Propagation and molecular characterization of fowl adenovirus serotype 8b isolates in chicken embryo liver cells adapted on cytodexTM 1 microcarrier using stirred tank bioreactor

ABSTRACT

Large volume production of vaccine virus is essential for prevention and control of viral diseases. The objectives of this study were to propagate Fowl adenovirus (FAdV) isolate (UPM08136) in chicken embryo liver (CEL) cells adapted to Cytodex™ 1 microcarriers using stirred tank bioreactor (STB) and molecularly characterize the virus. CEL cells were prepared and seeded onto prepared Cytodex™ 1 microcarriers and incubated first in stationary phase for 3 h and in STB at 37 °C, 5% CO_2, and 20 rpm for 24 h. The CEL cells were infected with FAdV isolate (UPM08136) passage 5 (UPM08136CELP5) or passage 20 (UPM08136CELP20) and monitored until cell detachment. Immunofluorescence, $TCID_{50}$, sequencing, alignment of hexon and fiber genes, and phylogenetic analysis were carried out. CEL cells were adapted well to Cytodex™ 1 microcarriers and successfully propagated the FAdV isolates in STB with virus titer of $10^{7.5}$ (UPM08136CELP5B1) and $10^{6.5}$ (UPM08136CELP20B1) $TCID_{50}/_{mL}$. These isolates clustered with the reference FAdV serotype 8b in the same evolutionary clade. The molecular characteristics remained unchanged, except for a point substitution at position 4 of the hexon gene of UPM08136CELP20B1, suggesting that propagation of the FAdV isolate in STB is stable and suitable for large volume production and could be a breakthrough in the scale-up process.

Keyword: Chicken liver cell; Cytodex 1™ microcarrier; Fowl adenovirus 8b; Bioreactor; PCR