Topic/Title (Norwegian)

Produksjon og molekylære analyser av hepatitt C virus (HCV) vaksineantigener med CRIPSR genomredigert *Nicotiana benthamiana*

Kortnavn: Grönn CRISPR anti-viral lösning GC-AVL

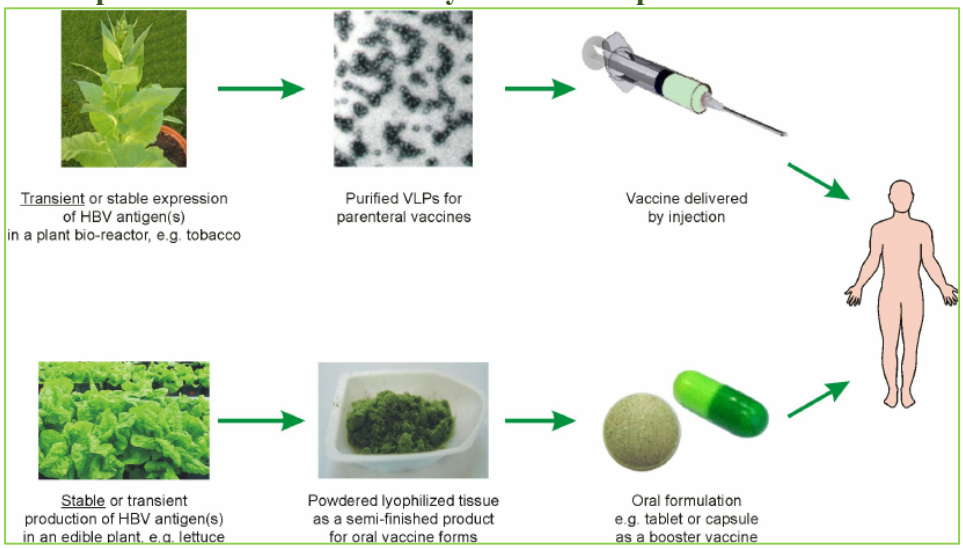
Topic/Title (English)

Production and molecular characterization of hepatitis C viral vaccine antigens derived from CRISPR genome edited *N. benthamiana* plants

Short name: Green CRISPR anti-viral leaves GC-AVL

Picture

Green plants are the ideal factory for low cost production of human vaccines



Summary (Describe the topic/thesis, type of thesis work: field work, laboratory work, literature study)

Chronic infection with hepatitis C virus (HCV) remains a leading cause of liver-related pathologies and is a global health problem, currently affecting more than 71 million people worldwide. The development of a prophylactic vaccine is much needed to complement the current antiviral treatment available and possibly achieve HCV eradication. Traditionally vaccine antigens are produced in bacteria, chicken eggs, and mammalian cells. The use of plants for the development and production of recombinant human vaccines was established about three decades ago and offers several advantages as following. A) Green plants use free solar energy and capture CO_2 making them environmentally friendly vaccine production platforms compared to the energy-demanding fermenter-based and greenhouse gas emission-burdened animal-based vaccine production platforms. B) Plant-based systems are more economical as plants can more readily be upscaled than other systems such as mammalian cells cultures. C) They lack the undesirable components found in conventional systems, e.g. endotoxins in bacteria, hyperglycosylated proteins produced by yeast, and human/animal pathogens in mammalian cell cultures and transgenic livestock. D) There are no limits to the production scale and the cost of scaling up is low, in contrast to mammalian cells where media costs increase in line with the production scale and eliminate any economic benefits of large-scale manufacturing. E) Plant-based systems are highly versatile. Plants also possess the ability to carry out post-translational modifications similar to naturally occurring systems. F) The

plant-based systems bypass the safety concerns inherent in live virus vaccines (Clark et al. 2017; Dobrica et al. 2021; Fausther-Bovende and Koblinger 2021).
Nevertheless, the HCV antigens to be produced are glycoproteins, and there are some differences between plant and mammalian glycosylation patterns. In particular, plants add an extra fucose and xylose to the core N-linked glycans. This may affect the immunological properties of the antigens. To overcome this potential challenge, a CRISPR/Cas9-mediated double knockout *N. benthamiana* line can be generated (Jansing et al. 2019), inactivate the glycosyltransferases that otherwise add the xylose and fucose residues to the core glycans. The resulting glycans should closely resemble the glycans produced in human hosts with an anticipated outcome of enhanced immunogenicity of HCV antigens.

The MSc thesis work aims to train the MSc candidate in plant biotechnology and molecular biology covering plasmid vector preparation, bacterial culture, *Agrobacterium*-mediated transient expression, DNA and RNA isolation, PCR and protein analysis. The thesis involves mainly laboratory work with some greenhouse work on *Agrobacterium*-mediated agroinfiltration and sample collection post agroinfiltration in addition to thesis writing.

Subject area (keywords)

Plant biotechnology, plant-made vaccine antigen, human health, CRISPR/cas9 gene editing

Language thesis (Norwegian and/or English)
English

Bachelor or Master thesis
MSc thesis

Credits
60

Project/company
NMBU and NIBIO

Please contact

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References:

1. Jihong Liu Clarke*, Lisa Paruch, Mihaela-Olivia Dobrica, Iuliana Caras , Catalin Tucureanu, Adrian Onu, Sonya Ciulean, Stavaru Crina, Andre Eerde, Hege Steen, Sissel Haugslien, Catalina Petrareanu, Catalin Lazar, Ioan Popescu, Ralph Bock, Jean Dubuisson, Norica Branza-Nichita (**2017**) Lettuce-produced hepatitis C virus E1E2 heterodimer triggers immune response in mice and antibody production post oral vaccination. ***Plant Biotechnology J.*** DOI: 10.1111/pbi.12743
2. Dobrica, Mihaela-Olivia; van Eerde, André; Tucureanu, Catalin; Onu, Adrian; Paruch, Lisa; Caras, Iuliana; Vlase, Ene; Steen, Hege; Haugslien, Sissel; Alonzi, Dominic; Zitzmann, Nicole; Bock, Ralph; Dubuisson, Jean; Popescu, Costin-Ioan; Stavaru, Crina; Clarke, Jihong Liu; Branza-Nichita, Norica (**2021**) Hepatitis C virus E2 envelope glycoprotein produced in Nicotiana benthamiana triggers humoral response with virus-neutralizing activity in vaccinated mice. ***Plant Biotechnology J*** doi: 10.1111/pbi.13631
3. Hugues Fausther-Bovende and Gary Koblinger (**2021**) Plant-made vaccines and therapeutics. ***Science*** 373 issue 6556, 13 August 2021

4. Julia Jansing, Markus Sack, Sruthy Maria Augustine, Rainer Fischer, Luisa Bortesi (**2019**) CRISPR/Cas9-mediated knockout of six glycosyltransferase genes in Nicotiana benthamiana for the production of recombinant proteins lacking β-1,2-xylose and core α-1,3-fucose. ***Plant Biotechnol J*** doi: 10.1111/pbi.12981