strategy for the formation of AAC vaccines that efficiently enhance immune responses.

This research was supported by the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (NRF-2017R1A2A2A0569788), and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1A6A3A11930180).

20. High-level expression of monoclonal antibodies from stable transgenic plants using ethanol-inducible viral replicons

Werner S, Havranek K, Engler C, Ehnert TM

Nambawan Biotech GmbH, Halle (Saale), Germany

Corresponding author: Dr. Stefan Werner <Werner@nambawanbiotech.com>

Currently, most plant-made recombinant proteins are produced using transient expression systems, i.e. the genetic information for producing the protein is not stably integrated into the plant genome but is introduced using the agrobacterial DNA transfer machinery. High yields are usually achieved by inserting the gene-of-interest into a viral replicon resulting in amplification of the genetic information and thus in high-level protein production. Transient expression has many advantages including flexibility and speed of the process. However, production at a large scale requires large quantities of agrobacteria, special equipment and, in case of an injectable drug, bacterial endotoxin has to be removed during the purification process.

In contrast, transgenic plants expressing gene under transcriptional promoter can be grown in large numbers but usually suffer from low expression levels. We have developed a system that overcomes this limitation by stably introducing viral replicons into the genome of *Nicotiana benthamiana* plants which are activated by a simple treatment with a dilute ethanol solution. We show that two no*N*-competing viral replicons (TVCV and PVX) can be introduced into the same plant giving high expression of a full-size antibody upon ethanol induction. Antibody yield is comparable to transient expression from agroinfiltration as demonstrated for the therapeutic antibodies Rituximab and Trastuzumab.

21. A tool for every job: Bluetongue Virus-like Particle production in plants

Thuenemann E, Lomonossoff G

John Innes Centre, Norwich, UK

Corresponding author: Eva Thuenemann (JIC) <eva.thuenemann@jic.ac.uk>

Bluetongue Virus-like particles (VLPs) are just one of the many types of VLP that can be efficiently produced in plants using the transient HyperTrans (HT) expression system. These heteromultimeric particles have previously been shown to be efficacious as a vaccine against Bluetongue disease in sheep (Sainsbury & Lomonossoff, 2008; Sainsbury, Thuenemann & Lomonossoff, 2009; Thuenemann et al., 2017).

The large internal space within bluetongue virus (BTV)-like particles also makes them ideally suited to nanotechnology applications. A thin shell of approx. 40 nm internal diameter is formed by only 120 copies of the innermost structural protein, VP3. Fusion of green fluorescent protein (GFP) to VP3 results in assembly of BTV-like particles with functional GFP on the inside, which still resemble wild-type particles on the outside (with co-expression of the other structural proteins) (Brillault et al., 2017). Similarly, fusion of a kinase enzyme to VP3 still allows assembly of BTV-like particles which have the potential to act as a nano-scale reaction vessel allowing diffusion of substrate into and out of the particles through pores. Such internally modified particles could find application in bioimaging and medicine, for instance.

To aid in development and scale-up of production of these particles, we are developing protocols which allow us to purify BTV-like particles using conventional floor-standing centrifuges.

References

Sainsbury, F., Lomonossoff, G. P. 2008. Extremely high-level and rapid transient protein production in plants without the use of viral replication. *Plant Physiology* 148: 1212-1218.

Sainsbury, F., Thuenemann, E. C., Lomonossoff, G. P. 2009. pEAQ: versatile expression vectors for easy and quick transient expression of heterologous proteins in plants. *Plant Biotechnology Journal* 7: 682-693.

Thuenemann, E. C., Meyers, A. E., Verwey, J., Rybicki, E. P., Lomonossoff, G. P. 2013. A method for rapid production of heteromultimeric protein complexes in plants: assembly of protective bluetongue Virus‐like Particles. *Plant Biotechnology Journal* 11: 839-846.

Brillault, L., Jutras, P. V., Dashti, N., Thuenemann, E. C., Morgan, G., ... Sainsbury, F. 2017. Engineering recombinant virus-like nanoparticles from plants for cellular delivery. *ACS Nano* 11: 3476-3484.

22. Anthocyanin bio-fortified colored tomato endowed with enhanced antioxidant capacity as a concept plant for space-based farming

Massa S[1], Bennici E[1], Cemmi A[1], Demurtas OC[1], Diretto G[1], Del Fiore A[1], De Rossi P[1], Spelt C[2], di Sarcina I[1], Koes R[2], Quattrocchio F[2], Baccaro S[1], Benvenuto E[1]

[1]ENEA Italian National Agency for New Technologies, Rome, Italy
[2]University of Amsterdam, 1012 WX Amsterdam, The Netherlands

Corresponding Author: Silvia Massa <silvia.massa@enea.it>

Human deep space exploration and the setting of Martian outposts is though to be possible especially in connection to the development of plant-based life support systems. This work deals with the design of novel plant systems directed towards the production of valuable bioactive compounds to tackle both health and food supply issues related to long-term manned space missions.

Subject of our experimental design for space-tailored approaches is tomato (*Solanum lycopersicum L*.), excellent source of bioactive molecules like carotenoids, ascorbic acid and other vitamins, but generally poor in anthocyanins. Anthocyanins are colored molecules involved in many plant physio-pathological mechanisms including cell oxidative stress. Through the optimization of the production of these natural anti-oxidants, we wanted to explore tomato plant-based systems as natural 'bioreactors' of anthocyanins improving, at the same time, the resilience of these systems to the harsh environmental conditions encountered in deep space flights or outposts. We started with "hairy root"cultures (HRCs), as a good test bed system for resilience to adverse aerospace conditions. Subsequently, we engineered whole tomato plants (cv. MicroTom). We introduced In both systems a permanent genetic switch of the anthocyanins pathway by the ectopic expression of a MYB-like transcription factor (PhAN4) of Petunia hybrida.

HRCs and plants harboring this additional gene showed a purple phenotype and an increased total polyphenol content as compared to controls. Anthocyanin content profiling was performed by liquid chromatography coupled to high resolution mass spectrometry and revealed 5 major derivatives of delphinidin and petunidin. Transcript profiling is currently under investigation.

The increased flavonoid content in these plant systems results in enhanced scavenging ability towards free radicals. In fact, Electron Spin Resonance Spectroscopy (ESR) experiments show that dried tissues are able to counteract reactive oxygen species generated upon gamma irradiation.

In conclusion, novel plant "ideotypes" were devised for space applications by a single gene engineering. These plants/organs could become natural bioreactors of molecules designed to counteract oxidative stress encountered in plant growing in non natural conditions and as nutraceutical and dietary supplement for the crew.

23. Innovative use of berry seeds in cosmetics

Puupponen-Pimiä R, Nohynek L, Honkapää K, Oksman-Caldentey KM

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Riitta Puupponen-Pimiä <riitta.puupponen.pimia@vtt.fi>

Natural, safe and healthy are the megatrends in global cosmetic industry. Many cosmetic ingredients are synthetic, especially the preservatives, which are associated with many negative or even toxic effects by the consumer. Plant based natural compounds and extracts have raised a lot of positive interest and they are screened at the moment by many cosmetic companies for their preservative characteristics and other positive activities.

The role of skin microbiome in skin health is one of the hot topics in cosmetic research. Skin is the largest organ of human body, harboring one billion microbes/cm^2, and cosmetic companies are just now starting to understand the importance of a healthy skin microbial community to the general health and wellbeing of the skin. New product category is just entering the market focusing on supporting and stabilizing healthy skin microbiota by limiting the growth of harmful and pathogenic microbes and supporting the growth of beneficial ones.

VTT has a long experience on berry research, especially on antimicrobially active phenolic compounds. The focus has been on the industrial berry side streams and the utilization of the whole berry raw material. We have developed berry and fruit based natural antimicrobial ingredients for the cosmetics originating from the berry and fruit side streams by novel technologies. Three technologies have been patented and they are based on 1) dry fractionation technologies using seed sanding, 2) mild and gently milling, and 3) novel extraction technologies.

In addition to antimicrobial activity the fractions provide several important technological advantages as cosmetic ingredients: totally water soluble, free of toxic solvents or reagents, good storage life.

24. Scalable production of proteins in plants

Holton N, Kellner F, Cater P

Leaf Expression Systems, Norwich, United Kingdom

Corresponding author: Nicholas Holton <holtonn@leafexpressionsystems.com>

Leaf Expression Systems is a manufacturing and contract development business specialising in the expression and production of proteins, metabolites and complex natural products for research and bio-medical applications using a proprietary, transient expression technology, Hypertrans®. Leaf Expression Systems is based in a purpose-built, state of the art facility on the Norwich Research Park that was opened in January 2017. It provides services to companies and research organisations by producing sufficient quantities of these valuable proteins and other natural products to enable research and product development. Hypertrans®, is efficient, safe and simple to use to quickly produce proteins in plants such as vaccines, antibodies or enzymes. Other potential uses include producing many proteins at the same time and so creating new biochemical pathways for producing complex ‘bioactive’ molecules such as novel anti-cancer drugs and anti-infectives. We are exploring the potential of the Hypertrans® to express therapeutic and diagnostic proteins. In addition, we are working on a number of grant funded projects to develop virus-like particle based vaccines against emerging diseases.

25. Production of chimeric Virus-like Particles with epitope display of an immunogenic dengue antigen in *Nicotiana benthamiana*

Pang EL[1], Peyret H[2], Pong LY[3], Hassan SS[3], Fang CM[1], Lai KS[4], Lomonossoff GP[2], Loh SHS[1]

[1]*University of Nottingham Malaysia Campus, Selangor, Malaysia*
[2]*John Innes Centre, Norwich, United Kingdom*
[3]*Monash University Malaysia, Selangor, Malaysia*
[4]*Universiti Putra Malaysia, Selangor, Malaysia*

Corresponding author: Sandy Loh Hwei San <Sandy.Loh@nottingham.edu.my>

In view of dengue disease which estimates to haunt 50% of the world population, several preventive and control measures have been employed in research which include Aedes vector suppression, antiviral drug discovery and vaccine development. As the existence of four antigenically distinct dengue serotypes can complicate the disease condition via antibody-dependent enhancement, the research on producing a safe and effective vaccine is prioritised. Virus-like Particles (VLPs) have always been known to confer stronger immunogenicity in vivo and hence represent a very appealing platform for vaccine development. In this context, the chimeric VLPs were designed by displaying dengue envelope protein domain III (cEDIII) on the hepatitis B core antigen (HBcAg) which served as the epitope carrier for expression using pEAQ-HT vector. These cEDIII VLPs were produced in *Nicotiana benthamiana*. The successful assembly of chimeric VLPs presenting the cEDIII antigen was verified by transmission electron microscopy and immunogold labelling. Moreover, specific antibody responses against cEDIII were also detected following the immunogenicity testing of the purified chimeric VLPs in BALB/c mice. Despite of the modest immune responses, current study has solidly shown that the insertion of a small protein (cEDIII) into the major immunodominant region of HBcAg would not disrupt the VLPs assembly. This potentiates further investigations towards the development of a VLP-based vaccine for dengue in near future.

26. Plant-made Dengue vaccine antigen, envelope protein domain III stimulates humoral and cell-mediated immune responses

Pang EL[1], Peyret H[2], Pong LY[3], Hassan SS[3], Fang CM[1], Lai KS[4], Lomonossoff GP[2], Loh SHS[1]

[1]*University of Nottingham Malaysia Campus, Selangor, Malaysia*
[2]*John Innes Centre, Norwich, United Kingdom*
[3]*Monash University Malaysia, Selangor, Malaysia*
[4]*Universiti Putra Malaysia, Selangor, Malaysia*

Corresponding : Sandy Loh Hwei San <Sandy.Loh@nottingham.edu.my>

Dengue outbreaks have spurred intriguing concerns in recent years, primarily affecting nations in the tropical and subtropical regions. As dengue virus co-circulates in the form of four antigenically distinct serotypes, the chance of acquiring secondary infection is relatively high, by which this can cause severe dengue and leads to life-threatening conditions. In order to prevent the risk of disease exacerbation by antibody-dependent enhancement, a dengue vaccine should provide a robust protection against the four serotypes simultaneously. In this study, a consensus sequence of the dengue virus envelope protein domain III (named as cEDIII) was expressed in *Nicotiana benthamiana* as the vaccine antigen of interest. A fusion with cholera toxin B subunit was strategised to boost the immunogenicity of the recombinant protein. Following the vector construction using pEAQ-HT, transient expression was achieved via agroinfiltration in host plants. The recombinant protein was purified and then subjected to BALB/c mice immunisation. Serum analyses showed that a successful stimulation of anti-cEDIII response was obtained along with its neutralisation activities against the four dengue serotypes. Furthermore, a mixed Th1/Th2 response was detected following the antigenic stimulation of mouse splenocytes. These findings have thereby provided an evidence that the development of a plant-based dengue vaccine is feasible and will be anticipated to make a significant impact in dengue control in the future.

27. Membrane binding domain fusion with a protein decreases expression of both a fusion protein and its co-expressed homolog

Shotland Y[1], Grinishin I[1], Popov M[2], Popov S[1]

[1]*Shamoon College of Engineering, Beer-Sheva, Israel*
[2]*Ben Gurion University of the Negev, Beer-Sheva, Israel*

Corresponding author: Yoram Shotland <yshotlan@sce.ac.il>

The expression of heterologous membrane proteins in *E. coli* might be challenging, some costly proteins (e.g. GDNF), are hardly expressed in this system. Although, most of the processes leading to expressing cloned proteins, are clarified already, in some specific cases, the explanations of reasons leading to low expression level of certain proteins are not trivial, e.g., the intramembrane serine protease cleavage (Erez & Bibi, 2009). It is known, that in proteins with low expression levels DNA codon usage in membrane-binding domains (MBD) is crucial (Claassens et al., 2017). Manipulation with the genetic code may lead to higher production of functionally active membrane proteins, but may lead to misfolded and unstable products as well (Nørholm et al., 2012). However, at the native expression level, it is a common practice to consider that co-expression of several proteins in one host does not affect the expression levels of each of the proteins and that the change in MBD affects only the protein they are attaching to. To get better understanding of the effect of MBD on protein expression, we examined simultaneously the effect of MBD on the expression of the attached protein and co-expressed protein in the same cell. The influence of the MBD attached to the C-terminal of a fluorescent protein (EGFP) on its expression level and on the co-expression of another fluorescent protein (mCherry) was investigated. We have found that MBD fusion dramatically decreases the expression level of EGFP. Moreover, MBD decreased the expression not only of its cis-fusion protein, but of the totally different trans-protein as well. Thus, we have elaborated a working model, which allows investigation of the influence of a membrane protein expression on the expression level of another no*N*-membranal protein.

References

Erez, E., Bibi, E. 2009. Cleavage of a multispanning membrane protein by an intramembrane serine protease. *Biochemistry* 48: 12314-12322.

Claassens, N. J., Siliakus, M. F., Spaans, S. K., Creutzburg, S. C., Nijsse, B., Schaap, P. J., ... Van Der Oost, J. 2017. Improving heterologous membrane protein production in *Escherichia coli* by combining transcriptional tuning and codon usage algorithms. *PloS One*, 12:9, e0184355.

