

Cloning and expression of highly pathogenic avian influenza virus full-length nonstructural gene in *Pichia pastoris*.

ABSTRACT

Avian influenza (AI) is a highly contagious and rapidly evolving pathogen of major concern to the poultry industry and human health. Rapid and accurate detection of avian influenza virus is a necessary tool for control of outbreaks and surveillance. The AI virus A/Chicken/Malaysia/5858/2004 (H5N1) was used as a template to produce DNA clones of the full-length NS1 genes via reverse transcriptase synthesis of cDNA by PCR amplification of the NS1 region. Products were cloned into pCR2.0 TOPO TA plasmid and subsequently subcloned into pPICZA vector to construct a recombinant plasmid. Recombinant plasmid designated as pPICZA-NS1 gene was confirmed by PCR colony screening, restriction enzyme digestion, and nucleotide sequence analysis. The recombinant plasmid was transformed into *Pichia pastoris* GS115 strain by electroporation, and expressed protein was identified by SDS-PAGE and western blotting. A recombinant protein of approximately ~28kDa was produced. The expressed protein was able to bind a rabbit polyclonal antibody of nonstructural protein (NS1) avian influenza virus H5N1. The result of the western blotting and solid-phase ELISA assay using H5N1 antibody indicated that the recombinant protein produced retained its antigenicity. This further indicates that *Pichia pastoris* could be an efficient expression system for a avian influenza virus nonstructural (NS1).

Keyword: Pathogenic avian influenza; Nonstructural gene; *Pichia pastoris*.