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VACCINE POTENTIAL FOR TICK ANTIGENS IN THE CONTROL OF TICKS AND TICK-BORNE DISEASES.

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Introduction

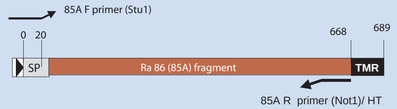
Ticks are important ecto-parasites of mammals, they transmit a number of pathogens both to animals and humans.
In humans they transmit bacterial, viral and protozoan diseases such as the lyme disease, tick-borne encephalitis and monocytic human ehrlichiosis to mention but a few.
Control of ticks is by intensive application of acaricides which are potentially hazardous to the environment therefore use of tick vaccine would offer an alternative method of control.

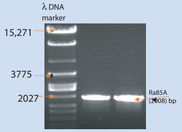
The successful use of Bm86 recombinant vaccine for the protection of cattle from Boophilus microplus tick species stimulated search for such proteins in other tick spp
The discovery of a homologous gene to Bm 86 with 85% identity in Rhipicephalus appendiculatus tick spp has lead to its cloning and eventual expression in the baculovirus insect cell system.
The aim of this study was to look at the vaccine potential of the secreted salivary gland cement protein in conjunction with the concealed gut glycoprotein for their use in combination with the anti-pathogen vaccine for the control of ticks and tick-borne diseases in the tropical and sub-tropical countries.

A feature of the arthropod vector/host interaction is that ticks secrete pharmacologically active salivary gland molecules into the mammalian host.

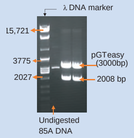
Therefore the hypothesis of this study is that these secreted tick peptides have potential for application as novel tick and tick-borne disease control agents and that they may enhance the protection induced by anti-pathogen vaccines.

Cloning of the variant Ra 85A cDNA clone into baculovirus transfer vector:
The full length sequence Ra 86 (85A) was amplified from the bluescript (kindly provided by R. Bishop ILRI, Kenya) using customer specific primers that introduced StuI and Not I restriction sites and a C-terminal 6x-His tag. This was first cloned into pGEM-T-easy plasmid before introduction into the pFastBac Dual: p10GFP donor vector. The original signal sequence was maintained in the construct for efficient secretion and possible glycosylation of the protein. However the putative trans-membrane region, 20 amino acids (668 - 689) was removed.



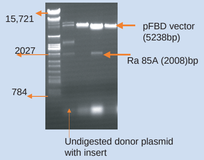


Ra 85A PCR fragments (2008bp) visualized products on 0.8% agarose, these were re-cloned in pGEMT easy.

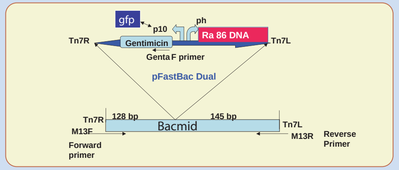


Ra 85A fragment cloned in pGEMT-easy vector were digested using Stu1/Not1

The digested Ra 85A fragment was re-cloned in pFBD:p10GFP donor vector (Kaba et al 2002). This donor palsmid has a gentamycin and ampicillin resistant gene which is used for selection of positive colonies.



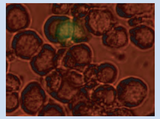
These were transformed into heat competent DH10 Bac delta-cc E.coli. This strain carries the baculovirus shuttle vector (Bacmid) with a mini-attTn7 target site and a helper plasmid.

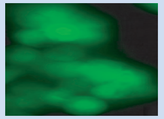


Transposition occurs between the mini-attTn7 site on the donor vector and the bacmid to generate recombinant bacmid. The helper plasmid produces enzymes which facilitates transposition.

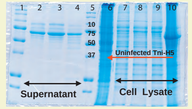
Expression of the recombinant proteins in insect cells

Ra 85A was transfected in Sf21 and Tni-H5 insect cells at a multiplicity of infection (MOI) of 10 tissue culture infective dose (TCID)50 units/cell and subsequently harvested 72hrs post infection. GFP (the green fluorescent protein) was used as a marker for gene expression in all construct and was monitored using fluorescein isothiocyanate (FITC) excitation.