Nørholm, M. H., Light, S., Virkki, M. T., Elofsson, A., von Heijne, G., Daley, D. O. 2012. Manipulating the genetic code for membrane protein production: what have we learnt so far? *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1818: 1091-1096.

28. Impact of plant and cell culture extracts on bacteria involved in microbial skin balance

Hanani C, Louaizil M, Nohynek L, Rischer H, Puupponen-Pimiä R

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Hanani Celine <celine.hanani@vtt.fi>

The skin is a complex and fragile ecosystem at the interface with the outside environment. The overall of microorganisms, which colonize the skin, is called the cutaneous microbiota. Numerous endogenous and exogenous disturbances can cause a change in the composition of this flora and affect the host–microorganism relationship. Indeed, the healthy skin microbiome functions as guardian of host defense, while an imbalance between bacteria (called dysbiosis) leads to skin inflammation and disease such as psoriasis, eczema and acne.

Consequently, we developed innovative experimental approach putting forward a way to regulate skin microbiome using plant and cell culture extracts. First, the inhibition capacity of several hydrothermal extracts prepared from plant and cell culture, separately and together, was screened on isolated strains by an optimized agar diffusion method. Then, in order to quantify the inhibition of bacteria growth, measurement was performed in liquid culture as a more accurate assay. Results show that extracts, by acting in a synergistic way, control growth of specific bacteria involved in microbial skin balance. This model highlights the capacity of plant and cell culture extracts to affect bacterial functions in order to regulate the balance of skin microbiome.

29. A protease derived from the potyvirus Sweet potato feathery mottle virus (SPFMV) as biotechnological tool

Ruiz T, López-Moya JJ, Coca M

Centre for Research in Agricultural Genomics Barcelona, Spain

Corresponding author: Tarik Ruiz <tarik.ruiz@cragenomica.es>

As the global recombinant protein market grows, and the platforms for protein production develops, biotechnological tools for protein purification are more necessary. Among them are included proteases required for processing of fusion proteins. Potyviruses are a rich source of proteases with potent activity and high specificity. These viruses are the largest family of RNA plant viruses (Wylie et al., 2017). Their genome expression strategy relies in the proteolytic cleavage of a large polyprotein mediated by specific viral proteases. A well-known and broadly exploited potyvirus-derived protease is the NIa-Pro from Tobacco etch virus (TEV).

In this work, we decided to design a simple system for screening other NIa-Pro proteases by combining in vivo visualization and molecular analysis. We started with the characterisation of NIa-Pro protease from the potyvirus Sweet potato feathery mottle virus (SPFMV). The viral gene product was cloned in a vector adequate for transient expression in leaves of *N. benthamiana* plants via agroinfiltration. Also, we generated a reporter construct containing the specific NIa-Pro cleavage sequence placed between an oleosin protein and the green fluorescent protein (GFP). Plant oleosins are known to target proteins to oil bodies as fusion proteins (Van Rooijen & Moloney, 1995; Montesinos et al., 2016). When agroinfiltrated alone, this reporter construct produced fluorescently labelled oil bodies, but when co-agroinfiltrated in the presence of the NIa-Pro construct, the cleavage of the reporter resulted in cytoplasmatic localization of the GFP fluorescence. This change was easily monitored using confocal laser microscopy. These observations were validated by Wester*N*-blot analysis of the produced intact or processed fusion proteins, confirming the visually detected activity of the NIa-Pro. Finally, the interest of this in vivo screening system, and the potential uses of the SPFMV NIa-Pro protease as a biotechnological tool in molecular farming will be discussed.

References

Montesinos Barreda, L., Bundó, M., Izquierdo, E., Campo, S., Badosa Romañó, E., ... Coca, M. 2016. Production of biologically active Cecropin A peptide in rice seed oil bodies. *PLoS One* 11: e0146919-e0146937.

Van Rooijen, G. J., Motoney, M. M. 1995. Plant seed oil-bodies as carriers for foreign proteins. *Nature Biotechnology* 13: 72.

Wylie, S. J., Adams, M., Chalam, C., Kreuze, J., López-Moya, J. J., Ohshima, K., ... Zerbini, F. M. 2017. ICTV virus taxonomy profile: Potyviridae. *Journal of General Virology* 98: 352-354.

30. Two reductases GRED1 and GRED2 may be involved in several polyketide biosynthetic pathways in Gerbera

Zhu L, Teeri TH

University of Helsinki, Finland

Corresponding author: Zhu, Lingping <lingping.zhu@helsinki.fi>

Polyketide derivatives are important secondary metabolites in plants with various bio-activities. Their biosynthesis is initiated by type III polyketide synthases (PKSs) catalyzing iterative decarboxylation condensation reactions, and then undergo a series of enzymatic reduction, cyclisation and aromatization reactions (Stewart Jr et al., 2013). In gerbera (Gerbera hybrida), the polyketide derivatives gerberin, parasorboside and 4-hydroxy-5-methylcoumarin (HMC) are highly produced in all tissues. Three gerbera specific PKSs have been identified accounting for synthesis of these compounds. Gerbera 2-pyrone synthase 1 (G2PS1) is responsible for the biosynthesis of gerberin and parasorboside (Eckermann et al., 1998) and G2PS2 and G2PS3 are involved in HMC biosynthesis (Pietiäinen et al., 2016). Two ancient anther-specific chalcone synthase like genes, GASCL1 and GASCL2, were also identified in gerbera. They encode the key PKSs in the biossyntheis of sporopollenin, the main component of the exine layer of mature pollen grains (Kontturi et al., 2017. Polyketide reductases are supposed to catalyze the reduction of intermediates in all of these polyketide biosynthetic pathways.

Coexpression analysis was conducted with transcriptomic data collected from a number of gerbera tissues and a tetraketide α-pyrone reductase 2 (TKPR2) like gene GRED1 was found highly co-expressed with G2PS1. Besides GRED1, there is one other gene, GRED2, belonging to the TKPR2 gene family in gerbera. TKPR2 (and TKPR1) are anther-specific genes involved in sporopollenin synthesis. However, the gerbera GRED1 and GRED2 are expressed almost everywhere. Gerbera GRED1 and GRED2 RNAi lines showed aborted pollen and significant reduction of HMC and parasorboside content was detected in GRED2 RNAi leaves and petals. Based on these observations, we speculate that GRED1 and GRED2 may be not only involved in sporopollenin biosynthesis but also catalyze the reduction steps in parasorboside and HMC biosynthesis in gerbera. In the near future, in vitro experiments will be done to provide more information.

References

Eckermann, S., Schröder, G., Schmidt, J., Strack, D., Edrada, R. A., ... Teeri, T. H. 1998. New pathway to polyketides in plants. *Nature* 396: 6709, 387.

Kontturi, J., Osama, R., Deng, X., Bashandy, H., Albert, V. A., Teeri, T. H. 2017. Functional characterization and expression of GASCL1 and GASCL2, two anther-specific chalcone synthase like enzymes from *Gerbera hybrida*. *Phytochemistry* 134: 38-45.

Pietiäinen, M., Kontturi, J., Paasela, T., Deng, X., Ainasoja, M., ... Teeri, T. H. 2016. Two polyketide synthases are necessary for 4-hydroxy-5-methylcoumarin biosynthesis in *Gerbera hybrida*. *The Plant Journal* 87: 548-558.

Stewart Jr, C., Vickery, C. R., Burkart, M. D., Noel, J. P. 2013. Confluence of structural and chemical biology: plant polyketide synthases as biocatalysts for a bio-based future. *Current Opinion in Plant Biology*, 16: 365-372.

31. Catch me if you can – How plant virus nanoparticles spy on functional molecules

Röder J, Commandeur U.

RWTH Aachen University, Aachen, Germany

Corresponding author: Juliane Röder <juliane.roeder@molbiotech.rwth-aachen.de>

In nature, cellulose is degraded by a combination of synergistically acting enzymes, which can be secreted or anchored in the cellulosomes of anaerobic microorganisms. Recently this multi-enzyme structure has served as a model for designer cellulosomes, mimicking the natural spatial organization of synergistically acting enzymes for technological purposes. In this respect, positional control is necessary for effective biomass degradation and can be achieved by immobilizing enzymes onto reusable artificial scaffolds to improve their stability and activity (Artzi et al., 2017). However, recombinant scaffolds are usually expressed from long open reading frames, providing multiple possibilities for the selective attachment of matching enzymes, and this can result in low expression levels and unstable mRNA (Feng & Niu, 2007). This disadvantage can be circumvented by using molecular farming to produce high yields of identical coat protein (CP) subunits assembled into plant virus nanoparticles (VNPs). Plant viruses feature a precisely ordered 3D structure, but examples of catalyst-decorated nanoparticles are rare. The functionality of VNPs can be tailored by chemical conjugation to exposed amino acid residues, genetic engineering or a combination of both (Le & Hu, 2017). However, these strategies suffer from limitations, most notably the genomic instability of large inserts, the need for certain amino acids as conjugation sites, and the usually poor overall coupling efficiency or fusion protein yield. A convincing solution would be a protein tag on the virus surface that can be generally applied for the attachment of different target proteins.

Because covalent binding strategies usually achieve more efficient functionalization than no*N*-covalent protein interactions, we used the *Streptococcus pyogenes* SpyTag/SpyCatcher (ST/SC) system. Using this system, we achieved the stable and rapid immobilization of whole enzymes onto Potato virus X (PVX) and Tobacco mosaic virus (TMV) scaffolds without increasing the genetic load, as demonstrated by the attachment of Trichoderma reesei endoglucanase Cel12A. PVX and TMV not only differ in their flexibility, but also in the helical arrangement of their CPs, which influences the catalytic activity of the displayed enzymes. The activity of Cel12A increased ~2.5-fold when it was immobilized on PVX rather than TMV particles, whereas more endoglucanase molecules were concentrated on the shorter TMV scaffold. PVX-ST and, when other functions are desired, also TMV-ST nanoparticles are universally applicable platforms allowing the dense and stable presentation of target peptides that usually require post-translational modifications.

Artzi, L., Bayer, E. A., Moraïs, S. 2017. Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides. *Nature Reviews Microbiology* 15: 83.

Feng, L., Niu, D. K. 2007. Relationship between mRNA stability and length: an old question with a new twist. *Biochemical Genetics* 45: 131-137.

Le, D. H., Hu, H., Commandeur, U., Steinmetz, N. F. 2017. Chemical addressability of potato virus X for its applications in bio/nanotechnology. *Journal of Structural Biology* 200: 360-368.

32. Future pharma: A Phase I dose escalation trial to evaluate the safety of single IV administration of P2G12 in healthy volunteers

Bartolf A, Cosgrove C, Ma JKC

St George's University of London

Corresponding author: Angela Bartolf <abartolf@sgul.ac.uk>

Despite progress in the development of quality of care and treatments to prolong life for those infected, HIV continues to be pandemic It is likely that a range of new strategies will be needed simultaneously to bring the epidemic under control and a HIV neutralising antibody could play an important role in post exposure including mothers in labour.

P2G12 is a human IgG1 class monoclonal antibody (MAb) which has been expressed in tobacco plants. The parent molecule is one of a handful of MAbs with potent neutralising activity against HIV-1 and a variant has demonstrated protection against SHIV challenge in macaques. It was also safe in repeated intravenous infusions (IVIs) in HIV-infected humans. The P2G12 has not raised any safety concerns in completed rat and human studies. Phase I, dose escalation in 3 ascending dose cohorts of 18 healthy subjects aged 18-45 years. This will be the first formal Phase I study in Europe, of a GMP compliant plant-derived MAb delivered by IVI.

The aim of this study was to establish the safety of P2G12 administered as IVI by assessing > grade 3 reactions. Secondary objectives will look at the concentration of P2G12 in the serum and any grade events post-IV. P2G12 in saline will be infused IV. Following no observed effects after 10 minutes at slow rate, this will be increased to complete the infusion in 30 minutes. There will be a delay of >4 hours between volunteer doses and escalation to the higher dose will not occur until 2 week safety data of the last volunteer.

Participants will undergo a health screening to assess eligibility to the inclusion/exclusion criteria prior to drug administration. Frequent medical monitoring (new medical concerns, routine laboratory parameters, ECG and vital signs) will continue up to 12 weeks post-IV. Serum immunogenicity and the associated plant-glycans will be measured at 11 time points using a specific ELISA and pharmacokinetic (PK) analysis will be completed. PK results will be compared with those from rats to determine if the rat model can be used to predict PK of plant antibodies. Favourable safety and immunogenicity results will be needed prior to a trial of P2G12 infusion in people who live with HIV infection.

33. Expression and characterization of milk-clotting cyprosin B from *Cynara cardunculus* and function of its plant specific insert in *Nicotiana benthamiana*

Kanagarajan S[1,2], Lundgren A[2], Kim SY[1], Zhu LH[1], Brodelius P[2]

[1]*Swedish University of Agricultural Sciences, Alnarp, Sweden*
[2]*Linnaeus University, Kalmar, Sweden*

Corresponding author: Selvaraju Kanagarajan <selvaraju.kanagarajan@slu.se>

The aqueous flower extracts of *Cynara cardunculus* are traditionally used for cheese-making in Mediterranean countries. The lack of availability of cardoon flowers in the region limits its production at industrial level. Hence, a study was undertaken to heterologously produce milk clotting enzyme, cyprosin (*CYPB*), a major component in the flowers of *C. cardunculus* and to confirm the role of their plant-specific insert (PSI) in the activity and localization of *CYPB* in *N. benthamiana*. In our study, *CYPB* and PSI deleted *CYPB (CYPBΔPSI*) genes were transiently expressed in *N. benthamiana*. Heterologously produced proteins were extracted from 9 days post infiltration and purified, which produced the yield of 80 mg kg-1 (*CYPB*) and 60 mg kg-1 (*CYPBΔPSI*) fresh weight of leaves, thus making it possible for large-scale industrial endeavors. The proteolytic activity of purified recombinant *CYPBΔPSI* was determined as 18.7 U, which was almost three times higher than the activity of *CYPB* (6.4 U). This suggests that deletion of PSI is not directly related to the activity of *CYPB*. Milk-clotting assay of purified *CYPB* and *CYPBΔPSI* showed that the time required to clot the milk was almost similar to that of wild type protein extracted from flowers of cardoon. To determine the significance of PSI in localization of CYPB, cDNAs of CYPB, *CYPBΔPSI* and PSI were fused with enhanced green florescent protein and transiently expressed in *N. benthamiana*. Subcellular localization analyses indicated that CYPB destined to vacuole, while *CYPBΔPSI* was trafficked in endoplasmic reticulum (ER) and vacuolar membrane i.e. tonoplast and PSI was observed in both ER and vacuole which suggests that *CYPB* with or without PSI was targeted into vacuoles. This indicated that the major function of PSI is vacuolar membrane permeabilization and absence of PSI (*CYPBΔPSI*) ended in tonoplast exemplifies existence of some vacuolar targeting signals in the regions of CYPB as well as PSI to target the vacuole.

34. Carnivorous plants as a potential new drug-producing system

Bonitz T, Kolano R, Fester K

University of Leipzig, Leipzig, Germany

Corresponding author: Tobias Bonitz <tobias.bonitz@uni-leipzig.de>

The digestive fluid of carnivorous plants contains a wide variety of well-defined hydrolytic enzymes. This ability to produce, secrete and transport substances into special compartments of the plant could be used to produce pharmaceutical relevant substances, e.g. recombinant proteins. Furthermore, by interfering signal transduction pathways we can gather information about the regulation of enzyme secretion in carnivorous plants. Here we present the transformation (Hirsikorpii et al., 2002) of several genera of carnivorous plants with the marker protein GFP (green fluorescent protein) using different transformational methods.

