Supplementary Information

Journal name: ***Biomolecules***

Title: Characterization of matrix metalloprotease-9 gene from Nile tilapia (*Oreochromis niloticus*) and its high-level expression induced by *Streptococcus agalactiae* challenge

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Supplementary figures

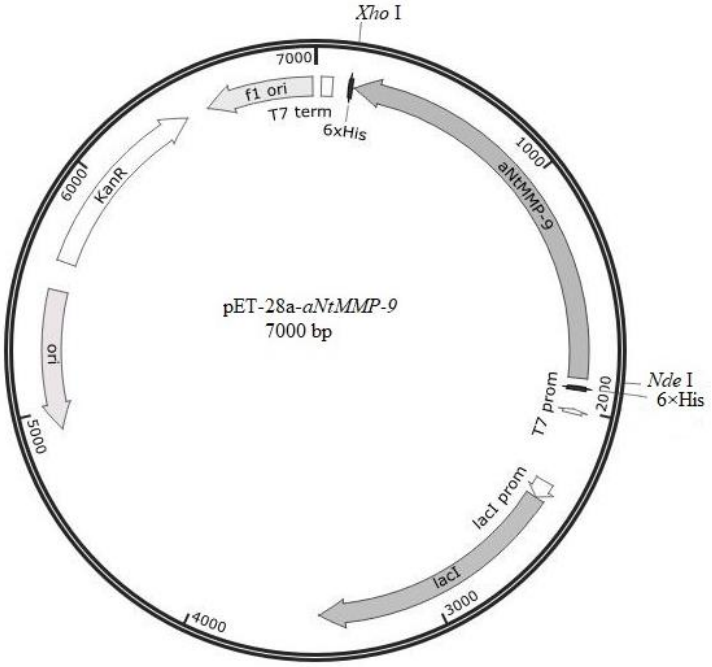

Figure S1. Reconstruction of the expression vector pET-28a/*aNtMMP-9*

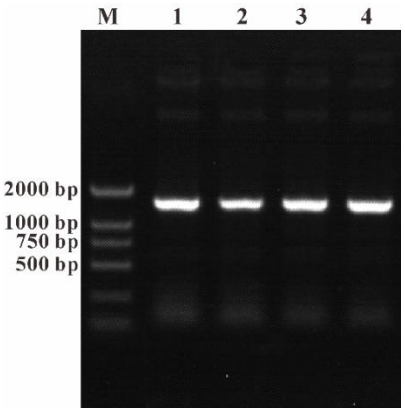

Figure S2. Identification of positive transformants containing pET-28a/*aNtMMP-9* vector amplified by PCR.

M, DNA marker DL2000; Lanes 1-4, the bands (1,716 bp) were amplified from different transformants.

