Antibody Engineering for Greenhouse Protection

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At A Glance

Pythium, *Phytophthora* and *Fusarium* fungi excrete toxins that account for 80% of epidemics in plants grown in greenhouses (Schuerger, 1998)

Increased disease risk due to new environmental law mandating nutrient solution recycling

Spacecraft & ALS systems are contaminated with many microorganisms, including *Pythium* (Novikova, 2001; Jenkins, 2000)

Small antibody fragments can bind the excreted toxins

Objectives

Develop antibodies to the *Pythium aphanidermatum* Necrosis inducing elicitor (PaNie) using ribosome display

Increase affinity of antibodies by directed *in vitro* molecular evolution

Insert antibody gene in greenhouse plants to protect plants from pathogens

Introduction

Root rot and blight results in considerable revenue loss for greenhouse growers

- Expected to rise due to 2002 Ontario Nutrient Management Act mandating nutrient recycling

Pythium aphanidermatum is the most common cause of root-rot and blight in Ontario.

- It spreads without notice and can form spores

A B C

Figure 1. Root rot and blight caused by *P. aphanidermatum* in pepper (A), tomato (B) and cucumber (C)

Pythium aphanidermatum Necrosis inducing elicitor (PaNie) causes cell death in plants such as pepper, tomato and cucumber (Veit, 2001)

PaNie is a protein with up to 71% homology to other elicitors from *Phytophthora* and *Fusarium*

Functional PaNie can be produced in *E.coli*

Ribosome Display

Blood from llama, containing antibody producing lymphocytes, was collected and antibody-DNA amplified

A 'library' of antibody-DNAs was constructed (Figure 2A)

Transcription and translation results in the production of a Antibody-Ribosome-mRNA (ARM) complex

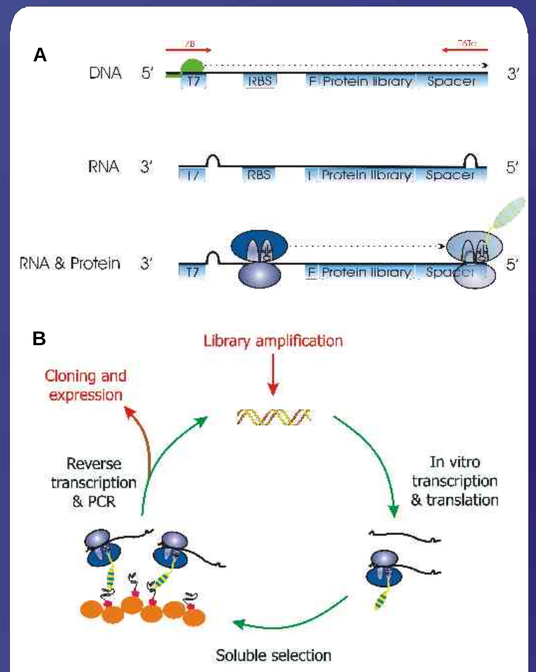


Figure 2. Principles of Ribosome Display. (A) The antibody repertoire of the llama inserted in a library framework containing elements necessary for transcription (T7) and translation (Ribosome Binding Site and spacer). (B) A round of soluble selection including formation of ARM complexes, selection on magnetic antigen coated beads and PCR-based recovery of an enriched library.

Soluble selection illustrated in Figure 2B:

- Stringent washing removes low affinity binders from beads coated with antigen (binding partner)

Genotype of binders recovered by reverse transcription

Cloning and expression is conducted in *E.coli*

Enzyme Linked Immunosorbent Assay (ELISA) to determine affinity

In vitro molecular evolution strategies to improve affinity

- DNA shuffling and/or error-prone PCR

Transgenics

Gene codon optimisation and promoter design

Agrobacterium-mediated transformation of tomato, pepper & cucumber

Characterisation of plant-borne resistance

Determination of transgenic stability

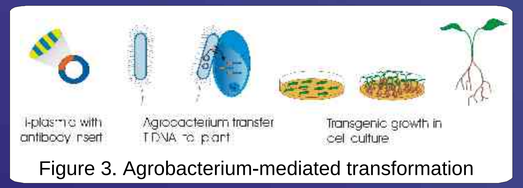


Figure 3. Agrobacterium-mediated transformation

Commercial Value

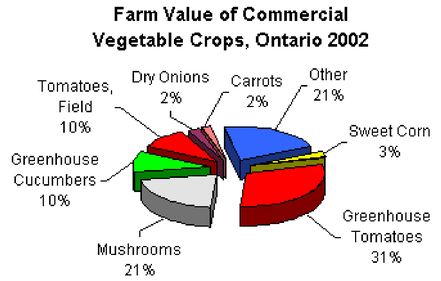
2002 Greenhouse crop sales exceeded $ 2B in Canada and $ 1B in Ontario alone (OMAF, 2003)

Antibodies can be used in an increasing level of sophistication

i. Pre-symptom disease detection
ii. Toxin filtering/chelation
iii. Plant-borne resistance by local production of antibodies
iv. Nanobody-fungicide fusion for very specific action

Flowers Canada and Toxin Alert can convert the technology into some or all levels above for various crops.

- Creating jobs in these areas of the crop protection industry
- Setting the stage for other companies to adapt the concept for fringe crops.



Greenhouse Tomatoes	$ 217M	Sweet Corn	$ 22M
Mushrooms	$ 150M	Carrots	$ 16M
Greenhouse Cucumbers	$ 75M	Dry Onions	$ 16M
Field Tomatoes	$ 71M	Other	$ 153M

Figure 4. Value of Vegetable crops in Ontario 2002 (OMAF, 2003)

Marketability

Existing partnerships each support specific stages of R&D and will help in marketing thereof

- Toxin Alert is a dedicated partner supporting the early stages of research; e.g. antibody production
- Flowers Canada will be involved in the development of later stages of research; e.g. transgenic development

In addition to greenhouse vegetables, flowers and plants, turfgrass is heavily affected by *Pythium* infections

- Links with Guelph Turfgrass Institute exist

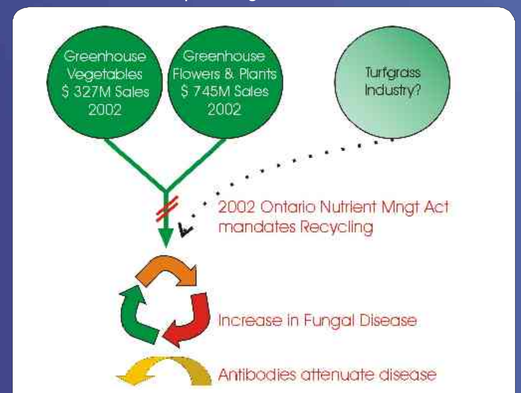


Figure 5. Markets affected by new environmental legislation and increasing fungal disease.

Results & Conclusion

Specific antibodies to elicitors or other plant toxins could be produced in a matter of weeks

PaNie has been successfully cloned and expressed in *E.coli*

Five rounds of successful Ribosome Display completed

- Specific binding of antibodies to PaNie observed through competitive selection
- Binders with high affinity have been isolated

>40% of vegetable crops can benefit from antibodies

Water and nutrient recycling regimes mandated by the government, impose greater need for protection

- Antibodies allow greenhouse growers to stay competitive, while adhering to environmental standards
- Antibodies can give reliability to space-based life support, where infections must be avoided at all costs

References

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