Reference

Hirsikorpi, M., Kämäräinen, T., Teeri, T., Hohtola, A. 2002. *Agrobacterium*-mediated transformation of round leaved sundew (*Drosera rotundifolia L.*). *Plant Science* 162: 537-542.

35. *N*-glycosylation pattern of secretomes of *Chlorella* species

Kim S-R[1], Choi J[1], Jeon JM[1], An HJ[2], Oh MJ[2]

[1]*Sogang University, Seoul, Korea*
[2]*Chungnam National University, Daejeon 34134, Korea*

Corresponding author: 김성룡 <sungkim@sogang.ac.kr>

Chlorella, unicellular green algae, has been spotlighted not only as a model organism to help study biological questions but also as a cell factory for various metabolites, biofuels and biopharmaceuticals. For using *Chlorella* as a biopharmaceutical source, it is required to understand *N*-glycosylation pattern of proteins. To this end, we have analyzed the *N*-glycosylation of secretomes from *C. vulgaris* UTEX395 and an arctic *Chlorella* species Arm0029B. By using ultra-high resolution 15T FT-ICR mass spectrometry, we found 427 and 53 proteins from the culture of UTEX395 and Arm0029B, respectively. Among the 427, 128 were predicted as secreted proteins. MS analysis of the secretome showed that a major form of *N*-glycan in UTEX395 was Man8, whereas that in Arm0029B was Man4_GluNAc2_Fucose1_Xylose1. The *N*-glycosylation pattern in both UTEX 395 and Arm0029B showed plant specific methylation. We believe that the findings in this study can extend our knowledge about the signaling and defense systems in *Chlorella*. Furthermore, it would contribute to the development of future biopharmaceutical production

36. Tissue specificity and genomic organization of bitter sesquiterpene lactone biosynthesis in *Cichorium intybus L.*

Cankar K[1], Bogdanovic M[2], Todorovic S[2], Dragicevic M[2], Simonovic A[2], van Houwelingen A[1], Schijlen E[1], Schipper B[1], Gagneul D[3], Hendriks T[3], Quillet MC[3], Bouwmeester H[4], Bosch D[1], Beekwilder J[1]

[1]*Wageningen Plant Research, Wageningen, The Netherlands*
[2]*University of Belgrade, Belgrade, Serbia*
[3]*Université Lille Nord de France, Villeneuve d'Ascq, France*
[4]*Wageningen University, Wageningen, The Netherlands*

Corresponding author: Katarina Cankar <katarina.cankar@wur.nl>

Chicory (*Cichorium intybus L.)* produces bitter sesquiterpene lactones (STLs). STLs form a class of compounds that predominantly occur in Asteraceae species. STLs have a variety of bioactivities, ranging from allelopathic activity to protective activity towards herbivorous insects in roots and flowers. In chicory, STLs provide bitterness, which gives the typical flavour to the belgian endive, and their presence in roots has been deployed to convert chicory root into a coffee substitute. STLs have a number of health associated properties, such as antimicrobial activity, and are used as chemotherapeutic agents. The initial dedicated enzymes in the biosynthetic pathway leading to costunolide have been characterized. However, the genetic organization and tissue specificity of their biosynthesis is largely unknown. Concentration of two sesquiterpene lactones in different chicory tissues was correlated to expression of genes involved in the first dedicated biosynthetic steps. BAC clones encoding different paralogs of the germacrene A synthase and germacrene A oxidase were sequenced, and revealed several tightly linked copies of these paralogs. Promoters of two germacrene A synthases and a germacrene A oxidase were fused to GFP and expressed in plants regenerated from chicory hairy root cultures. Highest gene expression was observed in epidermal tissues of leaf and root. This work opens the possibility to select for chicory germplasm diversified in STL content, and to study their role in chicory in defence and physiology.

37. Assessment of lectin microarray-based carbohydrate profiling for proteins transiently expressed in plants

Gerlach J[1], Steele J[2], Cunningham S[1], Peyret H[2], Lomonossoff G[2], Joshi, L[1]

[1]*National University of Ireland, Galway, Ireland*
[2]*John Innes Centre, Norwich Research Park, UK*

Corresponding author: Jared Gerlach <jared.gerlach@nuigalway.ie>

Proteins which require mammalia*N*-type glycosylation for proper folding or efficacy present additional hurdles for plant production systems. This is largely due to the potential for incomplete and/or immunogenic oligosaccharide structures (no*N*-mammalian or no*N*-human residues and configurations) to be added to proteins of pharmaceutical interest by expression host plants. Assessment of oligosaccharides attached to proteins is normally performed with chromatographic or chromatography-hyphenated spectroscopic methods (e.g. LC-MS) which are time consuming, expensive and often specialist in nature. In this work, we have explored the use of a lectin microarray approach, coupled with carbohydrate-specific enzymatic treatment, to rapidly assess the structures attached to proteins ectopically-expressed within the leaves of *Nicotiana benthamiana*. A proprietary lectin microarray consisting of fifty lectins sourced from plant, fungal and bacterial sources was used to analyse transiently expressed proteins after fluorescent labeling. Signatures for untreated recombinants were compared to signatures for proteins treated with glyco-active enzymes. Evidence of the contribution of typical plant substituents was observed through the selective release of structures. This approach is relatively inexpensive and provides a means of rapidly validating the glycosylation profiles of transiently expressed, high value proteins for end-line human biomedical and health applications.

38. Not shown

39. Tobacco BY-2 - a highly economic production host for recombinant proteins

Häkkinen ST, Reuter L, Nuorti N, Joensuu JJ, Rischer H, Ritala A

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Suvi Häkkinen <suvi.hakkinen@vtt.fi>

Plant cell culture technology is a fascinating tool for various biotechnological applications offering possibilities for optimizing production, independently of climatic or environmental effects. However, often encountered hindrance in using plant cells in biotechnological platforms is the economic burden, i.e. the contained cultivation of plant cells is expensive mainly due to the specialized medium requirements and low multiplication rate of the cells. Tobacco BY-2 (Nicotiana tabacum cv. 'Bright Yellow') cell line is highly synchronisable and thus desirable for investigation of various aspects of plant cell biology and metabolism. Rapid accumulation of biomass and easy transformation has made the BY-2 cells the most frequently used plant cell line for protein production. However, when it comes to industrial production two factors must be specifically ensured: stability of the production host and the cost efficiency of the production process. The aim of this work was to identify most cost-relevant nutrients in the culture medium and to determine culture medium compositions resulting in cheaper production of either biomass and/or recombinant protein.

A cost estimation was performed to identify the most expensive nutrients in the culture medium. This involved the evaluation of costs of macro- and microsalts, vitamins and plant hormones. Price evaluation of the culture medium was performed based on the list prices of the nutrients provided by SigmaAldrich. No other factors such as production costs were included in the evaluation. Evaluation resulted in altogether 7 compounds, which were selected as studied factors based on their final cost and relevance. The screening of the factors was performed with BY-2 clone 'Hulk' against biomass production and GFP-HFBI accumulation using DoE1.

The most promising medium composition in respect of biomass production was subjected to more detailed examination, i.e. the cost of the selected media were compared and for the most cost-efficient media, components were reduced from 100 % to 50 % levels in 10 % intervals. With the optimized culture medium, 43-55 % cost reduction in regards to biomass and up to 69 % reduction in regards to recombinant protein production was achieved. In order to determine the whole cost structure of the production of plant-based protein, TEA or LCA should be conducted with actual desired recombinant protein of commercial interest. However, this study shows that significant cost savings can be achieved with reducing the consumption of most expensive components in the culture medium.

Häkkinen, S. T., Reuter, L., Nuorti, N., Joensuu, J. J., Rischer, H., Ritala, A. M. 2018. Tobacco BY-2 media component optimization for a cost-efficient recombinant protein production. *Frontiers in Plant Science* 9: 45.

40. Plant-derived fusio*N*-protein based affinity ligands as an alternative to mAb purification using Protein A

Buyel JF[1,2], Rühl C[1], Knödler M[2]

[1]*RWTH Aachen University, Aachen, Germany*
[2]*Fraunhofer IME, Aachen, Germany*

Corresponding author: Johannes Buyel <johannes.buyel@ime.fraunhofer.de>

Monoclonal antibodies (mAbs) are currently the dominating type of proteinaceous biopharmaceuticals on the market and well-established production routines are available for their manufacture. In most cases the purification of mAbs starts with, or at least incorporates at some point, a Protein A affinity step, which has resulted in substantial improvements of the ligand. However, resin prices are still high and can be a major cost factor during production. Several alternative ligands have therefore been developed but often lack the selectivity/specificity of Protein A. Here we report the development of a fusio*N*-protein affinity ligand consisting of a base scaffold fused to the linear epitope of a target mAb. This will facilitate a rapid adaptation of the method to other mAbs with linear epitopes in the future. We cost-effectively produced the ligand in plants, one-step purified it and used a design of experiments approach to optimize the coupling to a sepharose base-resin. The resulting affinity resin was used to capture the epitope-specific mAb from another crude plant extract in a column format. We screened and optimized the elution conditions and evaluated the dynamic binding capacity as well as ligand stability for comparison with Protein A.

41. Production of an anti-Human Immunodeficiency Virus antibody in the barley endosperm

Hensel G[1], Floss DM[1,2], Rutten T[1], Conrad U[1], Kumlehn J[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany
[2]Heinrich-Heine-University, Düsseldorf, Germany

Corresponding author: Goetz Hensel <hensel@ipk-gatersleben.de>

Molecular farming is a useful option for the production of valuable recombinant proteins. As a major prerequisite, highly efficient and cost effective expression systems are required. The cereal grain is an attractive vehicle for producing recombinant proteins, because its evolution as a protein storage organ assures an environment in which proteins accumulate to high abundance and remain stable over long periods without any need for refrigeration or aseptic conditions. To establish efficient recombinant protein accumulation in the barley endosperm, the reporter gene Gfp was expressed under control of the oat GLOBULIN1 promoter. Transgenic barley grain accumulated up to 1.2 g/kg dry weight of recombinant protein (GFP), deposited in small roundish compartments assumed to be ER-derived protein bodies. The molecular farming potential of the system was tested by generating doubled haploid transgenic lines engineered to synthesize the anti-Human Immunodeficiency Virus antibody 2G12, which is a well-studied model and had been used in many different expression systems including plants. The 2G12 antibody was produced in a functional and soluble form and its level of accumulation exceeded those achieved in transgenic tobacco, maize and rice.

42. Construction of a Zera®-adjuvanted Human papillomavirus E7-based DNA vaccine delivered in plant-made HPV pseudovirions

Chabeda A[1], van Zyl AR[1], Hendrikse M[1], Öhlschläger P[2], Moravec T[3], Rybicki EP[1], Hitzeroth II[1]

[1]*University of Cape Town, Rondebosch, South Africa*
[2]*Aachen University of Applied Sciences, Aachen, Germany*
[3]*Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*

Corresponding author: Aleyo Chabeda <aleyo.chabeda@gmail.com>

Human papillomavirus (HPV) infects mucosal and cutaneous basal epithelial cells after tissue microtrauma. Most infections are cleared by the immune system; however, some benign cervical lesions progress to invasive cervical cancer, caused predominantly by high-risk HPVs. There are currently 3 licensed vaccines on the market, but these vaccines have limitations due to their type-restricted prophylactic efficacy, a lack of therapeutic efficacy and the high costs associated with production. Plant-based expression has significant potential to facilitate reduction in vaccine costs due to lower cost of goods, and its rapid scalability in comparison to other systems. The HPV E6 and E7 early gene products are ideal targets for use as therapeutic vaccines due to their role in disruption of the cell cycle and their constitutive expression in premalignant and malignant tissues.

To address the lack of therapeutic efficacy of current vaccines, we aimed to develop a novel E7-based DNA vaccine delivered by plant-made HPV pseudovirions (PsVs). A geminivirus-derived self-replicating plasmid encoding a shuffled E7 (E7SH) sequence that has no transformation ability but contains natural cytotoxic T-lymphocyte epitopes, was constructed using GoldenBraid technology and co-expressed in plants with HPV-16 or HPV-35 L1- and L2- encoding expression vectors. GoldenBraid assembly was successful and the pseudogenome was encapsidated into HPV-16 and -35 plant-made PsVs. HEK 293TT cells were transfected with the PsVs to examine potential E7SH expression. The PsVs were capable of infecting mammalian cells resulting in E7SH expression. This shows the promise for use of plant-made HPV PsVs to encapsidate and deliver candidate therapeutic DNA vaccines for HPV infections and HPV-associated lesions as well as for other applications.

43. Cloning and functional characterization of glycosyltransferases from *Nicotiana benthamiana*

Sun G, Härtl K, Schwab W

Biotechnology of Natural Products, Freising, Germany

Corresponding author: Guangxin Sun <sgx_here@hotmail.com>

Glycosylations have a major impact on the physicochemical properties of bioactive natural products. They increase the stability of labile small molecules; decrease the toxicity of potential hazardous compounds, and influence the bioactivity of pharmaceuticals. Hence, targeted glycosylation of acceptor molecules is of high scientific significance. However, regio- and stereoselective chemical synthesis is challenging and expensive. A promising approach is the enzymatic glycosylation via glycosyltransferases (GTs). They catalyze the transfer of a sugar moiety from an activated donor, usually UDP-glucose, to a broad range of acceptor molecules. Thus, GTs are a powerful tool for the biotechnological glycosylation of bioactive natural products. Investigations on novel GTs from new plant sources will enable the modification of a diverse range of value chemicals currently applied in the food and cosmetics industry.

Therefore, we identified and functionally characterized GTs from tobacco (Nicotiana benthamiana). A substrate screening and kinetic analysis were performed, and the enzyme specificities determined. In doing so, we uncovered novel enzymatic activities towards ionols. Overexpression of the GTs in tobacco plants verified the in vivo activity. Furthermore, we employed whole cell biotransformation to produce these interesting glucosides on a large scale to test them in various technical applications.

