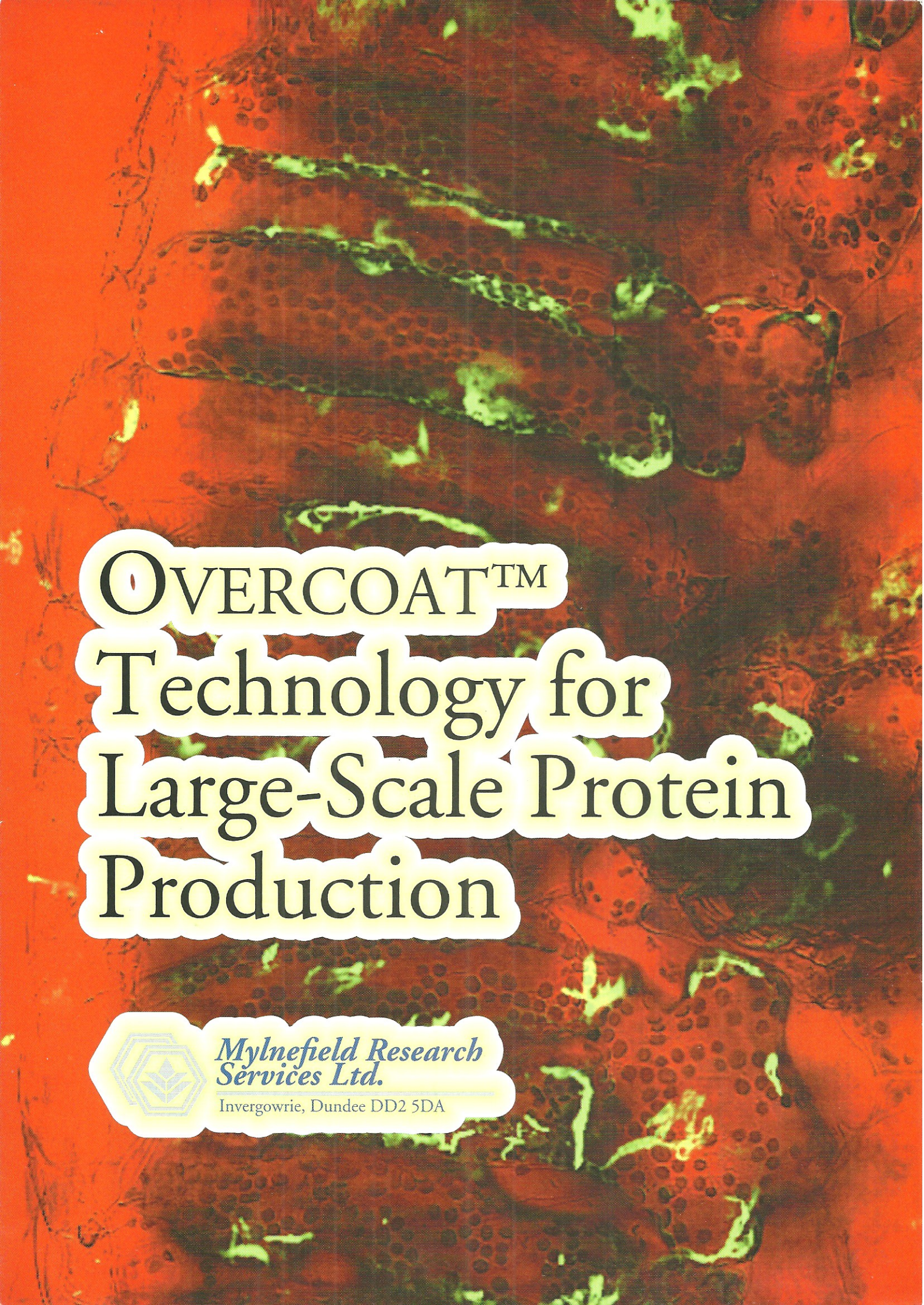


OVERCOAT™ Technology for Large-Scale Protein Production

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The Scottish Crop Research Institute

The Scottish Crop Research Institute (SCRI) is a major international centre for research on agricultural, horticultural and industrial crops.

A broad multidisciplinary approach to research and development is a special strength of the Institute. The range of skills available, from fundamental studies on genetics, molecular biology and physiology, through agronomy and pathology to glasshouse and field trials, is unique within the UK research service.

Introduction

Many plant viruses, and the protein products which they encode, accumulate rapidly and to very high levels following infection of host plants. The speed with which virus infections become established throughout a plant, and the high yield of virus-encoded proteins, makes virus-based vector systems an attractive and cost-effective route for the over-production of valuable proteins.

We have shown recently that potato virus X (PVX; Figure 1a), a filamentous plant virus, can be used as a vector for the production of foreign proteins in plants.

PVX is a single-stranded positive-sense RNA virus that infects over 200 plant species. The virus particle comprises a helical array of identical coat protein (CP) subunits wound around the viral nucleic acid. For wild-type virus, approximately 1300 CP subunits are incorporated into each virus particle. Electron microscopic analysis of PVX-infected tissues reveals the presence of organised, crystal-like aggregates of virus particles occupying a large proportion of the cytoplasm in infected cells. These structures are important in allowing high level accumulation of the virus away from cellular proteases which might otherwise degrade them.