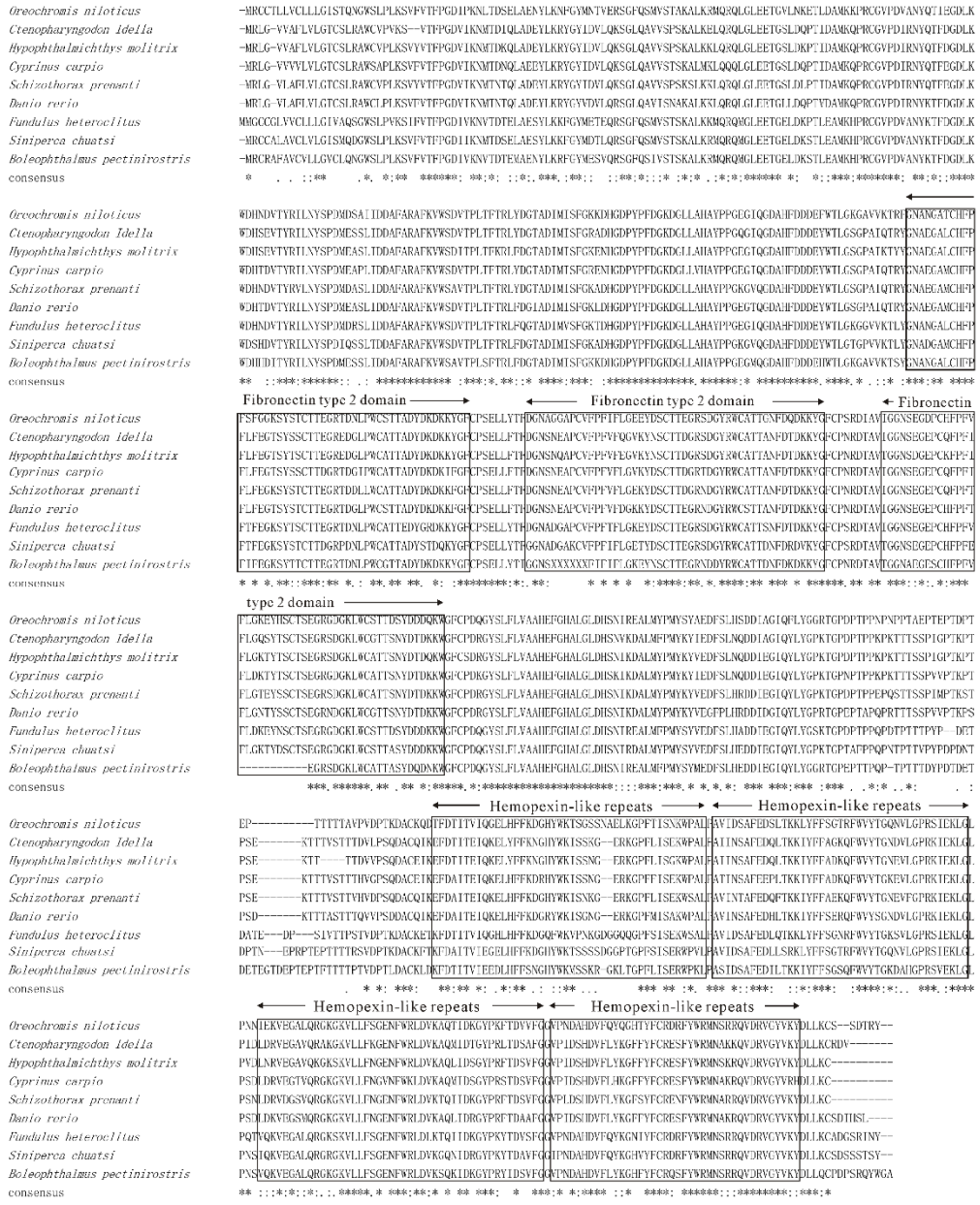

Figure S3. Multiple amino acid sequences alignment of MMP-9 from Nile tilapia and other species.

Amino acid sequences of MMP-9s from other species, including *Ctenopharyngodon idella* (Accession No. ADU34085.1), *Hypophthalmichthys molitrix* (APO13601.1), *Cyprinus carpio* (BAB39390.1), *Schizothorax prenanti* (AMB72630.1), *Danio rerio* (AAI60656.1), *Fundulus heteroclitus* (XP_012726094.1), *Siniperca chuatsi* (AKA66298.1), and *Boleophthalmus pectinirostris* (XP_020785344.1).

