Transient Recombinant Protein Production in Glycoengineered Plant Cell Suspension Cultures

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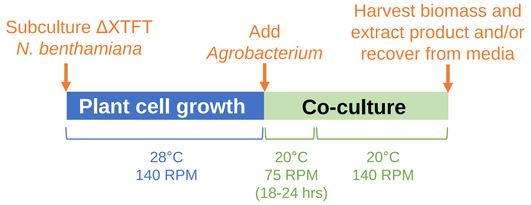
Background

- **Transient recombinant protein production systems are well-suited for emergency situations where rapid production of novel protein therapeutics is needed**
  - In plants, *Agrobacterium tumefaciens* can be used as a gene delivery vector for transient expression
  - *Agrobacterium* vectors can be made in as little as two weeks, reducing the initial development time required before large-scale production of novel recombinant proteins
- **Controlling glycosylation patterns can enhance the efficacy and safety of therapeutic proteins**
- To reduce plant-specific glycans, β1,2-xylosyltransferase and α1,3-fucosyltransferase knockdown (ΔXTFT) *Nicotiana benthamiana* plants have been generated
  - Removing plant-specific glycan patterns reduces the risk of an adverse patient immune response
  - In animal studies, antibodies produced in ΔXTFT *Nicotiana benthamiana* plants also showed enhanced efficacy compared to antibodies produced in Chinese hamster ovary (CHO) cells[1]

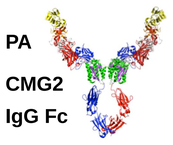
Materials and Methods

Project objectives:
1) Generate cell suspension cultures from ΔXTFT *Nicotiana benthamiana* plants
2) Transiently produce and characterize an anthrax toxin receptor-Fc fusion protein (CMG2-Fc) using glycoengineered plant cell cultures

Co-culture process overview



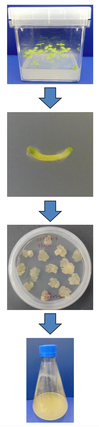
CMG2-Fc mechanism of action:
- Inhibits binding of anthrax protective antigen (PA) to endogenous CMG2 receptors on human cells
- Soluble CMG2 has been shown to protect CHO cells from cell death resulting from PA treatment[2]
- CMG2 was fused to Fc region of human IgG to increase circulatory half-life, enable clearance of PA by the immune system, and simplify purification



Model of CMG2-Fc protein dimer, bound to anthrax protective antigen (PA)

Source: Planet Biotechnology

Generation and characterization of ΔXTFT *Nicotiana benthamiana* plant cell suspension cultures



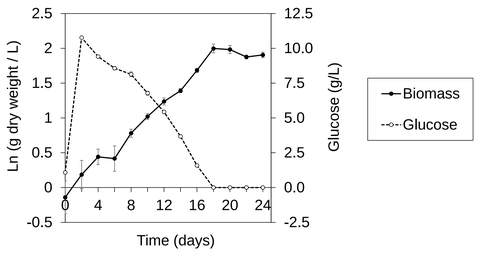
Aseptically grown ΔXTFT *N. benthamiana* plants

Explant on semi-solid media

Dedifferentiated callus grown on semi-solid media

ΔXTFT *N. benthamiana* cells in suspension culture

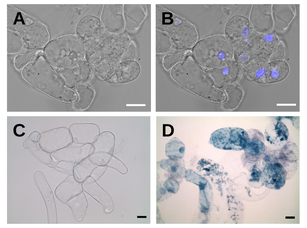
Growth Kinetics



Initial biomass concentration[1] (g dry weight/L)	Maximum biomass concentration[1] (g dry weight/L)	Maximum specific growth rate[2] (day^{-1})	Yield coefficient[2] (g dry weight biomass/ g glucose)
0.9 ± 0.2	7.4 ± 0.5	0.113 ± 0.004	0.55 ± 0.04

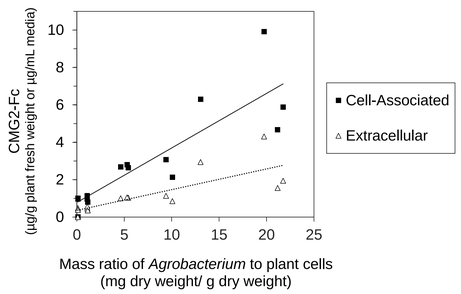
[1] Average ± standard deviation of triplicate samples.
[2] Value ± standard error for linear regression coefficient.

