A vaccine prototype using baculovirus expression system for the control of Avian Influenza Virus

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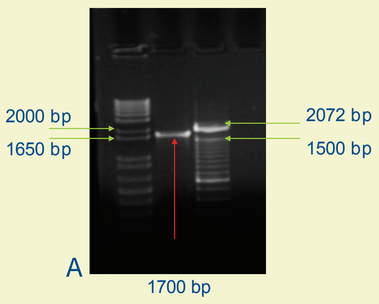
Introduction

Influenza virus causes an important disease worldwide. Avian Influenza LP and HP represent a high risk for poultry producers, and its control is a major burden according to sporadic outbreaks which are related to the distribution of HP variants. The virus causes disease in several continents. The control of AIV, H5 subtype, remains as a main challenge and different kind of vaccines are available. The use of insect cells and baculovirus to produce the H5 HA offers the versatility, simplicity, scale-ability and yields of a very efficient vaccine producing process.

Materials and Methods

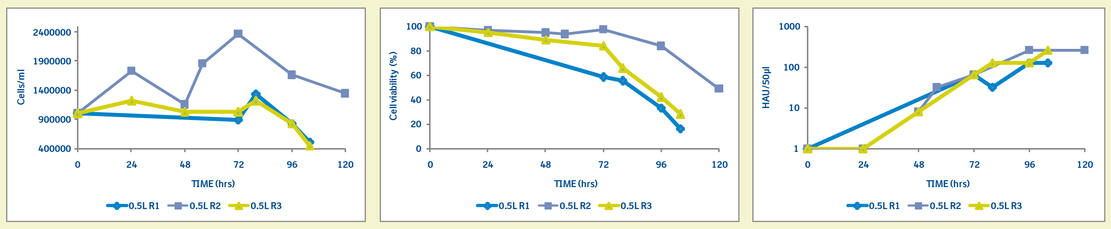
A clade 1 sequence of H5 haemaglutinin from an Asian Avian H5N1 isolate was used as a template to chemically synthetize a codon-optimized version for expression in insect cells. When insect cells are infected with the mutK + H5 HA Baculovirus, the polyhedrin promoter will subsecuently direct a high level of expression of the mutK+H5 HA protein. After plaque purification and subsecuent scale-up in SF(+) cells, the supernatant of the recombinant baculovirus containing the mutK + H5 HA gene was put down as a MSV and designated as “mutK + H5 HA Baculovirus DB Master Seed Virus”.

Characterization



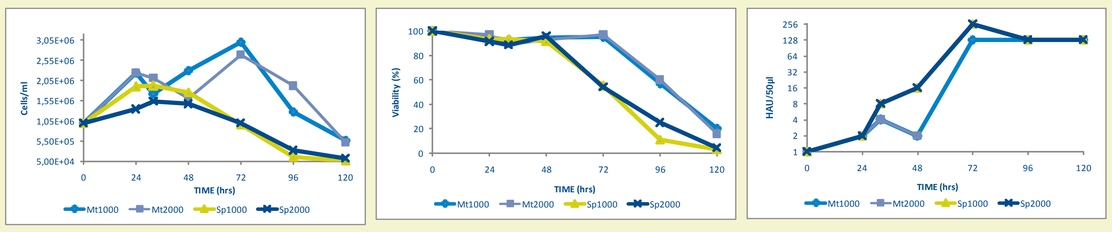
Stability analysis shows that the genetic construct and the protein are possible to be detected after 5 serial passages of the Master Seed virus. H5-specific primers were used to detect the 1.7 kb fragment (A), sequencing is ongoing. A monoclonal H5 HA-specific antibody was used to detect the mono, di, and trimeric forms of the protein (B) whether from the first, fifth passage, or a from a representative harvest (lane 2, harvest amount used to be obtained after 4 dpi).

Results



A B C

Cell density (A), cell viability (B), and antigen yield (C) using MSV+4 master seed virus at MOI of 0.1. Cell viability dropped down after 48 hpi. and the antigen accumulation did not change after 4 dpi.



A B C

Infection with MOI of 0.5 (Green, purple) or 0.1 (Blue, Red). Cell density (A), cell viability (B), and antigen yield (C). Cell population reaches densities above 2x10E6, and the antigen yields is the same, suggesting that the Optimal Peak Cell Density (OPCD) must be between 2 and 3x10E6.

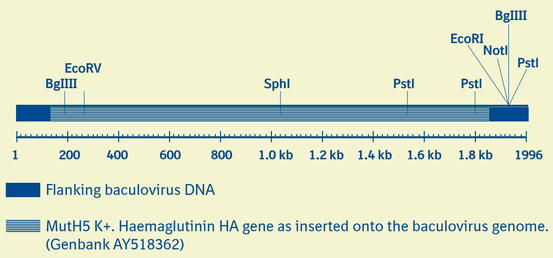
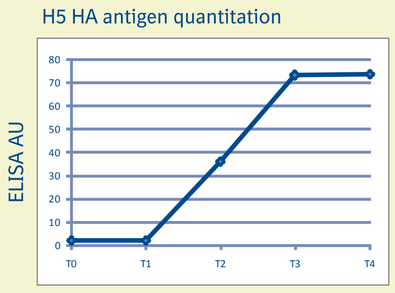
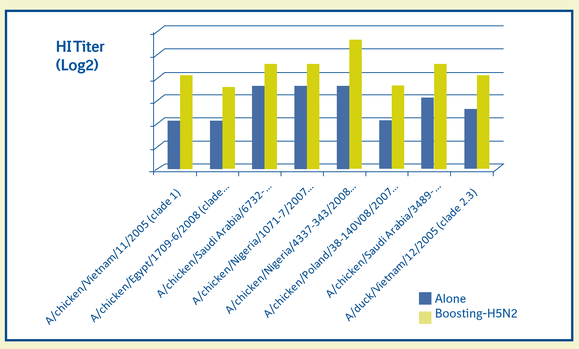


Diagram depicting the genetic construct inserted into the baculovirus genome. Stability testing after 5 passages have shown the sequence is stable and no further changes where detected.