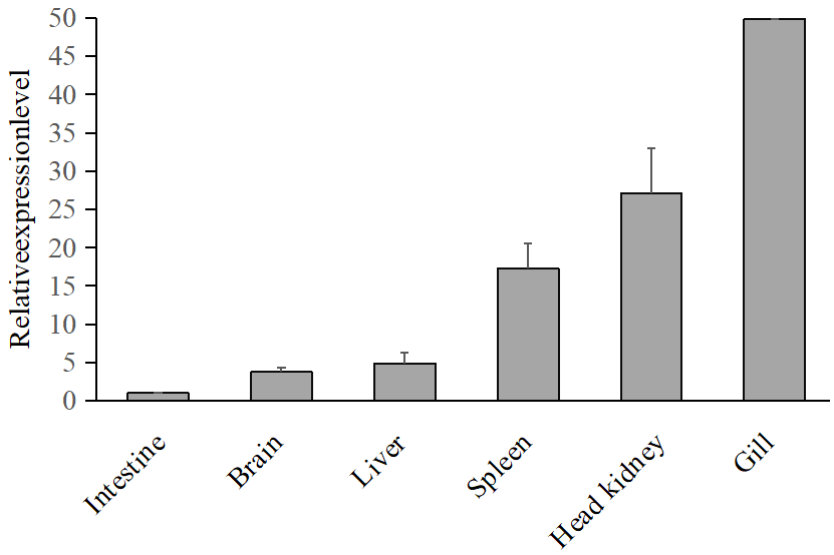

Figure S4. Relative transcriptional levels of *NtMMP-9* in different tissues from healthy fish. The transcriptional value of *NtMMP-9* in the intestine was used as the internal reference.

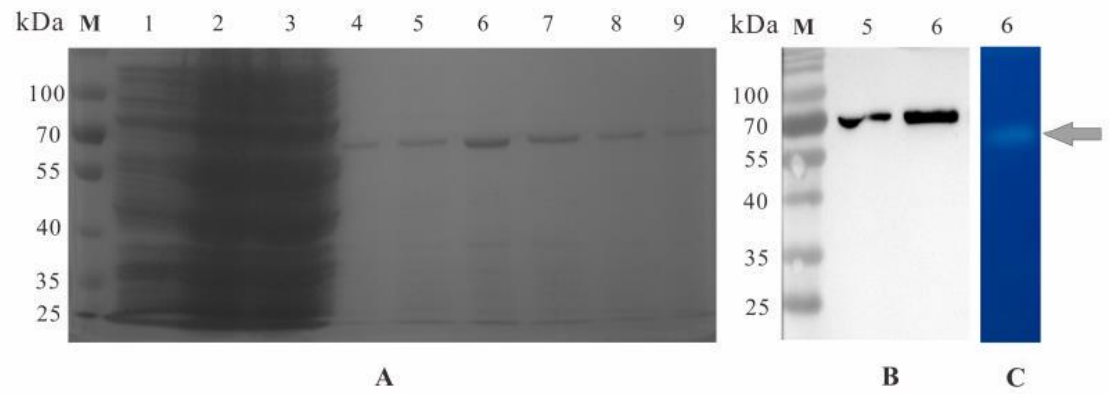

Figure S5. Electrophoretic analysis of recombinant aNtMMP-9 expressed from Rosetta-gami 2 (DE3) competent cell.

(A) SDS-PAGE of aNtMMP-9 expressed by Rosetta-gami (DE3) at 16 °C. M, protein maker; Lane 1, the total protein expressed without IPTG; Lane 2, the total protein expressed after IPTG (0.5 mM) induction for 12 h; Lane 3, unbound protein; Lane 4, the protein from last washing with imidazole (200 mM); and Lanes 5–9, the proteins collected using an elution buffer (0.5 mL, 200 mM imidazole) and repeated five times. (B) Western-Blotting analysis for the proteins obtained from lanes 5 and 6 in Figure S4A. (C) Gelatin zymography of the protein harvested from that of Figure S4B. The molecular weight (63.4 kDa) of the obtained aNtMMP-9 was denoted by an arrow.

Supplementary tables

Table S1. Information of experimental fish used in this study

Fish	Fish number per tank	Tank number	Fish number in different time points after *S. agalactiae* challenge for collecting their tissues			
			4 h	24 h	48 h	72 h
Healthy group	10	1	Three healthy fish for collecting tissues and identifying the transcriptional levels of *NtMMP-9* gene			
Control group	10	3	3	3	3	3
Challenged group	10	3	3	3	3	3

Notes: Eighty fish were purchased, and 76 of them were used in this study. Three fish was used to perform the pathogen-free detection. Three fish was used to extract the total RNA and synthesize cDNA. Others of the 76 fish were divided into three groups according to those described in Materials and Methods.