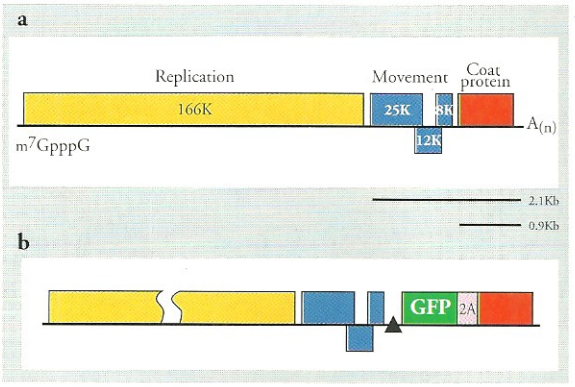


Figure 1a Genetic map of potato virus X. **b** GFP- CP potato virus X vector carrying the GFP - coat protein fusion.

The Experiment

In our novel experimental system we first fused the green fluorescent protein (GFP) from the jellyfish *Aqueorea victoria via* its C-terminus to the N-terminus of the PVX coat protein using the 2A 'protease' from foot-and-mouth disease virus (FMDV) as a linker between the passenger ('OVERCOAT™') protein and the PVX CP (Figure 1b).

Fusion of GFP to the viral coat protein affected neither the ability of the virus to replicate, nor to move systemically throughout the plant. Inoculation of plants with the modified virus (PVX.GFP-CP; Figure 1b) led to complete infection of both inoculated and systemic leaves (Figure 2).



Figure 2 Virus-infected plant expressing GFP visible under UV light.

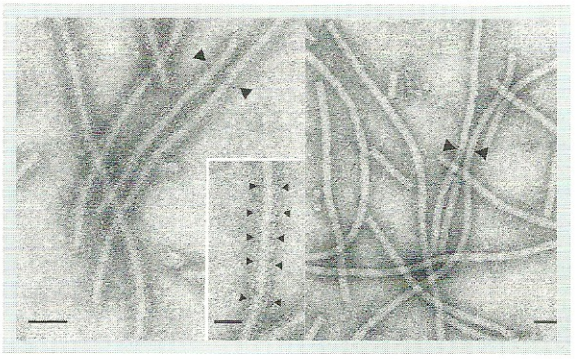


Figure 3 OVERCOAT™ virions (left panel) are over twice the diameter of normal PVX (right panel).

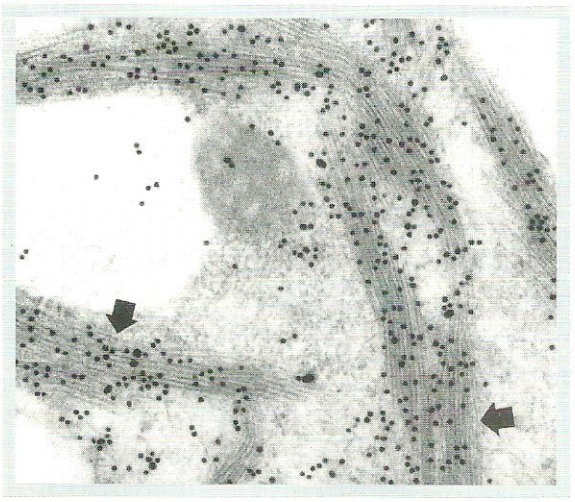


Figure 4 Paracrystalline virus arrays decorated with GFP antibodies.

Under the confocal microscope, the fluorescent virus was aggregated into organised, paracrystalline arrays (viroplasms) which were easily detected within individual cells (see cover and Figure 4).

In the electron microscope, the PVX.GFP-CP virus particles were shown to be over twice the diameter (29.6 nanometres) of normal PVX (12.6 nanometres) due to the outer layer of GFP over the protein coat (Figure 3, left panel). Immunochemistry, using antisera specific to either PVX CP or GFP, confirmed that the assembled virus coat had indeed been altered to accommodate the GFP (Figure 4).

The above studies, using GFP, illustrate the potential for decorating the coat of filamentous plant viruses with a broad range of foreign proteins (or peptides) and accumulating these to **very** high concentrations within plants. Over a thousand copies of the foreign OVERCOAT™ protein can be harvested from each virus particle.