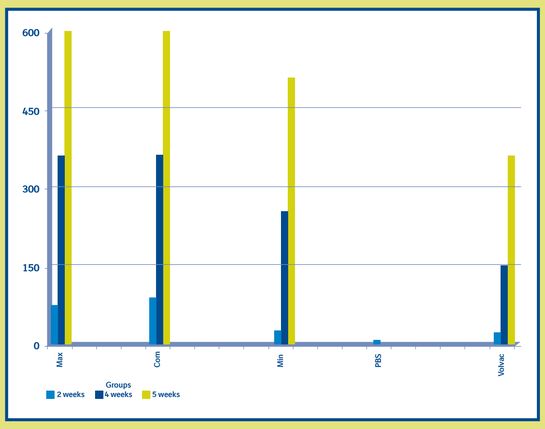


Estimation of antigen yield using a commercial ELISA assay. Values did not change after 96 hpi (T4), in agreement with previous HAU results.



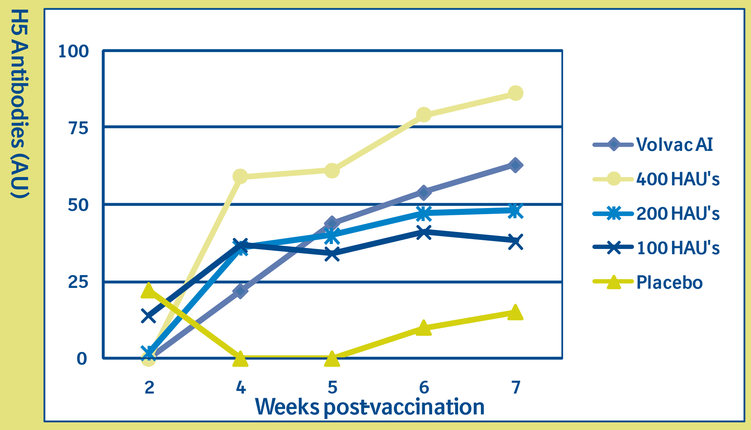
Antibody levels (HI titres) elicited by 0.5 ml administered subcutaneously in 3 weeks old SPF chickens. Representatives of several clades and subclade of HPAI H5N1 virus were used as antigens. Seroconversion was demonstrated in all cases and titres were above 4 Logs base 2 (1:16). A comparative priming/boosting application was included for comparison (DOA for BES H5 and 10 days old for H5N2 vaccine).

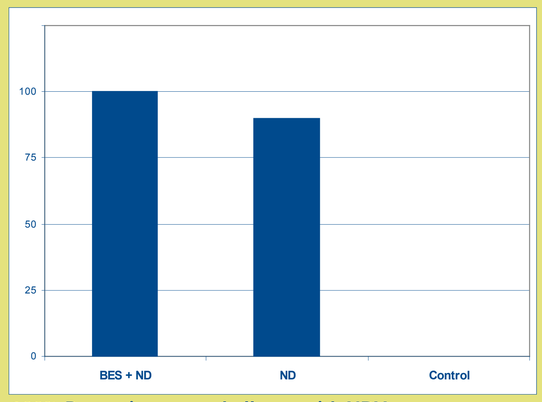
In order to test the biological activity of the expressed protein and its ability to trigger an immune response, oil/water emulsions of different amounts of the protein were administered to SPF chickens and antibodies levels were detected using an ELISA-based system and HI titration. Both approaches were able to demonstrate seroconversion and a dose-response curve was observed among the different doses. Altogether, results support the feasibility of the genetic contruct and the expression platform to produce bulk amounts of biologically active protein.



Antibody levels (HI titres) elicited by 0.5 ml administered subcutaneously in weeks old SPF chickens. The prototype included several amounts of haemaglutinin per dose as indicated. Chickens were bled at 2, 4 and 5 weeks post vaccination to show development of the immune response.

Antibody levels (ELISA) elicited by 0.5 ml administered subcutaneously in weeks old SPF chickens. The prototype included several amounts of haemaglutinin per dose as indicated.
Chickens were bled at 2, 4, 5, 6, and 7 weeks post vaccination to show development of the immune response.





100% Protection upon challenge with NDV

Vaccinated chickens were administered with a combinated prototype including 400 HAU of H5 HA and 128 HAU of ND KV (both BEI-inactivated). 3 weeks post vaccination the animals were challenged with NDV and mortality recorded during two weeks. The presence of the H5 HA antigenic fraction did not affected the protection conferred by the ND fraction as shown in the figure.

Conclusions

- A vaccine prototype based on the H5 HA sequence expressed in the baculovirus/insect cells system is available.
- Process parameters have been optimized to achieve reproducible and relieable yields in the scale of 0.5 L.
- The expressed antigen triggered an immune response when administered as an inactivated oil-emulsion to 3 weeks old chickens.
- The antigen was stable upon combination with BEI-inactivated NDV and elicited a protective immune response demoNstrated by challenge with NDV infectious virus.

References

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2. Crawford, J. et. al.(1999): Baculovirus-derived hemagglutinin vaccines protect agains lethal influenza infections by avian H5 and H7 subtypes. Vaccine,17: 2265-2274.
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