Cell Culture Morphology

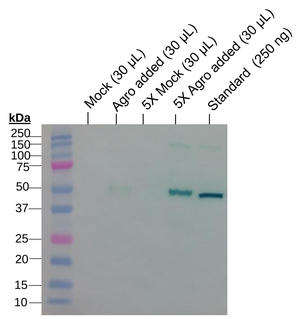


Scale bars indicate 20 µm.
(A, B) Same field of view
(B) Nuclear staining with DAPI
(C) Cells stained with Evans blue
(D) Cells treated with 70% ethanol prior to Evans blue staining

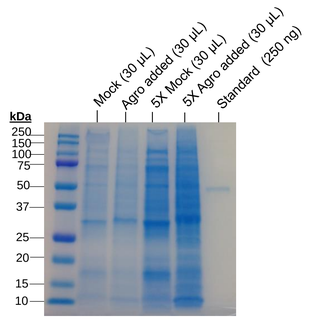
Transient production of CMG2-Fc in glycoengineered plant cell suspension cultures



- After 7 days of co-culture, CMG2-Fc expression was quantified by ELISA
- Co-culture was performed in 3 separate flasks for each mass ratio target
- Extracellular CMG2-Fc observed despite addition of an ER retention signal



Western Blot



SDS-PAGE

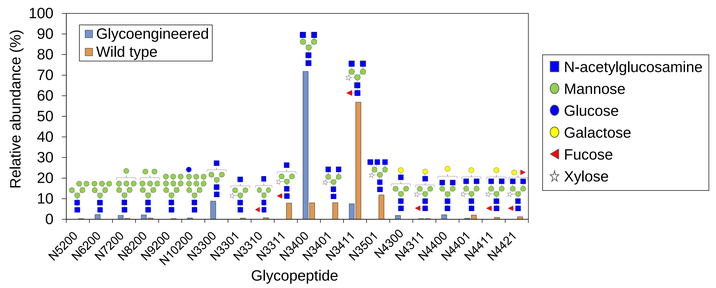
- Strong band at expected molecular weight observed after 5 fold concentration (5X) of biomass extract from highest expressing flask

N-glycan analysis of CMG2-Fc produced in glycoengineered plant cell suspension cultures

Transient Production
- An *Agrobacterium* vector targeting CMG2-Fc extracellularly was used to allow more complex glycoforms

Purification
- Cell-associated CMG2-Fc was purified using Protein A chromatography

N-glycan Analysis
- CMG2-Fc has only one N-glycosylation site, which is located on the Fc portion
- N-glycan analysis was performed on cell-associated CMG2-Fc using LC-MS/MS



- **Glycoengineered** = CMG2-Fc produced in ΔXTFT *N. benthamiana* cell cultures
- **Wild type** = CMG2-Fc produced in wild type *N. benthamiana* plants
- Glycopeptide labels indicate the number of monosaccharides in N-linked glycans in the following order: hexose (mannose, glucose, and galactose), N-acetylglucosamine, fucose, and xylose.

Conclusions

- Plant cell suspension cultures generated from ΔXTFT *Nicotiana benthamiana* plants had a maximum specific growth rate of 0.113 day^{-1}
- An anthrax toxin receptor – Fc fusion protein was transiently produced in glycoengineered plant cell cultures
  - Expression levels up to 10 µg/g plant fresh weight were observed
  - Increasing mass ratio of *Agrobacterium* to plant cells enhanced expression levels
- Reduced levels of plant-specific glycans were observed on CMG2-Fc produced in the glycoengineered cell suspension cultures

Future Work

- Strategies to increase expression levels will be implemented, such as using a bioreactor to better control and optimize plant cell growth
- Simulating the process at commercial scale using SuperPro Designer software will identify target yield and recovery levels for cost-effective manufacturing
- To demonstrate the flexibility of this platform, other recombinant proteins will be produced by using different *Agrobacterium* vectors
- Additional genetic engineering of the host cell line could further enhance a product's safety and efficacy by tuning its N-glycan distribution

Acknowledgments

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- Nomad Bioscience GmbH provided ΔXTFT *N. benthamiana* seeds
- Planet Biotechnology, Inc. provided the CMG2-Fc standard and the ER retained CMG2-Fc *Agrobacterium* construct

References

1. Zeitlin, L. et al. Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc. Natl. Acad. Sci. U. S. A.* 2011, *108* (51), 20690-20694.
2. Scobie, H. M. et al. Human capillary morphogenesis protein 2 functions as an anthrax toxin receptor. *Proc. Natl. Acad. Sci. U. S. A.* 2003, *100* (9), 5170-5174.