Prospects for PVX-OVERCOAT™ technology

- Vaccines (continuous, discontinuous or multiple epitopes)
- Therapeutic proteins
- Industrial enzymes
- Phyto-bioremediation
- Biocatalysts
- Added nutritional value
- Crop protection

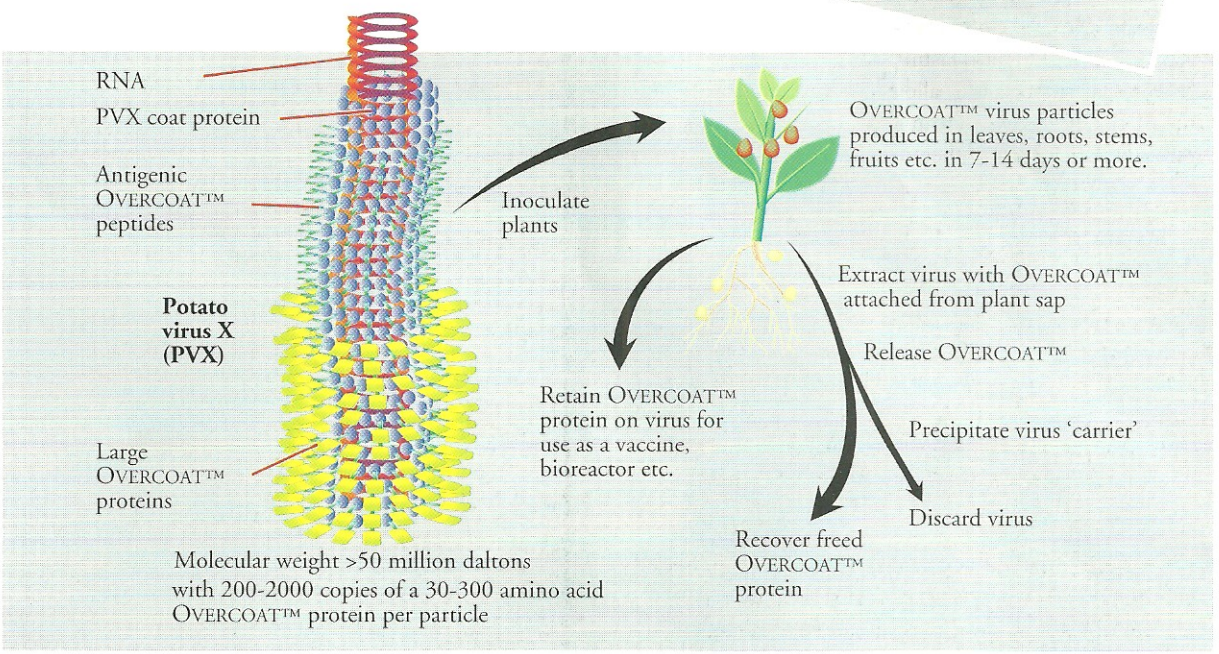


Figure 5 A sketch depicting the main features of this novel, generic platform technology to convert plants into cheap and efficient protein factories.

The Advantages

The OVERCOAT™ strategy has a number of attractive advantages over conventional approaches for the production of novel proteins.

- PVX has a broad host range allowing a choice of local or edible plants for the production of unique proteins.
- PVX accumulates to high levels in infected plants (OVERCOAT™ protein levels can exceed 20% of total soluble leaf protein) and yields of virus particles can exceed 1-2 grams per kilogram fresh (wet) weight of plant tissue.
- Purification of the foreign OVERCOAT™ protein from infected plant sap can be achieved simply through selective precipitation and low speed centrifugation of the carrier virus. The OVERCOAT™ protein could then be cleaved and separated from the virus, if desired.
- Because PVX is a filamentous rod there is no *a priori* constraint on the length of the modified RNA genome to be encapsidated, thus large OVERCOAT™ gene sequences can be inserted.
- PVX virus particles are stable over a wide pH range, resistant to a variety of chemical treatments and offer the possibility of extensive surface presentation of large discontinuous epitopes for use as multivalent vaccines.

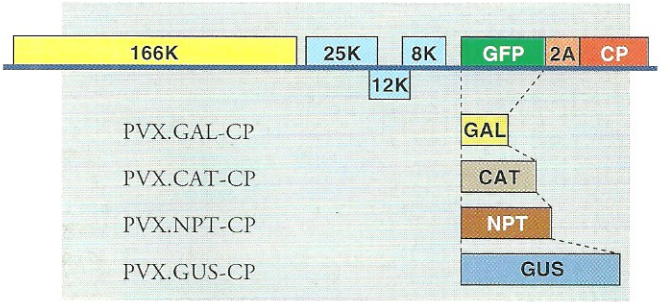


Figure 6 Genome organisation of PVX.GFP-CP and some OVERCOAT™ - coat protein fusion vectors.

- Engineering of the FMDV 2A protease allows the ratio of fused (OVERCOAT™) to unfused (CP) protein produced during virus infection to be controlled. We have shown that a mixed pool of large OVERCOAT™ fusion protein and free CP is a prerequisite for the successful assembly of virus particles. Virus particle formation from fusion protein alone is hindered by steric effects. Thus OVERCOAT™ proteins so far up to 32,000 daltons can be fused to 50% of the PVX CP (25,000 daltons) subunits in a single virus particle.
- So far, four unrelated OVERCOAT™ proteins (Figure 6) and two antigenic OVERCOAT™ peptide constructs have proved genetically and structurally stable in this unique PVX vector system, through successive infection cycles on host plants.

To forge stronger relationships with customers and partners, Mylnefield Research Services (MRS) Ltd. was established in 1989 as the commercial arm of SCRI. As a technology transfer company, MRS Ltd. is able to market the scientific expertise and resources of SCRI, and to promote it as a centre of scientific excellence.

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