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Serum samples of pigs from southern China were examined by hemagglutination-inhibition (HI) tests and ELISA using formalinized viruses as antigens. By HI tests, the results were not reproducible. By ELISA, antibodies specific to the HAs were not detected due to the presence of common antigens. Then, ELSIA using HA antigens expressed on 293T cells transfected with recombinant plasmids was performed. By the use of this method antibodies specific to the HA were clearly detected in serum samples of immunized chicken, experimentally infected mice and experimentally infected pigs. By using this established method, antibodies specific to H4 and H5 HAs were detected in the sera from pigs in southern China, in addition to H1 and H3 HAs, that are known to prevail in pig population in this area. These results suggest that pigs are infected with H4 and H5 influenza viruses in southern China.

It is necessary to examine these sera for other subtypes of HA than H1, H3, H4 and H5 that have been prepared and used in the present study. Further seroepidemiological study using the present method with HA antigens of avian influenza viruses should provide information on the next pandemic influenza virus strain.

Expression of mammalian genes encoding biologically active proteins or peptides with potential pharmaceutical applications in transgenic plants

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Expression systems of foreign genes in transgenic plants have large advantages of production at a low cost and storage of expressed products, compared to *Escherichia coli* and yeast expression systems which have been used for mass production of biologically active proteins or peptides of potential pharmaceutical importance including vaccines, immunomodulators, growth factors, and enzymes. In addition to these advantages, the products expressed in vegetable foods can be orally administrated to. For these reasons, considerable efforts in the medical field have been made to express biologically active molecules of pharmaceutical potentials in transgenic plants in order to produce inexpensive vaccines and drugs. A strategy to produce "edible vaccines" is especially suitable for vaccinating children in the developing countries. Although these features of the expression systems in plants are also suitable for livestock industries, little attention has been directed to application of plant biotechnology to the veterinary field until now.

In order to develop techniques for improving livestock production through potentiation of host defense mechanisms against infectious diseases with feed as orally deliverable pharmaceuticals, the cDNAs encoding human interferon α (IFNα) which is a immunomodulator of multiple biological activities, and a lingual antimicrobial peptide (LAP) which is secreted by bovine oral epithelial cells, were introduced into potato (*Solanum tuberosum*) using the Agrobacterium tumefaciens-mediated transformation system. DNA insertion, and mRNA and protein expressions in the potato transformants obtained by the intro-

duction of IFNα cDNA, were confirmed but the expression levels of IFNα were too low to demonstrate the biological activity as measured by anti-viral effects. In the case of LAP cDNA-introduced potatoes, no transformants were obtained because of mutations in the vector plasmid.

The low expression levels of IFNα in the transformants could be explained by that (1) the expression levels of foreign genes depend on the sites of their insertion because the foreign DNAs are randomly inserted into the plant chromosomal DNA, (2) the translation of the transcript in plant cells is inefficient because of the differences in the codon usages between plant and mammal genes. The results obtained in this study suggest that mammalian genes being introduced into the plant cells should be converted into suitable forms to achieve high expression in plant cells.

Molecular cloning and characterization of *Babesia caballi* antigen genes for development of serodiagnosis for equine babesiosis

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Babesia caballi and *Babesia equi* which are tick-transmitted intra-erythrocytic protozoan cause equine babesiosis. The complement fixation test (CFT) and indirect immunofluorescent antibody test (IFAT) which are currently used for detecting *B. caballi* infection are not sensitive and specific enough. Therefore, in this study, aiming at more simple, sensitive and specific serodiagnostic methods for equine babesiosis on enzyme-linked immunosorbent assay (ELISA) using recombinant antigens, molecular cloning and characterization of *B. caballi* antigen genes were performed.

B. caballi cDNA library was constructed and screened with a serum of an experimentally infected horse with *B. caballi*. Several clones obtained by immunoscreening, out of which two clones, i.e. B.cA1 and B.cA2 were further analyzed.

B.cA1 contained a 2307bp insert with an open reading frame of 1887bp, which encoded polypeptide of 629 amino acids. Homology search revealed that B.cA1 was a member of heat shock protein 70 (hsp70) gene family. Horse antibodies affinity-purified with B.cA1 products reacted with not only 69kDa protein of *B. caballi*, but also 67kDa protein of *B. equi* antigens. This suggests that cross-reactivity between *B. caballi* and *B. equi* which ELISA using merozoite lysates sometimes encouters is partly due to the presence of common epitopes between hsp70s.

B.cA2 containing a 729bp insert encoded a polypeptide of 212 amino acids which showed high homology to the C-terminal sequences of rhoptry associated protein-1(RAP-1) of several *Babesia* species. Southern blot analysis showed that *B. caballi* genome contained multiple copies of this gene as is the case in other Babesia species. Horse antibodies affinity-purified with B.cA2 products strongly reacted with 53 and 55kDa proteins of *B. caballi* but not with *B. equi* antigens. Moreover, on immunoblot analysis, B.cA2 recombinant proteins expressed in *E. coli*