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# Mutation of VP2 capsid protein of Foot-and-mouth disease virus and its expression in the insect cell system for increased thermostability

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## Abstract

FMD is an economically devastating livestock disease of global importance. The disease is caused by FMD virus of genus *Aphthovirus* of family *picornaviridae*. It affects both domestic and wild cloven-hoofed animals. Many alternate vaccine strategies such as peptide vaccines, DNA vaccines, viral-vectored vaccine, VLPs are attempted to date. The limited success of alternate strategies was attributed to either poor immune response or instability of antigen during storage. The FMDV capsid is reported to be thermo- and acid-labile. In this study, we report engineering and evaluation of FMDV capsid for enhanced thermostability.

In this study, five mutations (TS1, TS2, TS3, TS4 and TS5) with a single amino acid change were introduced in VP2 region, by PCR-mediated site-directed mutagenesis and generated recombinant bacmids by transposition method, transfected in insect cells to produce recombinant baculovirus expressing FMDV empty capsids. The expressed VLPs were analyzed for antigenic thermostability, by storing the samples at -20$^{o}$C, 4$^{o}$C, 24$^{o}$C and 37$^{o}$C and subjecting to s-ELISA at 15 days interval. Purified VLPs were also investigated based on $T_m$ from DSF stability assay after preheating at 37$^{o}$C for 1hr, 45$^{o}$C and 56$^{o}$C for 30 min and varying pH conditions like 6, 7 and 8. The expressed protein purified by 15 to 60% sucrose gradient, on analysis by TEM showed the intact empty capsid particles of ~25nm. The s-ELISA of stored samples revealed the consistency in reactivity of TS1, TS2 and TS3 samples stored at -20$^{o}$C, 4$^{o}$C, 24$^{o}$C and 37$^{o}$C for more than 75 days, while the drop in antigen reactivity was noticed for wild-type after 30 days and for TS5 after 45 days when stored at the different temperatures. TS4 showed stability when stored at -20$^{o}$C, 4$^{o}$C for more than 75 days but the sudden decrease in antigen reactivity was seen after 45 days at 24$^{o}$C and after 30 days at 37$^{o}$C. In DSF stability assay, TS3 exhibited a constant high $T_m$ after heating at 37$^{o}$C for 1hr, 45$^{o}$C for 30min and under pH conditions 6, 7 and 8 in comparison to other mutants and wild-type. However, the complete dissociation of capsid was seen in all mutants and wild-type when heated at 56$^{o}$C for 30 min. TS3 capsids exhibited relatively more stability compared to all other mutant capsids. Further studies evaluating TS3 mutant capsids in eliciting a protective immune response are needed to investigate its potential as a vaccine candidate.

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