Table S2. Interactions between NtMMP-9 and other proteins in Nile tilapia under *S. agalactiae* infection.

Abbreviation	Protein/Accession number	Abbreviation	Protein/Accession number
HB-EGF	Proheparin-binding EGF-like growth factor isoform X1 (XP_019200773.1)	MMP-14	Matrix metalloproteinase-14 (XP_003457484.1)
CYBA	Cytochrome b-245 light chain (XP_003442297.1)	MMP-15	Matrix metallopeptidase-15 (XP_003437629.1)
TIMP-2(1)	Metalloproteinase inhibitor 2 (XP_003450177.1)	DCN	Decorin precursor (NP_001266536.1)
TIMP-2(2)	Metalloproteinase inhibitor 2 (XP_003441786.1)	PTK	Proto-oncogene tyrosine-protein kinase Src isoform X4 (XP_003438972.1)
TIMP-4	Metalloproteinase inhibitor 4 (XP_003454588.1)	TIMP-3	Metalloproteinase inhibitor 3 (XP_005939292.1)
Collagenase 3	Collagenase 3 (XP_013129643.2)	Thbs2	Thrombospondin-2 isoform X3 (XP_005474097.1)
IGF-I	Insulin-like growth factor I (AAC17494.1)	Plat	Tissue-type plasminogen activator isoform X1 (XP_005473584.2)
ITGB4	Integrin beta-4 isoform X2 (XP_013129071.1)	Plasminogen	Plasminogen (XP_005461555.1)
Thbs1b	Thrombospondin-1b isoform X1 (XP_005453322.1)	Lama4	Laminin subunit alpha-4 (XP_005457978.1)
Thbs1a	Thrombospondin-1a precursor (NP_001266664.1)	LYVE1	Lymphatic vessel endothelial hyaluronic acid receptor 1 isoform X4 (XP_005471040.1)

Note: The data of Nile tilapia were from the STRING 10.5 (http://string-db.org/) of Nile tilapia.

Table S3. Putative binding sites of NtMMP-9 interacting with DCN

Interaction	NtMMP-9 aa	DCN aa	Length (Å)
Hydrophobic interaction	Cys-376, Cys-561	Trp-335	
Hydrophobic interaction	Phe-538, Trp-540, Val-551, Gly-552, Tyr-553	Leu-327, Phe-328, Val-332, Pro-333, Leu-353	
Cation-π interaction	Lys-377	Trp-335	
Anion-π interaction	Asp-557	Tyr-334	
CH-π interaction	Phe-538, Trp-540	Phe-328	
Hydrogen bond	Lys-377	Glu-336	3.1
	Tyr-553	Leu-327	2.3
	Val-551	Leu-353	2.4

Table S4. Putative binding sites of NtMMP-9 interacting with TIMP-2

Interaction	NtMMP-9 aa	TIMP-2 aa	Length (Å)
Hydrophobic interaction	Phe-183, Phe-209, Trp-202	Ala-197	
Hydrophobic interaction	Phe-111, Phe-168, Gly-170, Ala-172, Tyr-200, Tyr-215, Phe-217	Met-150, Val-194, Tyr-204, Val-207, Ala-208	
Cation-π interaction	Tyr-215	Arg-196	
CH-π interaction	Tyr-215	Tyr-204	
Hydrogen bond	Asp-39	Gln-84	2.7
	Ala-31	Thr-83	2.5
	Tyr-12	Gly-81	3.2
	Tyr-49	Gln-149	3.4
	Ser-23	Gln-146	3.5
	Arg-196	Met-150	2.8