44. *N*-Glycoproteomics of the Human Cytomegalovirus glycoprotein B produced in *Nicotiana tabacum* BY-2 suspension cells

Navarre C[1], Smargiasso N[2], Nader J[1], Mercx S[1], De Pauw E[2], Boutry M[1], Chaumont F[1]

[1]*Université de Louvain, Louvain, Belgium*
[2]*Université de Liège, Liége, Belgium*

Corresponding author: Catherine Navarre <catherine.navarre@uclouvain.be>

Human Cytomegalovirus (HMCV) represents a major cause of congenital disease when infecting the fetus. Glycoprotein B (gB), which is a crucial viral membrane protein involved in host cell fusion, contains 18 potential *N*-glycosylation sites. The ability to characterize and control the glycosylation pattern of recombinant gB represents a major goal before its use as a vaccine. The aim of our study consisted of obtaining plant cell lines that express gB and of evaluating its glycosylation profile. We expressed the large soluble ectodomain of HCMV gB in *Nicotiana tabacum* BY-2 cells. gB was secreted in the culture medium at a concentration of 60 mg/L. The protein was purified directly from the culture medium by ammonium sulfate precipitation and size exclusion chromatography. We analyzed the glycosylation status of the gB protein produced in three independent transgenic BY-2 lines. First, the occupancy rate of each *N*-site was determined. We then identified and quantified the *N*-glycans present on 17 out of the 18 *N*-sites by multienzymatic proteolysis and mass spectrometry (LC-MS/MS). Interestingly, the glycosylation profile differed on each site, some sites being occupied exclusively by oligomannose type *N*-glycans and others by complex bi-antennary *N*-glycans processed in some cases with additional Lewis-a structures. These data were compared to those obtained in parallel for the same HCMV gB variant produced in CHO cells. Apart from the typical plant-specific *N*-glycans, the profiles were strikingly similar between BY-2 and CHO-produced gB, indicating that, overall, the gB conformational structure is conserved when expressed in plant cells and that this structure influences the glycosylation profile at each site. However, the processed *N*-sites displayed much more heterogeneity in CHO cells with the presence of bi-, tri- and tetra-antennary structures that can be further elongated with sialic acids

This work was supported by a grant from the Service Public de Wallonie in Belgium (WBHealth 1318062).

45. Development of *Nicotiana benthamiana* lines with increased biomass production

Kopertekh L, Schiemann J.

Julius Kühn-Institut, Quedlinburg, Germany

Corresponding author: Kopertekh, Lilya <lilya.kopertekh@julius-kuehn.de>

Two main approaches are available to produce recombinant proteins in plants: stable transformation and transient expression. In recent years the transient expression technology has made rapid and impressive progress. High level of target protein expression, speed and scalability, reduced environmental concerns due to the production in contained facilities are major advantages of this method (Lomonossoff & D'Aoust, 2016). Transient expression is a two-component system consisting of expression vector and host plant. Therefore, both factors - expression vector used and host genome modification - might have a positive impact on quality and quantity of plant-produced recombinant proteins. *Nicotiana benthamiana* is a favourite host for transient expression because of its high susceptibility to viral and agrobacterial infection and fast growth rate under indoor conditions.

This study was aimed at developing *N. benthamiana* lines with increased biomass production, with a view to reduce the operation costs for plant host manufacturing at a large scale. To this end we generated transgenic *N. benthamiana* plants harbouring the CycD2 gene from Arabidopsis thaliana. The CycD2 gene is a positive regulator of the cell cycle and is known to influence the transmission from G1 phase to S phase (Cockcroft et al., 2000). The T1 progeny of CycD2 transgenic plants was evaluated in the greenhouse for above-ground biomass accumulation. We could select several lines with boosted biomass production that were morphologically indistinguishable from wild type plants. To check their suitability for foreign protein expression we agroinfiltrated these lines with a TMV-based vector carrying a gfp gene. The accumulation level of GFP protein in CycD2 transgenic plants was comparable to that in no*N*-transformed plants suggesting suitability of these lines for breeding new *N. benthamiana* genotypes with increased biomass production for molecular farming.

References

Cockcroft, C.E., den Boer, B.G.W., Healy, J.M.S., Murray, J.A.H. 2000. Cyclin D control of growth rate in plants. *Nature* 405: 575-579.

Lomonossoff, G.P., D'Aoust, M.A. 2016. Plant-produced biopharmaceuticals: A case of technical developments driving clinical deployment. *Science* 353: 1237-1240.

46. Production and characterization of glyco-engineered anti-CD20 tumour-targeting scFv-Fc antibodies and interleuki*N*-2 conjugates in whole plants and hairy root cultures of *N. benthamiana*

Donini M, Marusic C, Lonoce C, Pioli C, Novelli F, Benvenuto E

ENEA Research Center, Rome, Italy

Corresponding author: Marcello Donini <marcello.donini@enea.it>

Anti-CD20 recombinant antibodies are successfully used for the treatment of B-cell malignancies such as no*N*-Hodgkin lymphomas. We demonstrated that recombinant anti-CD20 scFv-Fc antibodies derived from C2B8 mAb (rituximab), can be efficiently produced in *Nicotiana benthamiana* leaves by agroinfiltration and that an immunocytokine (IC) obtained by fusing the scFv-Fc to the human interleukin 2 (hIL-2) showed a CD20 binding activity comparable to that of rituximab and was efficient in eliciting antibody-dependent cell-mediated cytotoxicity (ADCC). Moreover, the IC devoid of the typical xylose/fucose *N*-glycosylation plant signature(IC-ΔXF) and the corresponding scFv-Fc- ΔXF antibody not fused to the cytokine, were obtained in a glyco-engineered ΔXylT/FucT *N. benthamiana* line. Purification yields from agroinfiltrated plants amounted to 20-35 mg/Kg of leaf fresh weight. When assayed for interaction with FcγRI and FcγRIIIa, IC- ΔXF exhibited significantly enhanced binding affinities if compared to the counterpart bearing the typical plant protein *N*-glycosylation profile and to rituximab. The glyco-engineered recombinant molecules also exhibited a strongly improved ADCC and complement-dependent cytotoxicity (CDC). Notably, our results demonstrate a reduced C1q binding of xylose/fucose carrying antibodies compared to versions that lack these sugar moieties.

In the attempt to find an alternative production system for the anti-CD20 antibodies, we expressed these molecules in hairy root cultures (HR). HR cultures represent an attractive platform for the production of heterologous proteins due to the possibility of secreting the molecule of interest into the medium. The aim of our study was to enhance the accumulation of the scFv-Fc with huma*N*-compatible glycosylation profile in HR culture medium. To this purpose we engineered *N. benthamiana* HR cultures expressing the red fluorescent protein (RFP) as a model for an easy screening of different auxins able to induce heterologous protein secretion into the medium. The 2,4-dichlorophenoxyacetic acid (2,4-D) was found to induce remarkable accumulation levels of RFP in the medium. The same protocol was applied for the tumor-targeting antibody from glyco-engineered ΔXTFT *N. benthamiana* HR cultures. Addition of 2,4-D determined an increased antibody accumulation up to ~16 mg/L while *N*-glycosylation profile revealed GnGn structures devoid of xylose and fucose residues. To our knowledge, this is the first example of the expression at high levels in HR of an engineered anti-CD20 antibody in a scFv-Fc format, paving the way towards organ cultures as contained production platform of recombinant biopharmaceuticals with a suitable glycosylation profile.

Marusic, C., Novelli, F., Salzano, A. M., Scaloni, A., Benvenuto, E., Pioli, C., Donini, M. 2016. Production of an active anti‐CD20‐hIL‐2 immunocytokine in *Nicotiana benthamiana. Plant Biotechnology Journal* 14: 240-251.

Marusic, C., Pioli, C., Stelter, S., Novelli, F., Lonoce, C., ...Donini, M. 2018. *N*‐glycan engineering of a plant‐produced anti‐CD20‐hIL‐2 immunocytokine significantly enhances its effector functions. *Biotechnology and Bioengineering* 115: 565-576.

47. Plant expression of a single chain variable region antibody fragment against Human papillomavirus L1 capsid protein

Meggersee RL, Rybicki EP, Meyers AE, Hitzeroth II

University of Cape Town, Rondebosch, South Africa

Corresponding author: Rosemary Meggersee <rosemary.meggersee@gmail.com>

Human papillomaviruses (HPV) are known to infect the genital epithelium and can produce lesions which can progress to invasive cervical cancer. More than 200 HPV types have been characterised based on their genome sequences, at least 12 of which cause cervical cancer. Cervical cancers are the 4th most common cancer in women and in 2012, an estimated 527,624 cases of HPV induced cervical cancer arose annually, of which 50.3% were fatal. In current diagnostic settings HPV serology is used to identify current and past-exposed individuals; however the balance between sensitivity and specificity of these assays are a problem. Many monoclonal antibodies have been developed against different HPV types to identify and characterize papillomavirus neutralizing epitopes, however these antibodies are often not commercially available, require the use of mice for production and are tedious and very expensive to make. Phage display represents an alternative approach to monoclonal antibody isolation. In this study anti-HPV L1 antibodies were generated from a mouse phage display library and bio-panned against HPV-16 L1 protein. The highest affinity binders were sequenced and cloned into a plant expression vector. The proteins were expressed in *Nicotiana benthamiana* plants and purified using nickel affinity chromatography. These single chain variable region antibody fragments showed specificity in binding and were able to detect HPV L1 in ELISA and western blot tests.

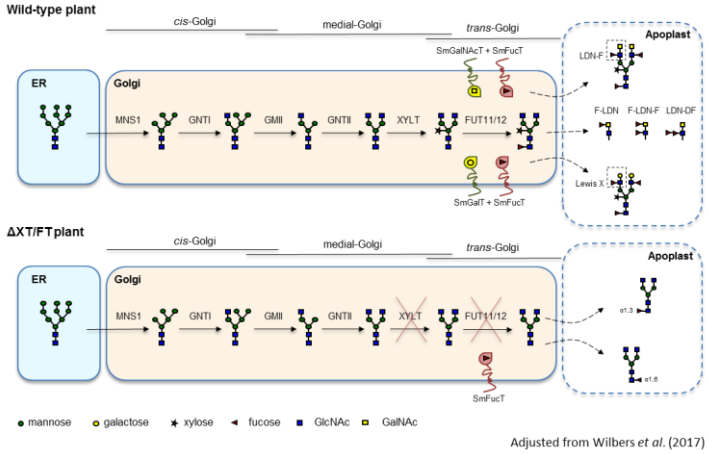
48. Characterisation of *Schistosoma mansoni* fucosyltransferases for glyco-engineering of 'native' helminth *N*-glycan structures *in planta*

van Noort K[1] Nguyen DL[2], Hokke CH[2], Schots A[1], Wilbers R[1]

[1]*Wageningen University & Research, Wageningen, The Netherlands*
[2]*Leiden University Medical Centre, Leiden, The Netherlands*

Corresponding author: Kim van Noort <kim.vannoort@wur.nl>

Clinical trials with live parasites and mouse model studies have shown the potential of helminths and their excretory/secretory (ES) proteins to dampen allergic reactions and autoimmune disorders. Moreover, glyca*N*-dependent mechanisms have been shown to be involved in several cases. To further develop helminth-derived ES glycoproteins as biopharmaceuticals, a large-scale expression system is required for the production of recombinant glycoproteins with defined and tailored glycosylation. The trematode Schistosoma mansoni produces highly fucosylated *N*-glycan structures on its glycoproteins, which cannot be synthesized in current production systems. Thereto, co-expression of specific fucosyltransferases in the expression host are required to introduce helminth-like *N*-glycan modifications. In the GeneDB database 20 different S. mansoni fucosyltransferase (SmFucTs) genes for *N*-glycosylation can be found. To date one α1,3 fucosyltransferase is characterised in vitro using glycan acceptors and shows to synthesise Lewis X. Thereto, we examined the function of ten selected SmFucTs by transient co-expression with model proteins in *Nicotiana benthamiana* plants. With this method we have identified SmFucTs that fucosylate LDN (forming LD*N*-F, F-LDN and F-LD*N*-F), synthesise Lewis X or are involved in α1,3 or α1,6 core fucosylation. These functionally characterised fucosyltransferases can immediately be applied to synthesise desired helminth-like *N*-glycan structures on recombinant glycoproteins in plants. Therefore, characterisation of SmFucTs, other glycosyltransferases and combinations of different glycosyltransferases expands our glyco-engineering toolbox and offers perspectives for large scale production of glycoproteins with functional helminth *N*-glycan structures in plants.



The authors kindly acknowledge the collaboration with Herta Steinkellner, Richard Strasser and Andreas Faissner.

49. Working towards a plant-produced Bovine papillomavirus vaccine: Expression of BPV1 virus-like particles and pseudovirions in plants

Hitzeroth II, Pietersen I, van Zyl AR, Rybicki EP

University of Cape Town, Rondebosch, South Africa

Corresponding author: Inga Hitzeroth <inga.hitzeroth@uct.ac.za>

Bovine papillomaviruses (BPV) are small DNA viruses implicated in several diseases and cancers of considerable veterinary and agricultural importance in cattle, horses and several wild animal species, leading to their economic depreciation and deterioration of their function and appearance. As BPV is widespread, easily transmissible, and infection often occurs asymptomatically, it is imperative that routine vaccinations are carried out to mitigate the threat of outbreaks occurring in livestock. There is a demand for prophylactic vaccines to immunise animals routinely and from a young age so as to prevent infection from occurring, as there is currently no fully effective treatment against established disease. Prophylactic VLP-based vaccines against BPV types 1, 2, and 4 have previously been produced in cultured animal cells. However, these are largely type-specific, expensive, and not widely available in developing countries, where food security and the use of cattle for meat, dairy, leather products, as well as draft animals, is often essential for survival.

Bovine papillomavirus has often been used as a model for the study of human (HPV) and other papillomaviruses (PV), and the use of a plant-produced BPV vaccine may set a platform for the development and testing of an HPV vaccine in an animal model. To address the lack of a cheap and readily-available prophylactic vaccine, we aimed to develop a plant-made VLP-based vaccine using the L1 and L2 capsid proteins of BPV1, the most prevalent, and one of the most virulent BPV types. We successfully expressed relatively high yields of both L1 and L1/L2 VLPs in N. benthamiana. Furthermore, we managed to encapsidate self-replicating plant geminivirus-derived reporter-gene plasmids into the L1/L2 capsids, demonstrating the first successful production of BPV pseudovirions (PsVs) in plants. These particles were also shown to infect and express the reporter gene in mammalian HEK293TT cell cultures. Particles of different sizes, probably corresponding to T=1 VLPs (~20-30nm) and T=7 PsVs (~50-60nm), were obtained in the production of PsVs. We were able to concentrate particles of different sizes by use of density gradient centrifugation, which is useful for separating the particles to study their functions, and in their use for different applications.

These findings highlight the potential use of BPV PsVs for a diverse range of functions, including their use as dual prophylactic-therapeutic vaccines capable of delivering therapeutics or DNA vaccines to specific tissues.

50. Discovery and biopharming applications of cyclotides as plant-produced pharmaceuticals

Craik DJ, Smithies B, Oguis QK, Qu H, Gilding EK, Jackson MA

The University of Queensland, Brisbane, Australia

Corresponding author: David Craik <d.craik@imb.uq.edu.au>

Naturally occurring peptides offer great potential as leads for drug design. This presentation will focus on a class of cyclic peptides from plants known as cyclotides. Cyclotides are topologically unique in that they have a head-to-tail cyclised peptide backbone and a cystine knotted arrangement of disulfide bonds. This makes them exceptionally stable to chemical, thermal or enzymatic treatments and, indeed, they are amongst nature's most stable proteins. Because of their exceptional stability and well-defined structures cyclotides make excellent templates for drug design applications. This presentation will give an overview of the discovery, distribution and evolution of cyclotides and will describe recent applications of cyclotides and related peptides in the design of drugs for cancer (Huang et al., 2015), pain (Castro et al., 2018) and thrombosis (Swedberg et al., 2016). Natural cyclotide-producer plants make cyclotides in high yield (~1g/kg fresh plant weight). Having designed therapeutically active cyclotides, we are now using crop plants to produce them. We describe the use of tobacco, *Arabidopsis* and petunia for the biopharming of designer cyclotides. We have established the Clive and Vera Ramaciotti Facility for the Production of Therapeutic Drugs in Plants to do this work. Cyclotides fill a gap in the pharmaceutical industry between 'small molecule' drugs and 'biologics' (antibodies and growth factors). Because they are naturally produced by plants cyclotides are ideal biopharming scaffolds.

