Development of a plant-based veterinary oral vaccine to combat avian influenza in Vietnam - BL/03/V14

Context and objectives

The most threatening avian flu strain H5N1 has now spread through over 40 countries in Asia, Middle East, Europe and Africa, and has already caused more than $10 billion in losses to poultry farmers. Vietnam has had more cases of H5N1 than any other country. Since late 2003 the H5N1 strain has killed or led to the culling of millions of poultry and killed 42 people. Considering vaccination is the most important measure to prevent pandemics, our work is focused on the development of the veterinary vaccine to combat avian influenza in Vietnam based on the recent achievement in plant based vaccine biotechnology, availability of HA sequence of H5N1 strains isolated from Vietnam and advanced knowledge on influenza vaccines. Therefore the main aims of this project were:

- To produce an influenza subunit vaccine in seeds of *A. thaliana* (as model plant for rapid evaluation) and soybean (as the ultimate large scale production system).
- To characterize the obtained lines at molecular and biochemical levels
- To evaluate immune responses of produced vaccines and their value for oral vaccination against avian influenza

Methodology

- **Characterization of HA genes of H5N1 viruses and construction of seed specific expression vectors**

HA genes of H5N1 viruses isolated in Vietnam were sequenced by Vietnamese partner. The expression vectors were designed by Belgium partner.

- **Transformation of the designed vectors into soybean and *A. thaliana***

The protocol applied for gene transformation of Vietnamese soybean varieties was developed by Vietnamese partner

- **Molecular and biochemical characterization of the transgenic lines**

SDS-PAGE and Western blot using c-myc tag antibody were developed by the Belgian partner (VUB)

- **Evaluation of the efficacy of influenza vaccines produced in the soybean and *A. thaliana* seeds**

Results

The HA genes of several strains of H5N1 isolated from infected domestic birds in Vietnam have been characterized and sequenced. The epitopes of HA gene were identified. The identified epitopes were used to construct the expression vector for plant transformation. Other vectors aiming to express full length viral proteins have been made.

In total 8 different constructs have been transferred into *A. thaliana* and some of them have been used to transform soybean. The T1 seeds of 76 *A. thaliana* lines transformed with different constructs have been harvested. The SDS-polyacrylamide gel electrophoresis was performed with protein extracted from the obtained lines (T1) and Western blot analysis has been done with several lines to determine whether the genes are expressed in seeds and to estimate expression levels of the transgenes. The segregation test was performed with the T1 positive lines and selected plantlets were grown in the greenhouse to produce the seeds in large quantity for further immunological evaluation. T2 seeds from these lines were harvested and the expression levels of the transgene were determined by SDS-PAGE and Western blot analysis.

Products and services

Database

Genes HA, NA and M1 of several strains of H5N1 isolated from infected domestic birds in Vietnam have been sequenced. Their sequences are available in Genbank database with following accessions:
AJ867074: HA gene, AJ867075: NA gene, AM040045: M1 gene

Papers

Son LV, Thu TT, Ha CH, Angenon G and Binh LT (2008): Expression of H5N1 antigen M1 in Arabidopsis seeds. J. Biotechnology 6: 563-568

Thu TT, Son LV, Binh LT and Angenon G (2008): Development of a plant-based veterinary oral vaccine to combat avian influenza in Vietnam. Abstract. BPBA Symposium "Secondary
Metabolites and Molecular Farming" 7 November 2008, Gent, Belgium

In preparation:

Thu TT, Son LV, Binh LT and Angenon G: Seed expressed avian influenza epitopes fused to LTB adjuvant protein for oral vaccination of poultry

Execution

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Discipline

Agrobiotechnology
Agriculture Environment
Medecine /Drugs (pharmacopy