Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Poster Abstract – E.20

POTATO VIRUS X-BASED PLANT-DERIVED VACCINE AGAINST AFLATOXIN B1

CATELLANI M.*, LICO C.*, CAPODICASA C.*, DONINI M.*, CONTI S.**, POLONELLI L.**, BENVENUTO E.*

*) ENEA, UTS Biotecnologie, Sezione Genetica e Genomica Vegetale, C.R. Casaccia, P.O. Box 2400, I-00100 Rome (Italy)
**) Università di Parma, Sezione di Microbiologia - Dipartimento di Patologia e Medicina di Laboratorio, Parma (Italy)

aflatoxin B1, vaccine, PVX, mimotope, Nicotiana benthamiana

Aflatoxin B1, a mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*, is a common contaminant and can occur in a wide range of important raw food commodities causing carcinogenesis and immunosuppression. Moreover, aflatoxins M1 is the hydroxylated form of aflatoxin B1 and this metabolite is produced when cows or other ruminants ingest feed contaminated with this mycotoxin. Aflatoxins M1 is then excreted in the milk and may subsequently contaminate other dairy products. Although the use of pesticides and good agronomic practices can reduce mycotoxin accumulation, its complete elimination is still a major challenge.

An effective strategy to limit the risks deriving from aflatoxin contamination in meat, milk and other derived products could be represented by preventive vaccination. This practice encounters several limitations such as the impossibility to directly administer the active mycotoxin both for its toxicity and the small size (~ 300 Da) which limits its immunogenic potential.

Our work is focused on the identification of a safe non-toxic vaccine against aflatoxin B1 to be tested on animal systems. To this aim, three previously identified peptides (Thirumala-Devi et al., 2001), that mimic the aflatoxin B1 molecule, were produced in plants using the transient expression system based on the potato virus X (PVX) viral vector. The epitope-display strategy using plant virus CP as carriers has been successfully tested for experimental vaccines (Marusic et al., 2001). In addition, the exploitation of plants for the production of therapeutic proteins offers several advantages such as absence of mammalian pathogens, cost effectiveness, large-scale production and relative ease in expression and purification.

All peptides were selected on the basis of specific aminoacid sequence features required for plant virus display (Lico et al., 2006). Hence, mimotopes were expressed on the virus surface as N-terminal fusion to the coat protein (CP) of a mutant PVX, expressing a truncated form of the CP.

The engineered viral vectors were used to inoculate *Nicotiana benthamiana* leaves and the capacity of the chimeric viruses to move systemically and infect the whole plant has been evaluated. Only one out of the three constructs tested, named 24-6_1, caused systemic infection and was further characterized by RT-PCR and Western.

The recombinant PVX carrying the 24-6_1 mimotope, purified from leaf tissue will be used to immunize mice and to evaluate its capacity to induce an immuno-response specific for the aflatoxin B1. The success of a preventive vaccination against aflatoxin B1 should also permit the reduction of aflatoxin M1 in milk, meat and derived products.

References

Thirumala-Devi *et al*.Phage-displayed peptides that mimic aflatoxin B1 in serological reactivity. J Appl Microbiol. 2001 Mar;90(3):330-6.

Marusic *et al.* Chimeric plant virus particles as immunogens for inducing murine and human immune responses against human immunodeficiency virus type 1. J Virol. 2001 Sep;75(18):8434-9.

Lico *et al.*, Peptide display on Potato virus X: molecular features of the coat protein-fused peptide affecting cell-to-cell and phloem movement of chimeric virus particles.J Gen Virol. 2006 Oct;87(Pt 10):3103-12.