References

Castro, J., Grundy, L., Deiteren, A., Harrington, A., O'Donnell, T., … Brierley, S. 2018. Cyclic analogues of α-conotoxin Vc1.1 inhibit colonic nociceptors and provide analgesia in a mouse model of chronic abdominal pain. *British Journal of Pharmacology* (n press).

Huang, Y.-H., Henriques, S. T., Wang, C. K., Thorstholm, L., Daly, N. L., Kaas, Q., Craik, D. J. 2015. Design of substrate-based BCR-ABL kinase inhibitors using the cyclotide scaffold. *Scientific Reports* 5: 12974.

Swedberg, J. E., Mahatmanto, T., Ghani, H. A., de Veer, S. J., Schroeder, C. I., Harris, J. M., Craik, D. J. 2016. Substrate guided design of potent FXIIa inhibitors based on the plant-derived Momordica cochinchinensis trypsin inhibitor-II (MCoTI-II) scaffold. *Journal of Medicinal Chemistry* 59: 7287-7292.

51. Nucleus specific degradation of GFP-tagged proteins in plants

Sorge E, Lermontova I, Helmold C, Conrad U

IPK Gatersleben, Stadt Seeland, Germany

Corresponding author: Eberhard Sorge <sorge@ipk-gatersleben.de>

Previous studies showed specific degradation of GFP-tagged proteins by alteration of 26S-proteasome pathway subunits. To recruit the cell´s own proteolytic machinery, the Fbox subunit of an E3-Ligase was fused to a nanobody with high specificity for GFP. With this chimeric construct, it was possible to rise experimental evidence for in vivo degradation of target proteins in insect and human cells (Caussinus et al., 2011). We have been able to replicate these results in tobacco plants, by addressing Full length EGFP as target protein (Baudisch et al., 2018).

Our latest experiments focus on specific subcellular protein degradation. Based on recent literature, we used a human BTB – anti-GFP nanobody fusion protein (Ju Shin et al., 2015) to channel the proteolytic activity to the nucleus of plant cells. This enabled us to observe strong reduction of GFP-fluorescence of target fusion proteins in nuclei.

References

Baudisch, B., Pfort, I., Sorge, E., Conrad, U. 2018. Nanobody-directed specific degradation of proteins by the 26S-Proteasome in Plants. *Frontiers in Plant Science* 9: 130.

Caussinus, E., Kanca, O., Affolter, M. 2011. Fluorescent fusion protein knockout mediated by anti-GFP nanobody. *Nature Structural & Molecular Biology* 19: 117-121.

Shin, Y. J., Park, S. K., Jung, Y. J., Kim, Y. N., Kim, K. S., ... Kee, Y. 2015. Nanobody-targeted E3-ubiquitin ligase complex degrades nuclear proteins. *Scientific Reports* 5: 14269.

52. Transient Production of an anthrax decoy fusion protein in glycoengineered *Nicotiana benthamiana* cell suspension cultures

Sukenik SC, Karuppanan KLQ, Lebrilla CB, Nandi S, McDonald KA

University of California, Davis, USA

Corresponding author: Sara Sukenik <scsukenik@ucdavis.edu>

Transient expression systems can enable large-scale production of novel recombinant proteins in shorter timeframes than stable transgenic systems. We are developing a transient plant cell culture expression platform that uses genetically engineered Agrobacterium tumefaciens for gene delivery. Since different proteins could be produced simply by using different Agrobacterium constructs in conventional bioreactor systems, this system is well suited for rapid response applications. To demonstrate the potential for production of human therapeutic proteins using this platform, glycoengineered, β(1,2)-xylosyltransferase and α(1,3)-fucosyltransferase knockdown *Nicotiana benthamiana* cell suspension cultures were used. An anthrax toxin receptor-Fc fusion protein (CMG2-Fc) was transiently produced by adding Agrobacterium to the glycoengineered *N. benthamiana* cell suspension cultures in shake flasks. Compared to CMG2-Fc produced through agroinfiltration of wild type *N. benthamiana*, CMG2-Fc produced in the glycoengineered transient cell culture system had dramatically reduced levels of β(1,2)-xylose and core α(1,3)-fucose. The impact of various process parameters (including the mass ratio of Agrobacterium added to plant biomass, plant cell growth stage, and co-culture media volume) on CMG2-Fc production will also be discussed.

53. Understanding the origin of cyclotide biosynthesis and re-tooling a plant genome to increase circular peptide production

Gilding EK, Jackson MA, Craik DJ

Institute for Molecular Bioscience, Brisbane, Australia

Corresponding author: Edward Gilding <e.gilding@imb.uq.edu.au>

Cyclotides are head-to-tail cyclized disulfide-rich peptides of roughly 30aa that have been implicated in plant defense. Their characteristic knotted and circular structure imbues these peptides with increased stability when challenged with temperature extremes and proteolytic environments. Because of these qualities they are actively being investigated as a scaffold for peptide based pharmaceuticals and agrichemicals. Five angiosperm families are known to produce cyclotides, however their distribution amongst taxa is inconsistent with phylogenetic relationships, suggesting the structure and machinery to synthesize these peptides are products of convergent evolution. Fortuitously, *Solanaceae* contains several taxa known to make cyclotides and, in addition it is a family with important plant expression systems (ex. *Nicotiana*). Key biosynthetic enzymes are asparaginyl endopeptidase (AEPs) isoforms that function as transpeptidases to create these circular peptides. No*N*-cyclotide producing plants without transpeptidase-type AEPs and induced to transiently express cyclotide precursors produce mostly misprocessed linear products, thus illustrating the importance of the biosynthetic machinery and precursor structure as being factors in efficient cyclotide production *in planta*. Here we aim to create a plant platform optimized for plug-and-play cyclotide production. The strategy is to trace the genetic origins of cyclotide precursor and biosynthetic genes to identify the best homologs in *Nicotiana benthamiana* to be replaced with cyclotide-attuned isoforms from various plants using CRISPR. Data is presented on the evolution of the gene families involved in producing these fascinating molecules and on efforts to create a strain of *N. benthamiana* competent as a plug-and-play circular peptide production platform.

54. Closing the ring: Reorganising cyclic peptide structure to enable expression of promising peptide pharmaceuticals in plants

Smithies B, Jackson MA, Huang YH, Gilding EK, Craik DJ

The University of Queensland, Brisbane, Australia

Corresponding author: Bronwyn Smithies <b.smithies@imb.uq.edu.au>

A plant-based production system for therapeutic peptides can offer a cheaper, greener alternative to traditional chemical synthesis. This work focuses on the production of a class of cyclic peptides called cyclotides, and overcoming one particular obstacle in their biosynthesis in plants – the cyclisation step. Cyclotides are ribosomally-synthesised peptides that are naturally occurring in plants. Head-to-tail cyclisation of these peptides render their C- and *N*- terminal residues inaccessible to enzymatic degradation, while the presence of three disulfide bonds strengthens the structure further. This ultra-stable cyclotide structure is adopted as a scaffold to stabilise smaller therapeutic peptide epitopes that would be otherwise unstable. Where in vitro synthesis of cyclotides has allowed laboratory scale studies to be carried out, it is not the most suitable method for producing cyclotides in large quantities for commercial production due to prohibitive costs and the use of harsh chemicals. This is where plant molecular farming comes in. However, the concepts used for in vitro synthesis of modified cyclotides cannot be applied to plant-based production. In fact, during chemical synthesis the cyclotide backbone can be cyclised at almost any position, but plant-produced cyclotides are cyclised enzymatically at a precise position. Incidentally, almost 75% of modified cyclotides interrupt the cyclisation point in the cyclotide sequence, meaning that recombinant expression of the mature cyclic product is not possible. A new cyclisation site needs to be engineered into the recombinant peptide in order to keep any new bioactive epitope in its optimal place within the backbone, and at the same time allow production of the stable, cyclic, product *in planta*. Through semi-rational design, and some trial and error, an alternative point of cyclisation was introduced into the cyclotide backbone, which was successfully processed into the mature cyclic product by the same enzymes found in cyclotide-producing plants. This breakthrough in cyclotide production should enable the most effective and promising cyclotide-based therapeutics to progress from small scale laboratory testing to large scale biosynthesis in plants.

55. Developing rice as a production system for cyclic therapeutic peptides

Qu H, Jackson MA, Gilding EK, Craik DJ

The University of Queensland, Brisbane, Australia

Corresponding author: Haiou Qu <h.qu@imb.uq.edu.au>

Plant-produced cyclic peptides range from 14~37 amino acids in length and are characterised by a head--to--tail cyclised backbone that is further strengthened by intramolecular disulfide bonds. They exhibit various bioactivities, including uterotonic, antimicrobial and anti--HIV activities, which are potentially exploitable for therapeutic uses. Moreover, due to their unique stable topological structure and tolerance to residue substitution, cyclic peptides have become promising molecular scaffolds for pharmaceutical protein-engineering applications. Currently, chemical synthesis is a common strategy for producing native and modified cyclic peptides, as well as recombinant synthesis and natural extraction. However, these strategies are limited for large-scale production by the high production cost, complexity of separation and deficient knowledge of the biosynthesis pathway. To address these issues, this project is aiming to investigate a non-native cyclic peptide producing species, rice, as a plant bioreactor to express cyclic peptides of interest. Rice represents a promising recombinant peptide production platform based on its proven stable accumulation of proteins, low bio-safety risk and cost-effectiveness. So far, no native cyclic peptides have been identified in any monocot species, however, several peptide precursor genes with homology to cyclic peptide genes have been identified, differing only in the absence of residues known to be important for *in planta* cyclisation. In this study, we aim to test the capacity of rice to produce cyclic peptides through transformation of a variety of precursor peptide genes together with or without Asparaginyl endopeptidase (AEP), a gene to aid cyclisation *in planta*. Native linear cyclic peptide-like precursor genes of rice were engineered with cyclisation-competent C-termini to further explore the mechanism of cyclisation *in planta* and the flexibility of cyclic peptide grafting in a monocot system.

56. Transient expression of prM-E and NS1 proteins of West Nile Virus *in planta* as a viable, low-cost method for vaccine and diagnostic reagent development

Wayland J, Meyers A, Rybicki E

University of Cape Town, Rondebosch, Cape Town, South Africa

Corresponding author: Jennifer Wayland <jennifer-wayland@hotmail.com>

West Nile virus (WNV) is widely disseminated, with a geographic range that now includes Africa, North America, Europe, the Middle East and West Asia. The virus is maintained in a bird-mosquito transmission cycle and mammals are considered accidental-dead-end hosts. WNV is a member of the Japanese encephalitis virus serocomplex, and in humans WNV infections can develop into febrile illness and severe meningoencephalitis. To date there is no human vaccine available.

Diagnostic reagents and vaccines are made by recombinant protein expression in a variety of systems, including bacteria, fungi and yeasts, and mammalian or insect cells. Transient plant-based expression systems are possibly the cheapest means of making complex proteins; have the ability to process proteins very similarly to mammalian cells, and do not require sterile incubation. Therefore, it is proposed that this system could be a viable means of making feasible, low cost reagents for West Nile virus for use as diagnostics, as well possibly being used to produce virus-like particles (VLPs) for use as vaccines in South Africa and elsewhere.

We aim to use the envelope (E) and membrane (prM/M) proteins of WNV to make no*N*-infectious empty VLPs as a candidate vaccine, and to use the no*N*-structural protein 1 (NS1) to make a diagnostic reagent for viral detection, *in planta*. The E, prM/M and NS1 genes were cloned into the plant expression vectors pTRAkc-ERH and pEAQ-HT, and infiltration time trials of the assembled constructs were conducted to determine the optimal OD600 for infiltration and the optimal sampling point for maximum protein yield. We investigated the results of co-expression of human derived chaperone proteins on viral protein expression. We report data on the yield of viral proteins and on the optimisation of their expression.

57. Developing butterfly pea as a production platform for cyclotide-based therapeutics

Oguis GK, Gilding EK Jackson MA, Craik DJ

University of Queensland, Brisbane, Australia

Corresponding author: Georgianna Oguis <g.oguis@imb.uq.edu.au>

Cyclotides are small, head-to-tail cyclised peptides with a knotted topology. They are of interest to many because they can be used as ultrastable scaffolds for grafting peptide-based therapeutics. To date, there are five angiosperm plant families that are known to produce them: *Violaceae, Rubiaceae, Solanaceae, Cucurbitaceae* and *Fabaceae*. This work focuses on butterfly pea (*Clitoria ternatea*), the only species in the *Fabaceae* family that is currently known to produce cyclotides. Combined with genome walking and fourth generation sequencing, the cis-regulatory elements upstream of butterfly pea cyclotides were scrutinised to determine the key factors that can enhance cyclotide production. Induction experiments on wild type butterfly pea seedlings showed significant increase in cyclotide production when exposed to numerous stress factors such as salicylic acid and ethylene biosynthetic precursor, among others. Being a native cyclotide producer equipped with the pre- and post-translational enzyme machinery to produce cyclotides, the study further looks into developing butterfly pea as a production platform for cyclotide-based therapeutics. A big step towards this endeavour is the establishment and optimisation of the hairy root transformation protocol in butterfly pea. With the knowledge gained from studying the cyclotide CREs in butterfly pea that can contribute to significant increase in cyclotide production, and the development of hairy root transformation protocol, butterfly pea can be an excellent biofactory for the production of cyclotide-based therapeutics.

58. Plant-derived avian flu vaccines: Potential applications for veterinary purposes

Phan HT[1,2], Ho TT[2], Schinkoethe A[1], Gresch U[1], Conrad U[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
[2]Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam

Correspondence: Phan Trong Hoang <hoang@ipk-gatersleben.de>

The H5N1 avian influenza A virus is a highly contagious, deadly pathogen in poultry, transmitted from poultry to humans causing high mortality (ca 60%) and able to pose a pandemic threat. Vaccination is the most effective approach to reduce illness and death from pandemic influenza. Influenza vaccines generally in use today are derived from viruses grown in hens' eggs. However, this approach exposes several obstacles such as complex processes, long preparation time and limited capacity. To address these points, the subunit vaccines are an alternative approach to meet the global demand for the influenza vaccines. However, the subunit vaccines generally induce low immunogenicity against influenza. To overcome this effect, we present new and innovative ways to generate highly immunogenic H5 oligomers from avian flu hemagglutinin *in planta*. H5 oligomers in the plant crude extract were highly active in hemagglutination resulting in high titers. Immunization of mice with two doses of plant crude extracts containing H5 oligomers caused strong immune responses and induced neutralizing specific humoral immune responses in mice. These results allow for the development of cheap influenza vaccines for veterinary application in future.

