World Academy of Science, Engineering and Technology
International Journal of Mathematical and Computational Sciences
Vol:14, No:12, 2020

Sheep Pox Virus Recombinant Proteins To Develop Subunit Vaccines

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Abstract : Sheep pox is a highly contagious infection that OIE regards to be one of the most dangerous animal diseases. It causes enormous economic losses because of death and slaughter of infected animals, lower productivity, cost of veterinary and sanitary as well as quarantine measures. To control spread of sheep pox infection the attenuated vaccines are widely used in the Republic of Kazakhstan and other Former Soviet Union countries. In spite of high efficiency of live vaccines, the possible presence of the residual virulence, potential genetic instability restricts their use in disease-free areas that leads to necessity to exploit new approaches in vaccine development involving recombinant DNA technology. Vaccines on the basis of recombinant proteins are the newest generation of prophylactic preparations. The main advantage of these vaccines is their low reactogenicity and this fact makes them widely used in medical and veterinary practice for vaccination of humans and farm animals. The objective of the study is to produce recombinant immunogenic proteins for development of the high-performance means for sheep pox prophylaxis. The SPV proteins were chosen for their homology with the known immunogenic vaccinia virus proteins. Assay of nucleotide and amino acid sequences of the target SPV protein genes. It has been shown that four proteins SPPV060 (ortholog L1), SPPV074 (ortholog H3), SPPV122 (ortholog A33) and SPPV141 (ortholog B5) possess transmembrane domains at N- or C-terminus while in amino acid sequences of SPPV095 (ortholog A 4) and SPPV117 (ortholog A 27) proteins these domains were absent. On the basis of these findings the primers were constructed. Target genes were amplified and subsequently cloned into the expression vector pET26b(+) or pET28b(+). Six constructions (pSPPV060ΔTM, pSPPV074ΔTM, pSPPV095, pSPPV117, pSPPV122ΔTM and pSPPV141ΔTM) were obtained for expression of the SPV genes under control of T7 promoter in Escherichia coli. To purify and detect recombinant proteins the amino acid sequences were modified by adding six histidine molecules at C-terminus. Induction of gene expression by IPTG was resulted in production of the proteins with molecular weights corresponding to the estimated values for SPPV060, SPPV074, SPPV095, SPPV117, SPPV122 and SPPV141, i.e. 22, 30, 20, 19, 17 and 22 kDa respectively. Optimal protocol of expression for each gene that ensures high yield of the recombinant protein was identified. Assay of cellular lysates by western blotting confirmed expression of the target proteins. Recombinant proteins bind specifically with antibodies to polyhistidine. Moreover all produced proteins are specifically recognized by the serum from experimentally SPV-infected sheep. The recombinant proteins SPPV060, SPPV074, SPPV117, SPPV122 and SPPV141 were also shown to induce formation of antibodies with virus-neutralizing activity. The results of the research will help to develop a new-generation high-performance means for specific sheep pox prophylaxis that is one of key moments in animal health protection. The research was conducted under the International project ISTC # K-1704 “Development of methods to construct recombinant prophylactic means for sheep pox with use of transgenic plants” and under the Grant Project RK MES G.2015/0115RK01983 "Recombinant vaccine for sheep pox prophylaxis".

Keywords : prophylactic preparation, recombinant protein, sheep pox virus, subunit vaccine
Conference Title : ICSRD 2020 : International Conference on Scientific Research and Development
Conference Location : Chicago, United States
Conference Dates : December 12-13, 2020