Molecular cloning and bioinformatic analysis of the *Streptococcus agalactiae* neuA gene isolated from tilapia

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ABSTRACT. Cytidine monophosphate (CMP) N-acetylneuraminic acid (NeuNAc) synthetase, which is encoded by the *neuA* gene, can catalyze the activation of sialic acid with CMP, and plays an important role in *Streptococcus agalactiae* infection pathogenesis. To study the structure and function of the *S. agalactiae* *neuA* gene, we isolated it from diseased tilapia, amplified it using polymerase chain reaction (PCR) with specific primers, and cloned it into a pMD19-T vector. The recombinant plasmid was confirmed by PCR and restriction enzyme digestion, and identified by sequencing. Molecular characterization analyses of the *neuA* nucleotide amino acid sequence were performed using bioinformatic tools and an online server. The results showed that the *neuA* nucleotide sequence contained a complete coding region, which comprised 1242 bp, encoding 413 amino acids (aa). The aa sequence was highly conserved and contained a Glyco_transf_GTA_type superfamily and an SGNH_hydrolase superfam-
ily conserved domain, which are related to sialic acid activation catalysis. The NeuA protein possessed many important sites related to post-translational modification, including 28 potential phosphorylation sites and 2 potential N-glycosylation sites, had no signal peptides or transmembrane regions, and was predicted to reside in the cytoplasm. Moreover, the protein had some B-cell epitopes, which suggests its potential in development of a vaccine against *S. agalactiae* infection. The codon usage frequency of *neuA* differed greatly in *Escherichia coli* and *Homo sapiens* genes, and *neuA* may be more efficiently expressed in eukaryotes (yeast). *S. agalactiae neuA* from tilapia maintains high structural homology and sequence identity with CMP-NeuNAc synthetases from other bacteria.

**Keywords:** Cloning; Bioinformatic analyses; *Streptococcus agalactiae*; *neuA* gene; NeuA amino acid sequences