59. Tackling emerging viruses – Plant made Virus-like Particles as candidate vaccine against Dengue virus

Ponnorf D[1], Alonso AD[1], Meshcheriakova Y[1], Stocks M[2], Peyret H[1], Lomonossoff GP[1]

[1]*John Innes Centre, Norwich, United Kingdom*
[2]*Plant Bioscience Ltd., Norwich, United Kingdom*

Corresponding author: Daniel Ponndorf <Daniel.Ponndorf@jic.ac.uk>

Dengue virus (Denv) is an enveloped virus, belonging to the family of *flaviviridae*, and emerged as a global threat in the last decades. It is transmitted by mosquitoes such as *Aedes albopictus* and Aedes aegypti and is currently present in Asia, Africa and the Americas and caused over 3 Mio infections in 2015 (WHO, 2016). Infection can be accompanied with fever and, in severe cases, neurological disorders, haemorrhagic fever and dengue shock syndrome (WHO, 2016). Prevention of infection can be achieved by vector control and vaccination. Dengvaxia®, a live attenuated recombinant vaccine, is currently the only approved vaccine against Denv in some Asian and South American nations, but raised concerns regarding to low vaccine efficiency and antibody depending enhancement of infection (WHO, 2016; Aguiar et al., 2016). Hence, a better and more immunogenic vaccine against Denv is of interest and might be achieved using virus like particles (VLPs). VLPs are self-assembled structures derived from viral components and antigens that mimic the native architecture of a virus, but lack the viral genome. Consequently, they are no *N*-pathogenic, but show a similar immunogenicity as the native virus, their size and repetitive structure contributing to their immunological efficacy (Chen & Lai, 2013). VLPs can be produced in yeast, bacteria, mammalian and insect cells and plants. Plants show several advantages compared to other expression systems regarding to safety, scalability and cost efficiency and have consequently become an attractive alternative expression system (Chen & Lai, 2013).

We present the successful production of Denv VLPs using the pEAQ-hyper trans expression system (Sainsbury, et al., 2009) in *Nicotiana benthamiana* by the co-expression of structural and no*N*-structural proteins of Denv and address the challenges we faced relating to the purification of enveloped VLPs from plants.

Aguiar, M., Stollenwerk, N., Halstead, S. B. 2016. The impact of the newly licensed dengue vaccine in endemic countries. *PLoS Neglected Tropical Diseases* 10:12, e0005179.

Chen, Q., Lai, H. 2013. Plant-derived virus-like particles as vaccines. *Human Vaccines & Immunotherapeutics* 9: 26-49.

Sainsbury, F., Thuenemann, E. C., Lomonossoff, G. P. 2009. pEAQ: versatile expression vectors for easy and quick transient expression of heterologous proteins in plants. *Plant Biotechnology Journal* 7: 682-693.

World Health Organization. 2016. Dengue vaccine: WHO position paper—July 2016. Weekly Epidemiology Record, 91:30, 349-364.

60. Evaluating VHH-Fc fusions as therapeutics

Bakshi S[1], Juarez P[1,2], Schepens B[1], Virdi V[1], Saelens X[1], Depicker A[1]

[1]*Ghent University, Ghent, Belgium*
[2]*Institute for Plant Molecular and Cell Biology (IBMCP), Valencia, Spain*

Corresponding author: Shruti Bakshi <shbak@psb.vib-ugent.be>

The variable domains of Heavy chain only antibodies (VHH) have many applications as injectable therapeutics but a limitation is the rapid elimination from circulation by renal filtration (De Meyer et al., 2014). A solution is the genetic fusion of VHH to the Fc part of a conventional antibody ensuring recycling to the bloodstream by the neonatal receptor FcRn. Here, we report the production by transient expression in infiltrated *Nicotiana benthamiana* leaves of VHH fusions to an Fc fragment derived from mouse IgG (VHH-IgG) and from mouse IgA (VHH-IgA) (Hultberg et al., 2011). This allowed us to demonstrate virus neutralizing activity of plant extracts with both IgG and IgA-based VHH fusions. Quality and functionality of these fusions were analyzed by western blot and ELISA, respectively. While being functional, VHH-IgA Fc fusions display only a weak band of the expected length (42kDa) and a pronounced band of about 25kDa, suggesting extensive proteolytic cleavage of the IgA-Fc part. The unavailability of commercial affinity ligands for purifying mouse IgAs currently presents a problem for the comparison of the protective activity in vivo of full-length VHH-IgA versus VHH-IgG antibodies. To circumvent this, the glycosylation site of the CH2-CH3 interface in mouse IgA-Fc was mutated to contain the paratope of the commercially available SSL7 resin (Wines et al., 2011). This novel adapted mouse IgA fusion could be purified by SSL7 affinity chromatography and was found to be functional in ELISA and neutralization assays. However, the copurification of the proteolytic fragment prevented us from comparing the VHH-IgG versus VHH-IgA efficacy in animal experiments.

References

De Meyer, T., Muyldermans, S., Depicker, A. 2014. Nanobody-based products as research and diagnostic tools. *Trends in Biotechnology* 32: 263-270.

Hultberg, A., Temperton, N. J., Rosseels, V., Koenders, M., Gonzalez-Pajuelo, M., ... Weiss, R. A. 2011. Llama-derived single domain antibodies to build multivalent, superpotent and broadened neutralizing anti-viral molecules. *PloS One* 6:4 e17665.

Wines, B. D., Ramsland, P. A., Trist, H. M., Gardam, S., Brink, R., Fraser, J. D., Hogarth, P. M. 2011. Interaction of human, rat, and mouse immunoglobulin A (IgA) with Staphylococcal superantigen-like 7 (SSL7) decoy protein and leukocyte IgA receptor. *Journal of Biological Chemistry* 286: 33118-33124.

61. Efficient production of antifungal proteins in plants using a simplified tobacco mosaic virus derived system

Coca M[1], Daròs JA[2], Marcos JF[3], Cordero T[2], Shi X[1]

[1]*CRAG Centre for Research in Agricultural Genomics, Cerdanyola del Vallès, Spain*
[2]*IBMCP CSIC Universitat Politècnica de València, Valencia, Spain*
[3]*IATA Institute of Agrochemistry and Food Technology (CSIC), Paterna, Spain*

Corresponding author: Maria Coca Lopez <maria.coca@cragenomica.es>

Fungi that infect plants, animals or humans pose a serious threat to human and animal health, food security and ecosystem resilience. Only a few classes of antifungals are currently available, which are questionable due to resistance, host toxicity and undesirable side effects. There is thus an urgent need for novel antifungals with improved properties and action mechanisms different from existing ones. Antifungal proteins (AFPs) secreted by filamentous fungi are promising biomolecules for the development of new antifungal therapies in medicine and agriculture (Meyer, 2008). They are small proteins, usually cationic, rich in cysteine, and folded in compact structures supported with disulfide bridges, which make them highly stable and resistant to heat, proteases and extreme pH. They exhibit specifically potent antifungal activity at very low concentrations against important human and plant fungal pathogens, and do not show toxic effects on plant or mammalian cells. The exploitation of AFPs, however, requires efficient, safe and economic production systems. Plants represent a good option for the production of these cysteine-rich proteins that require formation of disulphide bridges and proper folding.

Here, we report the development of an easy-to-manipulate viral vector for the production of large amounts of these small AFPs in *Nicotiana benthamiana* plants. The viral vector derives from a tobacco mosaic virus (TMV) infectious clone in which part of the sequences coding for the coat protein are replaced by the gene of interest. The engineered viral replicons are introduced into the biofactory host via agroinfiltration. Using this simple viral vector, we efficiently produced the Aspergillus giganteous AFP (Vila et al., 2001) and the Penicillium digitatum AfpB (Garrigues et al., 2017). High accumulation levels were reached when these bioactive small proteins were targeted to the apoplast, although low accumulation and toxic effects in the host plants were observed when targeted to intracellular compartments. Moreover, AFPs were easily purified from the apoplastic fluids by one-single step chromatography. We also demonstrate that this production system renders AFPs fully active against target pathogens, thus supporting the idea that plants are suitable biofactories to bring these antifungal proteins to the market.

Garrigues, S., Gandía, M., Popa, C., Borics, A., Marx, F., ... Manzanares, P. 2017. Efficient production and characterization of the novel and highly active antifungal protein AfpB from *Penicillium digitatum*. *Scientific Reports* 7: 14663.

Meyer, V. 2008. A small protein that fights fungi: AFP as a new promising antifungal agent of biotechnological value. *Applied Microbiology and Biotechnology* 78: 17-28.

Vila, L., Lacadena, V., Fontanet, P., del Pozo, A. M., Segundo, B. S. 2001. A protein from the mold Aspergillus giganteus is a potent inhibitor of fungal plant pathogens. *Molecular Plant-Microbe Interactions* 1411: 1327-1331.

62. Poster presented in number 19 – see abstract N:o 19

63. Biopharmaceuticals production in *Nicotiana benthamiana* - Axillary stem leaves as a key determinant of total protein yields

Goulet MC[1], Maltais AM[1], Gagné M[1], D'Aoust MA[2], Michaud D[1]

[1]*Université Laval, Québec, Canada*
[2]*Medicago Inc., Québec, Canada*

Corresponding author: Marie-Claire Goulet <Marie-Claire.Goulet@fsaa.ulaval.ca>

Modelling recombinant protein yields in higher plants used as production hosts for biopharmaceuticals requires a careful monitoring of protein rates as a function of leaf age, while the plant is growing and developing (Robert et al., 2013). We here characterized the accumulation patterns of different cytosol- and cell secretory pathway-targeted proteins in agroinfiltrated leaves of the transient expression host Nicotiana benthamiana. Accumulation rates were determined seven days post-infiltration in young, mature and older leaves to establish the relative importance of each leaf age group on protein yield at the whole plant level. Our data highlight the importance of young and mature leaves on overall yield, in contrast with older leaves representing almost 30% of the whole biomass but contributing less than 5% of total protein yield. Our data also highlight the relative importance of axillary stem leaves, which account for 35 to 50% of total recombinant protein yield depending on cultural conditions before agroinfiltration. These findings underline the importance of considering the relative importance of axillary stem and main stem leaves in protein map models for the *N. benthamiana* expression host. A general trend to come out of this work is the relation between recombinant protein yield per plant and the 'main stem leaf to axillary stem leaf' ratio, and hence the relevance of defining cultural conditions, physical treatments (e.g. pruning) or chemical (e.g. hormonal) treatments that promote axillary stem growth towards an optimal plant architecture for maximal productivity (Michaus et al, 2017). Examples along this line will here be discussed in terms of impact on leaf biomass production, recombinant protein yield and metabolic impact at the leaf cell proteome scale.

Reference

Michaud, D., Pepin, S., Ethier, G., Goulet, M. C., Gaudreau, L., ... Gosselin, A. 2017. U.S. Patent Application No. 15/324,926.

Robert, S., Khalf, M., Goulet, M. C., D'Aoust, M. A., Sainsbury, F., Michaud, D. 2013. Protection of recombinant mammalian antibodies from development-dependent proteolysis in leaves of *Nicotiana benthamiana*. *PLoS One* 8: e70203.

64. Improving the binding of the anti-HIV broadly neutralising antibody 10-1074 to the neonatal receptor to prolong in vivo half-life

Grandits M, Teh AYH, Ma JKC

St. George's, University of London, London, United Kingdom
Corresponding author: Melanie Grandits <p1607569@sgul.ac.uk>

35 years after the human immunodeficiency virus (HIV) was identified as the cause of Acquired Immune Deficiency Syndrome (AIDS), there is still no cure, only drugs to control it. Life-long antiretroviral therapy is costly, which limits availability to many patients, and its effectiveness may be compromised by viral escape. Broadly neutralising antibodies (bNAbs) offer a different perspective to pre-exposure prophylaxis (PrEP) and early post-exposure therapy. Promising results could be achieved by a combination of the bNAbs 10-1074 and 3BNC117, which was able to induce long-lasting virus control in macaques (Nishimura et al., 2017) and is currently undergoing a phase 1b clinical trial in HIV-1 infected and no*N*-infected individuals (NCT02825797).

To improve the efficacy of the bNAb 10-1074, we introduced a YTE mutation into the Fc region. This mutation has been shown to improve the half-life of monoclonal antibodies by increasing the affinity to the neonatal receptor (FcRn) (Dall'Acqua et al., 2002), which is responsible for IgG recycling. We used ΔXF *N. benthamiana* as an expression platform to improve binding to FcγRIIIa (Ferrera et al., 2011), thus possibly enhancing antibody-dependent cell-mediated toxicity (ADCC). We showed that the introduction of the YTE mutation into bNAb 10-1074 had a significant impact on yield, but no effect on affinity to gp120 nor its ability to potently neutralise HIV-1. As expected, 10-1074 carrying the YTE mutation exhibited a significantly higher affinity to FcRn than the no*N*-mutated 10-1074, suggesting an enhancement in its in vivo half-life. Our data also confirmed that removal of the core α1,3-fucose may lead to an improvement in ADCC.

References

Dall'Acqua, W. F., Woods, R. M., Ward, E. S., Palaszynski, S. R., Patel, N. K., ... Langermann, S. 2002. Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *The Journal of Immunology* 169: 5171-5180.

Ferrara, C., Grau, S., Jäger, C., Sondermann, P., Brünker, P., ... Umaña, P. 2011. Unique carbohydrate–carbohydrate interactions are required for high affinity binding between FcγRIII and antibodies lacking core fucose. *Proceedings of the National Academy of Sciences USA* 108: 12669-12674.

Nishimura, Y., Gautam, R., Chun, T. W., Sadjadpour, R., Foulds, K. E., ... Donau, O. K. 2017. Early antibody therapy can induce long-lasting immunity to SHIV. Nature 543: 559.

65. Expression of different Hemaglutinin proteins of Influenza Virus as Virus-like particle in tobacco plant

Ehsani P, Fotouhi F, Farahmand B, Alikhani A, Khorsandi S

Pasteur Institute of Iran,Tehran, Iran
Corresponding author: parastoo ehsani <p_ehsani@yahoo.com>

More than three decades passed from production of first vaccine candidate proteins in plants. Transgenic plant produced Hepatitis B surface antigen showed to have a suitable conformation for induction of antigenicity and immunity in experimented animals. However, low expression level and time needed for growth of transgenic plant enhanced the improvement of the system such as applying transient expression.

Each year, Influenza virus results in five million cases of severe illness and about 250,000 to 500,000 deaths (WHO 2014). It has been shown that Influenza vaccines could protect human against sickness, hospitalizations and deaths caused by these viruses. Because of the rapid change of virus, new version of the vaccine is developed twice a year and highly needed by at risk people such as pregnant women, the elderly, children between six months and five years of age. The intrinsic characteristic of some viral proteins to self-assemble as a Virus like Particle (VLP) in plant has made it a good system for expression of VLPs as in Influenza virus (Medicago, 2016). The repetitive nature of antigen in Virus like particle while there is no infectious genome has made VLP a good candidate to substitute the present inactivated influenza virus vaccines. One of the major concerns in production of VLP in plant is its assembly and stability during purification. Byrd-Leotis et al (2015) have shown that different mutations affect the resistance of virus to environmental factors. Their results showed that HA mutation in Lysin 58 to Isoleusin which is located at the membrane distal tip of the short helix of the hairpin loop structure, improved the stability and conferred the acid-stable phenotype for nearly all HAs examined. Considering the success, these two characteristics of K58I are interesting for potential use of the mutation in the development of stable Influenza vaccine candidates.

