

Expression of sheep pox viral A27L and L1R proteins in prokaryotic and eukaryotic systems

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Sheep pox is a severe illness causing mass death among small ruminants. Epizootics in different countries including Republic of Kazakhstan leads to great economic losses. Sheep pox virus (SPPV) belongs to *Capripoxvirus* genus of the *Poxviridae* family. Attenuated strains are used as vaccines for prophylactic means of the disease. Such vaccines are effective but has crucial disadvantage as virions could revert to virulent form by recombination. Genome sequencing and genetic engineering methods give opportunities to produce save vaccine with less expense. Nucleotide sequence of the SPPV “NISKHI” strain used in our work was analyzed to find orthologs of the immunogenic structural proteins of vaccinia virus. *sppv-niskhi-117* gene coding A27L ortholog (17.3 kDA) and *sppv-niskhi-060* gene coding L1R ortholog (20.3 kDA) were used for expression in various systems. Genes were cloned in bacterial pET-19b expression vector. Bacterially produced (*E. coli* BL21 strain) and purified proteins were used for rabbit immunization. Antibodies raised to recombinant proteins showed virus neutralizing activity. pACT2 vector was used to express proteins in *Saccharomyces cerevisiae* cells (CG 1945 strain). Only A27L protein successfully expressed in yeast cells. Transgenic *Nicotiana tabacum* and *Brassica napus* plants expressing viral proteins were obtained after transformation by pCambia 2300 vectors containing viral genes, translational enhancers and sequences for signal peptides to target recombinant proteins into chloroplast or endoplasmic reticulum. A higher recombinant protein yield was achieved when using transient expression (magnification) in *Nicotiana benthamiana* leaves. Maximal viral protein level was obtained in transplastomic *N. tabacum* plants carrying viral genes in the chloroplast genome. L1R protein tend to form di- and trimeric forms in *E. coli* and plants. Synthesized proteins may be used for development efficient recombinant subunit vaccine against sheep pox.