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EFFICIENT STABLE EXPRESSION OF NUCLEAR H5N1 AVIAN INFLUENZA VIRUS HA2 TRANSGENE IN *CHLAMYDOMONAS REINHARDTII*

Ausama Yousif[1], Mahmoud Bayoumi[1], Mahmoud Samir[2], Mahmoud Elgamal[1] and Haitham Amer[1, 3]

[1]Cairo University, Egypt
[2]Animal Health Research Institute, Egypt
[3]King Saud Universities, Saudi Arabia

Green biotechnology is the future of biopharmaceuticals production. The use of algae to produce biopharmaceuticals is of interest in vaccine-based control programs because of cost and environmental safety considerations. An attempt was made to express avian influenza virus (AIV) immunogens in algae because the virus is a serious economic, veterinary and public health threat. A commercial system was modified to allow expression of H5N1 AIV hemagglutinin subunit 2 (HA2) in the microalga *Chlamydomonas reinhardtii*. Codon-optimized AIV H5N1 HA2 (coHA2) sequences were synthesized and cloned into the transfer vector pChlamy_3/D-TOPO® (3DcoHA2). 3DcoHA2 plasmids were used to transform C. reinhardtii strain cc-125 by electroporation. Proper nuclear integration was confirmed in 16% of screened transformants selectively amplified in Hygromycin-containing TAP media. coHA2 mRNA transcription was confirmed using RT-PCR. AIV HA2 expression was confirmed using Western Blot analysis utilizing mono-specific AIV H5N2 polyclonal chicken antisera. Expressing transformants were maintained on Hygromycin-containing TAP agar for 26 weeks (15 subcultures). Expressing transformants maintained cell shape, motility and, growth characteristics similar to non-transformed C. *reinhardtii* cc-125. A coHA2-C terminus GFP was used to visualize HA2 expression in vivo using confocal microscopy. Background-normalized GFP-specific fluorescence of transformants was 15 % of the total cellular fluorescence. Fluorescence in GFP channels 508, 518, 528 and 538 nm was 3.9% of the total cellular fluorescence of non-transformed algae. Taken together, results indicate efficient stable expression of the AIV HA2 transgene and, warrant further investigation into immunogenic potential of the algae-expressed HA2.

ayousif198@gmil.com