We have prepared original, plant codon optimized and mutated forms of Influenza A virus (A/Indonesia/5/2005(H5N1)) hemagglutinin (HA) gene. To produce the mutated form of HA, its coding sequence was subjected to site directed mutagenesis by PCR using two sets of primers carrying the K58I mutation. The overlap PCR was done to assemble the HA gene. The genes were cloned in EcoR1 and BamH1 site of pTRAKc-ERH to remove KDEL fragment and introduced into *Agrobacterium*. Co-agroinfiltration of the above agrobcteria with agrobacteria carrying p19(silencing suppressor) gene cloned in pcambia into *Nicotiana benthamiana* plant were done. The VLP Purification and mouse immunogenicity experiments will be further investigated.

Byrd-Leotis, L., Galloway, S. E., Agbogu, E., Steinhauer, D. A. 2015. Influenza hemagglutinin (HA) stem region mutations that stabilize or destabilize the structure of multiple HA subtypes. *Journal of Virology* 89: 4504-4516.

Medicago Announces Phase 3 Study of VLP Quadrivalent Influenza Vaccine, MEDICAGO Sep 26, 2017, at: https://www.prnewswire.com/news-releases/medicago-announces-phase-3-study-of-vlp-quadrivalent-influenza-vaccine-647905263.html

WHO. 2014. Influenza (Seasonal) Fact sheet No. 211. Archived from the original on 30 November 2014.

66. Lingonberry cell culture as a food source - “Goodies” from bioreactor

Kärkkäinen E, Sumyk MS, Puupponen-Pimiä R, Reuter L, Rischer H.

VTT Technical Research Centre of Finland Ltd, Espoo, Finland

Corresponding authors: Kärkkäinen Elviira <elviira.karkkainen@vtt.fi> and Sumyk Solomia <ext-solomia.sumykmaletych@vtt.fi>

Plant cell culture (PCC) technology is an interesting new approach for production of plant based food. Even though plant cells have been considered for food production previously, studies and applications have concentrated on extracts and high value compounds instead of the plant cell mass itself. It has been proven that lingonberry (Vaccinium vitis-idaea) cell suspension cultures have great potential for food purposes based on both the visual appearance and from nutritional point of view. (Nordlund et al., 2018)

To achieve deeper understanding about the crucial factors affecting the biomass formation, lingonberry cell cultures have been studied under laboratory and pilot scale conditions. At small-scale experiments in 250mL flasks, the induced variation in starting pH of the media have shown a positive effect on fresh biomass increment. To optimize the physical growth conditions of lingonberry cell cultures, stirred tank cultivations (2 L wv) were carried out. Based on collected data the importance of lighting conditions and dissolved oxygen for example, were analysed in relation to biomass production and visual appearance of cell mass.

Due to its facility in use and appealing look, lingonberry cells are a good source of creativity. These cells have already been used for making jams, jellies and other sorts of prototype gastronomic "berry-experiences", where they are applied as the main ingredient.

Reference

Nordlund, E., Lille, M., Silventoinen, P., Nygren, H., Seppänen-Laakso, T., ... Rischer, H. 2018. Plant cells as food – A concept taking shape. *Food Research International* 107: 297-305.

67. Cellular agriculture of plants

Rischer H

VTT Technical Research Centre of Finland Ltd., Espoo, Finland

Corresponding author: Heiko Rischer <heiko.rischer@vtt.fi>

The production of agricultural products from cell cultures of bacteria, yeasts, fungi, animals and plants, collectively coined Cellular Agriculture, has received a lot of public attention for its potential to tackle some of the grand challenges in sustainable food production. Particularly *in vitro* meat is hyped because it could make animal husbandry obsolete. However, a plant-based diet is generally considered healthier than animal-based food and therefore the potential of plant cell cultures (PCCs) is extremely promising, too. A major advantage is that the technical processes for the production of phytochemicals for pharmaceuticals, pigments, cosmetics and additives by PCCs are already established, even at large scale. However, the value of the whole biomass as food has been neglected. First investigations confirm that PCCs contain nutritionally relevant combinations of proteins, carbohydrates and lipids, enriched with vitamins and health-promoting compounds. Additionally, they exhibit technological processability that influences digestibility and sensory attributes

68. Expressing and processing of recombinant Human β- Pre-pro-Nerve Growth Factor in *Nicotiana benthamiana*

Ofoghi H[1], Zangi M[1], Amini-Bayat Z[1], Ehsani P[2]

[1]*IROST Iranian Research Organization for Science and Technology, Tehran, Iran*
[2]*Pasteur Institute of Iran, Tehran, Iran*

Corresponding author: hamideh ofoghi <ofoghi@irost.ir >

The b-NGF (Beta Nerve Growth Factor) gene encode pre-pro-NGF consists of the signal peptide, pro domain and mature NGF protein. NGF is secreted mainly in its mature form. The pro domains of the neurotrophins have an important role in intracellular processing and the proper folding of NGF (Rattenholl et al., 2001). Also, the pro domain of neurotrophins is essential for intracellular trafficking and secretion, therefore it can significantly affect neurotrophins's function (Lu, 2003). The pro-domain of NGF is most likely removed by the serine protease furin in the trans-Golgi network and mature NGF with biologically active form is secreted. Active b-NGF is a dimeric protein of ~15 kDa in which three disulfide bridges connect the two monomers (Seidah et al., 1996). In this study, we have investigated the possibility of Processing of transiently expressed human pre-pro-NGF in Nicotiana benthamiana. Human pre-pro- NGF gene sequence was optimized according to *N. benthamiana* codon usage and cloned in PVX viral vector. To express the hNGF in *N. benthamiana* leaves, recombinant PVX-hNGF plasmids were electroporated to Agrobacterium tumefaciens strain GV3101. Leaves of infiltrated plants were vacuum-infiltrated to isolate apoplastic fluids.. Detection of rhNGF was done by western blot analysis, hNGF protein concentration were determined by reverse phase-high performance liquid chromatography (RP-HPLC). By Western blot analysis on crude extracts, Pro-NGF migrated at the expected size about 27 kDa. As predictable, the mature rhNGF was detected at ~15 kDa in the isolated apoplastic fluids. The rhNGF protein concentration was calculated about 70 µg/g fresh leaves by RP-HPLC analysis. Here, we show that expression of biologically active NGF protein without the requirement of chemical refolding is feasible in plants. Using *N. benthamiana* for producing recombinant proteins, as a plant-based expression system, has advantages over other organisms.

Lu, B. 2003. BDNF and activity-dependent synaptic modulation. *Learning & Memory* 10: 86-98.

Rattenholl, A., Ruoppolo, M., Flagiello, A., Monti, M., Vinci, F., ... Rudolph, R. 2001. Pro-sequence assisted folding and disulfide bond formation of human nerve growth factor 2. *Journal of Molecular Biology* 305: 523-533.

Seidah, N. G., Benjannet, S., Pareek, S., Savaria, D., Hamelin, J., ... Murphy, R. A. 1996. Cellular processing of the nerve growth factor precursor by the mammalian pro-protein convertases. *Biochemical Journal* 314:Pt 3, 951.

69. Not shown

.

70. Optimisation of *Brassica rapa rapa* (turnip) hairy root culture media for the production of biomass and secretion of recalcitrant recombinant proteins

Gutierrez Valdes N[1], Häkkinen ST[1], Oksman-Caldentey K-M[1], Lemasson C[2], Ele Ekouna JP[3], Boitel-Conti M[3], Cardon F[2], Ritala A[1]

[1]VTT Technical Research Centre of Finland Ltd. Espoo, Finland
[2] Root Lines Technology SA, Amiens, France
[3]Université de Picardie Jules Verne, Amiens, France

Corresponding author: Anneli Ritala <anneli.ritala@vtt.fi>

The increasing demand for recombinant proteins used as therapeutic agents has influenced the development of different production platforms. Plant systems are attractive for recombinant protein production as they offer safety (no risk of human-threatening viral contamination), low upstream costs, complex glycosylation, and high scalability. Among the plant-based systems, hairy root cultures present several advantages such as rapid propagation, genotypic and phenotypic stability, and among others, possible extracellular secretion of expressed proteins in a well-defined medium. The optimization of the hairy root cultures to produce sufficient yields of secreted and easy-to-purify recombinant proteins is an on-going endeavor as it usually depends on the protein of interest. As part of the H2020 PharmaFactory project, several strategies are being assessed to maximize the productivity of “hard-to-produce” recombinant proteins in hairy root cultures. In this regard, we are evaluating the impact of selected culture medium agents on productivity. The aim is to identify, from a range of culture medium additives, those that when used in combination or alone, increase the productivity of recalcitrant recombinant proteins. The potential of such agents has been evaluated based on statistical experimental design. Subsequently, with the biomass values and recombinant protein yields retrieved, a model is created to predict culture media conditions giving rise to the best recombinant protein yield. Currently, sucrose and calcium chloride have demonstrated to be important for the biomass production. More research is still required in order to create models with a higher prediction power.

71. In-solution antibody capture with plant-made hydrofobin-Protein A fusions

Kurppa K[1], Reuter L[1], Ritala A[1], Linder MB[1,2], Joensuu JJ[1]

[1]VTT Technical Research Centre of Finland Ltd. Espoo, Finland
[2] Aalto University, Espoo, Finland

Corresponding author: Jussi Joensuu <Jussi.joensuu@vtt.fi>

There is urgent need to reduce production and processing costs for monoclonal antibodies (MAbs). The current industrial standard for MAb purification involves use of initial affinity chromatography step with a column that consists *Staphylococcus aureus* Protein A / G that has been chemically immobilized to solid support beads. Aqueous two-phase systems are an interesting and scalable alternative to column chromatography for protein purification. We have previously shown that hydrophobins (HFBs), surface-active fungal proteins can be utilized as purification tags, enabling efficient capture of target proteins using a surfactant-based aqueous two-phase system (ATPS).

Here, antibody binding domains of Protein A were genetically fused with fungal hydrophobins and expressed transiently in *Nicotiana benthamiana* and in transgenic tobacco BY-2 cell suspension cultures. Protein A-HFB fusion expression was scaled up and the target protein was purified from plant extracts. The binding of these proteins to human antibodies was confirmed. Furthermore, the functionality of Protein A-HFB fusions was tested for in-solution capture of a MAb from hybridoma culture supernatant. The demonstrated ATPS process offers alternative to Protein A column chromatography and creates an exciting alternative for MAb purification.

72. Chloroplast produced antimicrobial peptide fusions for pharma and plant protection

Hoelscher M, Forner J, Bock R

Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

Corresponding author: Matthijs Hölscher Hoelscher@mpimp-golm.mpg.de>

Antimicrobial peptides (AMPs) are an important mechanism for combatting microbes and are produced by animals, plants, fungi and bacteria. AMPs are considered promising alternatives for current antibiotics (Fjell et al., 2012). Tobacco chloroplasts might be a useful platform for the production of these peptides due to their capacity for high protein accumulation (Bock 2015). Also expression of these peptides could provide a strategy for protecting plants against bacteria and fungi. In order to protect short single AMPs from potential degradation by the Presequence Protease (Kmiec et al., 2013) and in order to create multi-functional molecules, several AMPs were fused by flexible linkers. The presented research shows that it is possible to accumulate antimicrobial peptides that are fused to each other in sets of 3 different peptids. Strongly constitutive production of fusion peptides leads to pale or white plants. A next generation of plants producing these peptides under the chloroplast encoded inducible RAmpER system (Emadpour et al., 2015) strongly reduced these plant phenotypes. Ongoing work is focused on the characterisation of plant resistance, peptide purification and characterising antimicrobial activity, optimization of induction and tighter control of expression through inducible nuclear encoded switches.

References

Bock, R. 2015. Engineering plastid genomes: methods, tools, and applications in basic research and biotechnology. *Annual Review of Plant Biology*, 66, 211-241.

Emadpour, M., Karcher, D., Bock, R. 2015. Boosting riboswitch efficiency by RNA amplification. *Nucleic acids research*, 43(10), e66-e66.

Fjell, C. D., Hiss, J. A., Hancock, R. E., Schneider, G. 2012. Designing antimicrobial peptides: form follows function. *Nature reviews Drug discovery*, 11:1, 37.

Kmiec, B., Teixeira, P. F., Berntsson, R. P. A., Murcha, M. W., Branca, R. M., Radomiljac, J. D., ... Lehtiö, J. 2013. Organellar oligopeptidase (OOP) provides a complementary pathway for targeting peptide degradation in mitochondria and chloroplasts. *Proceedings of the National Academy of Sciences*, 110:40, E3761-E3769.

73. Production of fish vaccines against salmon pancreas disease using *Nicotiana benthamiana* and tobacco BY-2 cell culture

Steele J[1], Polinder E[2], Rojas M[2], Berthold T[1], van Gorkom S[2], Ritala, A[2], Lomonossoff GP[1]

[1] *John Innes Centre, Norwich, UK*
[2] *VTT Technical Research Centre of Finland Ltd, Espoo, Finland*

Aquaculture is the fastest growing food-production sector worldwide, recently overtaking wild-capture as the major provider of fish for human consumption and contributing over US$160bn to the global economy. Like many agricultural systems, a key concern for the sustainability of aquaculture is the effect of virus-borne diseases on livestock. With relatively low profit margins and the price sensitivity of the aquaculture market, fish farmers require low-cost interventions to protect against viral diseases.

Plant-based expression systems have a proven track record in producing vaccine candidates for use against human and animal diseases. The simple growing conditions and ease of candidate screening position plant-based vaccine production as a low-cost alternative to existing technologies, and an attractive avenue of research for the aquaculture industry.

To date there have been few reports of plant-produced vaccine candidates targeted at enveloped viruses. Enveloped viruses are typically more complex than their non-enveloped counterparts, requiring multiple post-translational modifications such as glycosylation during maturation. We have used Salmonid alphavirus (SAV), the causal agent of salmon pancreas disease, to explore the use of multiple plant-based expression systems to produce vaccine candidates against this enveloped virus.

Using both whole-plant expression in *Nicotiana benthamiana* and tobacco BY-2 cell culture we have produced vaccine candidates based on the major glycoprotein E2 of SAV. These include previously characterised immunogenic regions that are known to elicit antibody responses. We have developed methods that allow scalable purification of the antigens, and show that the plant-derived proteins cross-reacts with neutralising antibodies regardless of expression system used. The glycosylation state of purified proteins has been explored, allowing us to investigate differences in post-translational modification between whole-plant and cell culture systems.

We have demonstrated the ability of these two systems to produce vaccine candidates in a scalable manner suitable for industry. However, the key determinant of whether such approaches will benefit aquaculture will be the ability of the plant-produce material to stimulate protect immunity. Thus, although we can determine the yield of the vaccine candidates produced by each expression system, further investigations into vaccine efficacy *in situ* are required for a full economic analysis.