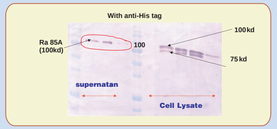


The recombinant protein was detected both in insect cells and in the culture medium after 48 hrs post infection using SDS-PAGE 12% gradient.

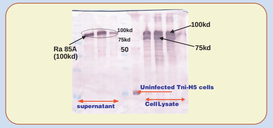


Lane 1/5 = All blue protein marker
Lane 2/3 = viral DNA transfection
Lane 4= virus passage 1
Lane 6= un-infected Tni-H5 insect cells
Lane 9= virus passage 1 lysate
Lane 10 = viral passage 2 of Sf21 insect cells.

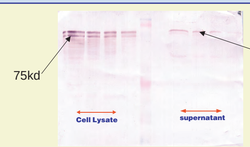
When probed on western blot analysis with anti-his antibodies detected a recombinant protein that was glycosylated.



Ra 85A was also detected using anti-Ra 86 serum

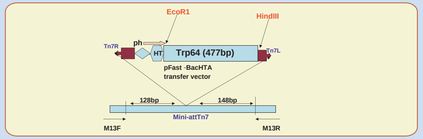


Probed with anti-Bm 86 serum from vaccinated rabbits showed cross-reactions with Ra 86 indicating presence of common epitopes.

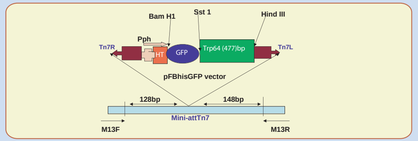


For the salivary gland cement protein (Trp64) two different approaches were used for cloning in the donor vectors.

Strategy 1. Cloned in pFB-HA under the baculovirus polyhedrin promoter

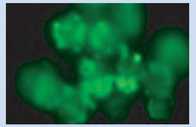


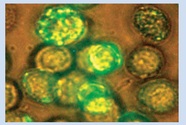
Strategy 2: Trp 64 sequence was cloned directly downstream in fusion to GFP sequence between the Sst1 and HindIII sites of the MCS of the vector.



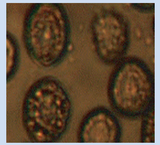
An enhanced GFP sequence had been cloned between the Bam H1 and Sst1 sites of the MCS of pFast Bac-HTa, downstream of the 6x his-tag sequence.

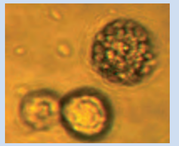
The Trp64 constructs appeared as insoluble aggregates in infected Sf21 and Tni-H5 cells, which may be due to the higher levels of expression.



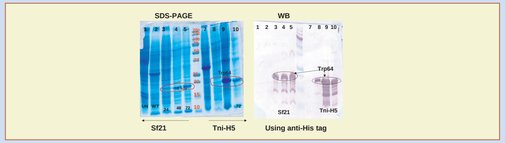


The non-fused Trp 64 recombinant protein also expressed in form of aggregates in the cytoplasm of the insect cells.





Lane 1= uninfected, Lane 2/7= infection with AcMNPV wild type,
Lanes 3/8,4/9,5/10= 24hrs,48,72hrs pi respectively.



Conclusion:

The Ra86 protein was expressed as a secreted and glycosylated protein detected both in media supernatant and cell lysate.

The molecular weight for Ra86 protein was predicted as 84.5kd from its protein sequence and when detected on SDS and WB was with his-tag and Ra 86 anti-serum fragments of 75kd and 100kd were visualized indicating be N-glycoslylation.

The Ra86 recombinant protein may be a good vaccine candidate since it's soluble and its amino acid sequence likely to have some hydrophilic patches which are likely to be exposed at the surface of a folded protein.

Both Trp64 constructs were expressed in form of aggregates in the cytoplasm of insect cells

References:

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Kaba SA., Nene V., Musoke AJ., Vlak J., Van Oers MM (2002). Fusion to the green fluorescent protein improves expression levels of Theileriaria parva sporozoite surface antigen p67 in insect cells, Parasitology, 125: 497-505.

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