Codon deoptimization of the viral capsid protein-encoding gene attenuates Macrobrachium rosenbergii nodavirus

ABSTRACT

The aquaculture production of giant freshwater prawn, Macrobrachium rosenbergii has been hampered by the outbreak of white tail disease, which is caused by Macrobrachium rosenbergii nodavirus (MrNV) infection, a virus from the family of Nodaviridae. It is a non-enveloped icosahedral RNA virus, which contains two segments of single-stranded positive-sense RNA genome; namely RNA1 and RNA2. To-date, there is no effective prophylactic or therapeutic agent available to control its outbreak. Current study aimed to attenuate MrNV through the manipulation of synonymous codon usage bias, in order to deoptimize the codon of the viral RNA2 segment, which encodes the viral capsid protein. A total number of 125 synonymous codon substitutions were introduced into the viral RNA2. Resulted in the designed mutant RNA2 to possess 84% nucleotide sequence similarity compared to the wild-type. Viral genes (wild type RNA1, RNA2, and mutant RNA2) that were harboured by respective pUC57 plasmid, were subsequently expressed through in-vitro transcription using T7 promoter and then, transfected into confluent Sf9 cells. The pathogenicity of these clones were compared between non-infected control cells, cells transfected with the wild-type and the attenuated clone, respectively. The comparison of pathogenicity was based on phenotypic examination of the transfected Sf9 cells. Sf9 cells transfected with the wild-type clone exhibited cytopathogenic effects, which include increased in size due to cytoplasmic swelling, and aggregation of the infected cells into clumps of various sizes. While Sf9 cells transfected with the mutant clone exhibited insignificant cytoplasmic swelling and cellular aggregations. The presence of viral genes in the transfected Sf9 cells at 92 h post transfection confirmed the infectivity of both wild-type and mutant clones. Results indicated that the mutant clone was attenuated.

Keyword: MrNV; White tail disease; Attenuated virus; Codon deoptimization; Vaccine