List of participants

Fabio Apone
Arterra Bioscience srl., Italy
fapone@arterrabio.it

Ingo Appelhagen
John Innes Centre, UK
ingo.appelhagen@jic.ac.uk

Shruti Bakshi
VIB - University of Gent, Belgium
shruti.bakshi@ugent.vib.be

Ani Barbulova
Arterra Bioscience srl., Italy
ani@arterrabio.it

Muriel Bardor
University of Rouen Normandie, France
muriel.bardor@univ-rouen.fr

Angela Bartolf
St. George's University of London, UK
abartolf@sgul.ac.uk

Eugenio Benvenuto
ENEA, Italy
eugenio.benvenuto@enea.it

Sheeba Bloodsworth
St. George's University of London, UK
sbloodsw@sgul.ac.uk

Lennard Bohlender
University of Freiburg, Germany
lennard.bohlender@biologie.uni-freiburg.de

Tobias Bonitz
University of Leipzig, Germany
tobias.bonitz@uni-leipzig.de

Luisa Bortesi
Maastricht University, The Netherlands
luisa.bortesi@maastrichtuniversity.nl

Dirk Bosch
Wageningen UR, The Netherlands
dirk.bosch@wur.nl

Frederic Bourgaud
Plant Advanced Technologies, France
frb@plantadvanced.com

David Brault
INNO-3B inc., Canada
dbrault@inno-3b.com

Inge Broer
University of Rostock, Germany
Inge.Broer@uni-rostock.de

Jennifer Bromley
British American Tobacco, UK
jennifer_bromley@bat.com

Johannes Felix Buyel
Fraunhofer IME, Germany
johannes.buyel@ime.fraunhofer.de

Katarina Cankar
Wageningen University, The Netherlands
katarina.cankar@wur.nl

Maria Teresa Capell
Universitat de Lleida, Spain
teresa.capell@pvcf.udl.cat

Aleyo Chabeda
University of Cape Town, South Africa
aleyo.chabeda@gmail.com

Qiang Chen
Arizona State University, USA
qiang.chen.4@asu.edu

Paul Christou
Universitat de Lleida, Spain
christou@pvcf.udl.cat

María Coca
CRAG, Spain
maria.coca@cragenomica.es

Udo Conrad
IPK Gatersleben, Germany
conradu@IPK-gatersleben.de

David Craik
The University of Queensland, Australia
d.craik@imb.uq.edu.au

Marc-Andre D´Aoust
Medicago Inc., Canada
daoustma@medicago.com

Philippe Dehottay
GSK, Belgium
philippe.dehottay@gsk.com

Christina Dickmeis
RWTH Aachen University, Germany
Christina.Dickmeis@molbiotech.rwth-aachen.de

Marcello Donini
ENEA, Italy
marcello.donini@enea.it

Parastoo Ehsani
Pasteur Institute of Iran, Iran
p_ehsani@yahoo.com

Arto Forsberg
VTT, Finland
arto.forsberg@vtt.fi

Noriho Fukuzawa
A.I.S.T, Japan
noriho-fukuzawa@aist.go.jp

Benjamin Bruno Gengenbach
Fraunhofer IME, Germany
benjamin.gengenbach@ime.fraunhofer.de

Jared Gerlach
National University of Ireland, Ireland
jared.gerlach@nuigalway.ie

Khadijeh Ghasemian
Rostock University, Germany
khadijeh.ghasemian@uni-rostock.de

Edward Gilding
The University of Queensland, Australia
e.gilding@imb.uq.edu.au

Anatoli Giritch
Nomad Bioscience GmbH, Germany
giritch@nomadbioscience.com

Marie-Claire Goulet
Université Laval, Canada
marie-claire.goulet@fsaa.ulaval.ca

Melanie Grandits
St George's University of London, UK
p1607569@sgul.ac.uk

Cornelius Gunter
University of Cape Town, South Africa
corriegunter@gmail.com

Noemi Gutierrez Valdes
VTT, Finland
ext-noemi.gutierrezvaldes@vtt.fi

Simone Hahn-Löbmann
Nomad Bioscience GmbH, Germany
hahn@nomadbioscience.com

Céline Hanani
VTT, Finland
celine.hanani@vtt.fi

Inga Hitzeroth
University of Cape Town, South Africa
inga.hitzeroth@uct.ac.za

Matthijs Hoelscher
MPIMP, Germany
hoelscher@mpimp-golm.mpg.de

Tanja Holland
Inofficial ISPMF Fanclub, Germany
Tanja-Holland@web.de

Nicholas Holton
Leaf Expression Systems, UK
holtonn@leafexpressionsystems.com

Hannu Hotti
VTT, Finland
ext-hannu.hotti@vtt.fi

Sandy Hwei-Sanloh
The University of Nottingham, Malaysia
sandy.loh@nottingham.edu.my

Suvi Häkkinen
VTT, Finland
suvi.hakkinen@vtt.fi

Katja Härtl
Technical University of Munich, Germany
katja.haertl@tum.de

Mark Jackson
The University of Queensland, Australia
m.jackson1@uq.edu.au

Julia Jansing
RWTH Aachen University, Germany
julia.zischewski@molbiotech.rwth-aachen.de

Jussi Joensuu
VTT, Finland
jussi.joensuu@vtt.fi

Selvaraju Kanagarajan
SLU, Sweden
selvaraju.kanagarajan@slu.se

YangJoo Kang
Chung-Ang University, South Korea
yangjoo33@naver.com

Franziska Kellner
Leaf Expression Systems, UK
kellnerf@leafexpressionsystems.com

Mi-Young Kim
Chonbuk National University, South Korea
mkim@jbnu.ac.kr

Seong-Ryong Kim
Sogang University Korea
sungkim@sogang.ac.kr

Deuk-Su Kim
Chung-Ang University, South Korea
verymana@naver.com

Kisung Ko
Chung-Ang University, South Korea
ksko@cau.ac.kr

Lilya Kopertekh
Julius Kühn-Institut (JKI), Germany
lilya.kopertekh@julius-kuehn.de

Elviira Kärkkäinen
VTT, Finland
elviira.karkkainen@vtt.fi

Eija Lehmuskallio
NatureGate Promotions Finland
eija@naturegate.net

Jouko Lehmuskallio
NatureGate Promotions Finland
jouko@naturegate.net

Camille Lemasson
Root Lines Technology, France
camille.lemasson@rootlines-tech.com

Efraim Lewinsohn
The Volcani Center, Israel
twefraim@agri.gov.il

George Lomonossoff
John Innes Centre, UK
george.lomonossoff@jic.ac.uk

Julian Ma
St. George's University of London, UK
jma@sgul.ac.uk

Maricarmen Martí
Universidad Politécnica de Valencia, Spain
mcmarti@ibmcp.upv.es

Coby Martin
The University of Western Ontario, Canada
cmart245@uwo.ca

Silvia Massa
ENEA, Italy
silvia.massa@enea.it

Luis Matias
Sequentia Biotech, Spain
lmatias@sequentiabiotech.com

Siv Matomaa
VTT, Finland
siv.matomaa@vtt.fi

Takeshi Matsumura
A.I.S.T. Japan
matsumura-t@aist.go.jp

Kouki Matsuo
A.I.S.T Japan
matsuo-kouki@aist.go.jp

Andrew Matthews
INNO-3B inc., Canada
amatthews@inno-3b.com

Sandiswa Mbewana
University of Cape Town, South Africa
sandiswa.mbewana@uct.ac.za

Rosemary Meggersee
University of Cape Town, South Africa
rosemary.meggersee@gmail.com

Rima Menassa
Agriculture and Agr-Food Canada, Canada
rima.menassa@agr.gc.ca

Sébastien Mercx
Université Catholique de Louvain (UCL), Belgium
sebastien.mercx@uclouvain.be

Ann Meyers
University of Cape Town, South Africa
ann.meyers@uct.ac.za

Dominique Michaud
Universite Laval, Canada
dominique.michaud@fsaa.ulaval.ca

Sissi Miguel
Plant Advanced Technologies, France
contact@plantadvanced.com

Dominik Mojzita
VTT, Finland
dominik.mojzita@vtt.fi

Cathy Moore
St. George's University of London, UK
cmoore@sgul.ac.uk

Kristiina Mäkinen
University of Helsinki, Finland
kristiina.makinen@helsinki.fi

Somen Nandi
University of California, USA
snandi@ucdavis.edu

Henrik Nausch
University of Rostock, Germany
henrik.nausch@uni-rostock.de

Catherine Navarre
Universite Catholique de Louvain, Belgium
catherine.navarre@uclouvain.be

Greta Nölke
Fraunhofer IME, Germany
greta.noelke@ime.fraunhofer.de

Hamideh Ofoghi
IROST, Iran
ofoghi@irost.ir

Georgianna Kae Oguis
The University of Queensland, Australia
g.oguis@imb.uq.edu.au

Kirsi-Marja Oksman-Caldentey
VTT, Finland
kirsi-marja.oksman@vtt.fi

Diego Orzaez
IBM CP-CSIC, Spain
dorzaez@ibmcp.upv.es

Sarunas Paskevicius
UAB Nomads, Lithuania
paskevicius.sarunas@gmail.com

Frank Petersen
Novartis Pharma AG, Switzerland
frank.petersen@novartis.com

Hadrien Peyret
John Innes Centre, UK
hadrien.peyret@jic.ac.uk

Trong Hoang Phan
IPK Gatersleben, Germany
hoang@ipk-gatersleben.de

Elizabeth Pinneh
St. George's University of London, UK
epinneh@sgul.ac.uk

Daniel Ponndorf
John Innes Centre, UK
daniel.ponndorf@jic.ac.uk

Riitta Puupponen-Pimiä
VTT, Finland
riitta.puupponen-pimia@vtt.fi

Haiou Qu
Institute for Molecular Bioscience, Australia
h.qu@imb.uq.edu.au

Emile Rage
ENEA, Italy
emile.rage@gmail.com

Kaewta Rattanapisit
Chulalongkorn University, Thailand
som_kaewza@hotmail.com

Nicole Raven
Fraunhofer IME, Germany
nicole.raven@ime.fraunhofer.de

Lauri Reuter
VTT, Finland
lauri.reuter@vtt.fi

Jaana Rikkinen
VTT, Finland
jaana.rikkinen@vtt.fi

Heiko Rischer
VTT, Finland
heiko.rischer@vtt.fi

Anneli Ritala
VTT, Finland
anneli.ritala@vtt.fi

Tarik Ruiz
CRAG, Spain
tarik.ruiz@cragenomica.es

Juliane Röder
RWTH Aachen University, Germany
Juliane.Roeder@molbiotech.rwth-aachen.de

Markus Sack
Pro-SPR GmbH, Germany
markus.sack@pro-spr.com

Kazuki Saito
Chiba University & RIKEN, Japan
ksaito@faculty.chiba-u.jp

Mattia Santoni
University of Verona, Italy
mattia.santoni@univr.it

Andreas Schaaf
Greenovation, Germany
aschaaf@greenovation.com

Joachim Schiemann
Julius Kühn Institut, Germany
joachim.schiemann@julius-kuehn.de

Stefan Schillberg
Fraunhofer IME, Germany
stefan.schillberg@ime.fraunhofer.de

Sara Selma García
IBMCP, Spain
saselgar@doctor.upv.es

Jun-Hye Shin
Université de Toulouse, France
junhyeshin@gmail.com

Yoram Shotland
SCE Shamoon College of Engineering, Israel
yshotlan@sce.ac.il

Samuel Smith
St. George's University of London, UK
sasmith@sgul.ac.uk

Bronwyn Smithies
Institute for Molecular Bioscience, Australia
b.smithies@imb.uq.edu.au

Eberhard Sorge
IPK Gatersleben, Germany
sorge@ipk-gatersleben.de

John Steele
John Innes Centre, UK
john.steele@jic.ac.uk

Åke Strid
University of Örebro, Sweden
ake.strid@oru.se

Sara Sukenik
University of California, USA
scsukenik@ucdavis.edu

Solomia Sumyk
VTT, Finland
ext-solomia.sumykmaletych@vtt.fi

Guangxin Sun
Technical University of Munich, Germany
guangxin.sun@tum.de

Tim Szeto
St. George's University of London, UK
tszeto@sgul.ac.uk

Teemu Teeri
University of Helsinki, Finland
teemu.teeri@helsinki.fi

Audrey Teh
St. George's University of London, UK
ateh@sgul.ac.uk

Tuuli Teikari
VTT, Finland
tuuli.teikari@vtt.fi

Arja Tervahauta
University of Eastern Finland, Finland
arja.tervahauta@uef.fi

Eva Thuenemann
John Innes Centre, UK
eva.thuenemann@jic.ac.uk

Oguz Top
University of Freiburg, Germany
oguz.top@sgbm.uni-freiburg.de

David Ullisch
Phyton Biotech GmbH, Germany
david.ullisch@phytonbiotech.com

Sophie van Gorkom
Janssen Vaccines and Prevention B.V.,
The Netherlands
sophie.vangorkom@gmail.com

Kim van Noort
Wageningen University, The Netherlands
kim.vannoort@wur.nl

Philippe Varennes-Jutras
University of Oxford, UK
philippe.varennes-jutras@plants.ox.ac.uk

Heribert Warzecha
Technische Universität Darmstadt, Germany
warzecha@bio.tu-darmstadt.de

Jennifer Wayland
University of Cape Town, South Africa
jennifer-wayland@hotmail.com

Stefan Werner
Nambawan Biotech GmbH Germany
werner@nambawanbiotech.com

Moonsik Yang
Chonbuk National University,
Republic of Korea
mskyang@jbnu.ac.kr

Lingping Zhu
University of Helsinki, Finland
lingping.zhu@helsinki.fi

Author index

E

F

G

H

J

K

L

M

N

O

P

Q

R

S

Series title and number

VTT Technology 342

Title	**The 3rd Conference of the International Society for Plant Molecular Farming** June 11-13, 2018: Book of abstracts
Author(s)	Kirsi-Marja Oksman, Anneli Ritala, Heiko Rischer, Jussi Joensuu, Suvi Häkkinen & Päivi Vahala (Eds.)
Abstract	The 3rd Conference of the International Society of Plant Molecular Farming will take place in the city of Helsinki, Finland, from the 11th to the 13th of June, 2018. The topics addressed by the ISPMF Conference will cover the whole palette of interests for the Plant Molecular Farming community, presenting all possible aspects of recombinant proteins and bioactive small molecules. Besides nine keynote speakers, we have selected 36 oral presentations and 73 poster presentations. The Conference will provide a delightful forum for 150 attendees to improve their scientific knowledge and explore the latest outcomes and trends in the field. It will offer plenty of networking opportunities, interaction with leading scientists, and stimulating creative exchange among researchers and people working in the respective industries. More information about ISPMF, please visit the website http://societyformolecularfarming.org
ISBN, ISSN, URN	ISBN 978-951-38-8674-5 (URL: http://www.vttresearch.com/impact/publications) ISSN-L 2242-1211 ISSN 2242-122X (Online) http://urn.fi/URN:ISBN:978-951-38-8674-5
Date	June 2018
Language	English
Pages	212 p.
Name of the project	
Commissioned by	
Keywords	
Publisher	VTT Technical Research Centre of Finland Ltd P.O. Box 1000, FI-02044 VTT, Finland, Tel. 020 722 111

The 3rd Conference of the International Society for Plant Molecular Farming

June 11-13, 2018: Book of abstracts

ISBN 978-951-38-8674-5 (URL: http://www.vttresearch.com/impact/publications)
ISSN-L 2242-1211
ISSN 2242-122X (Online)
http://urn.fi/URN:ISBN:978-951-38-8674-5