

Oral delivery of *Bacillus subtilis* spores expressing *Clonorchis sinensis* paramyosin protects *grass carp* from cercaria infection

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Table S1 Genes and primers sequences used for RT-PCR

Gene		Sequences of primers	Accession number
ZO-1	F	AAACTGTCGAGAGAGAAGCCT	KJ000055
	R	AGAGCCTGGTCCTCATGTTCTT	
Occludin	F	ATGAGCTGCCTGTGCGTAG	KF193855
	R	GGCGTAGTCTGTGTCACGAT	
Claudin b	F	AGCCAACGTCGTCATCCAAG	KF193860
	R	GCATCAGAGCGAGGGGTAGC	
Claudin c	F	GTACCCGCCACAAAGTCGTA	KF193859
	R	ACAATGGACACCGAACAAAACAA	
IgM	F	AGAGAAGGAACGCTGACGTG	DQ417927
	R	GTCTCCATGGGCTGTGGTAG	
IgZ	F	GGCTGTCTGGCCTCTGATTC	GQ429175.1
	R	TGGTACGCTGCATCACAAGT	
TNF- α	F	CAAACCTGCTCACCGAGACGA	HQ696609.1
	R	TGCAGTGGAAGCTCAGGAAA	
IL-8	F	CCCTACTGCTCCCTGGGTTA	JN663841
	R	AACCAAGCAGAATGGTGCAG	

Table S2 LC-MS/MS identification results of the protein from B.s-CotC-CsPmy spores

Sample	Score	Mass	Matches	Sequence	emPAI	Accseeion Number	Protein name
B.s-CotC-CsPmy	5048	99443	1144(305)	145(77)	83.54	gi 374434365	Paramyosin(<i>C. sinensis</i>)

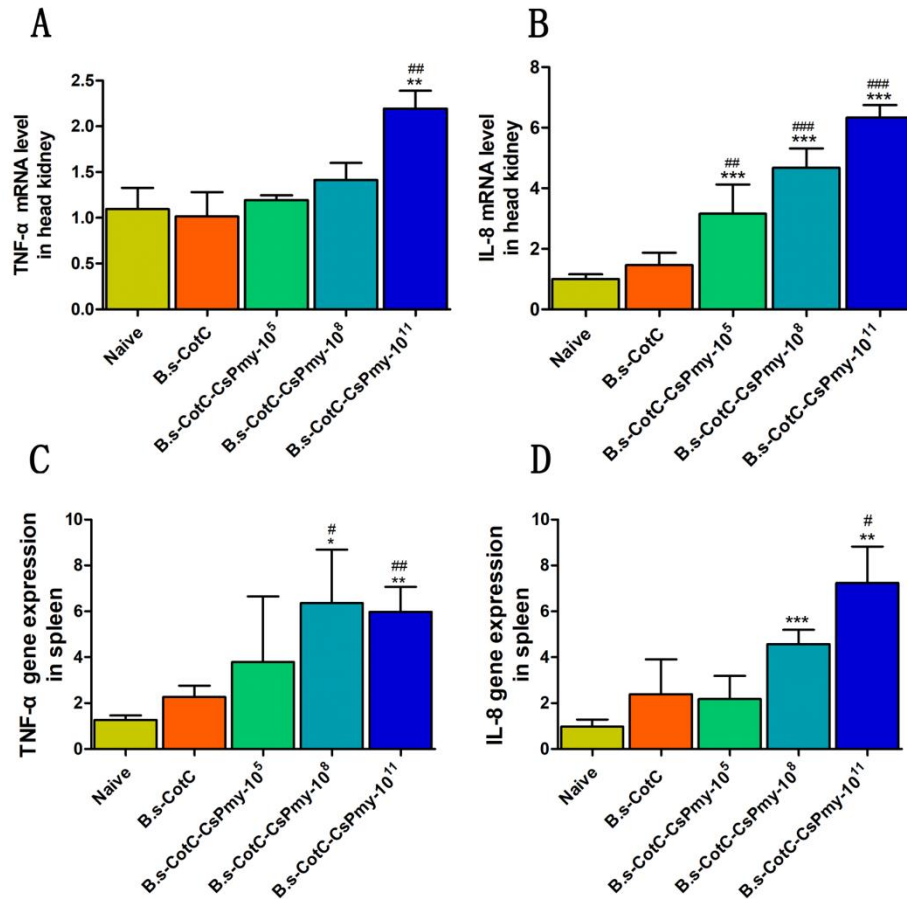


Fig. S1 Relative mRNA levels of TNF- α and IL-8 in head kidney and spleen 6 weeks after the beginning of the immunization. (A) TNF- α in head kidney; (B) IL-8 in head kidney; (C) TNF- α in spleen; (D) IL-8 in spleen. Naive, basal diet group; B.s-CotC-10⁸: basal diet plus 1 \times 10⁸ CFU/g B.s-CotC spores. B.s-CotC-CsPmy-10⁵, 10⁸, 10¹¹, basal diet plus 10⁵, 10⁸, 10¹¹ CFU/g B.s-CotC-CsPmy spores, respectively. * P < 0.05, ** P < 0.01, *** P < 0.001 (compared with naive group); # P < 0.05, ## P < 0.01, ### P < 0.001 (compared with B.s-CotC-10⁸ group).

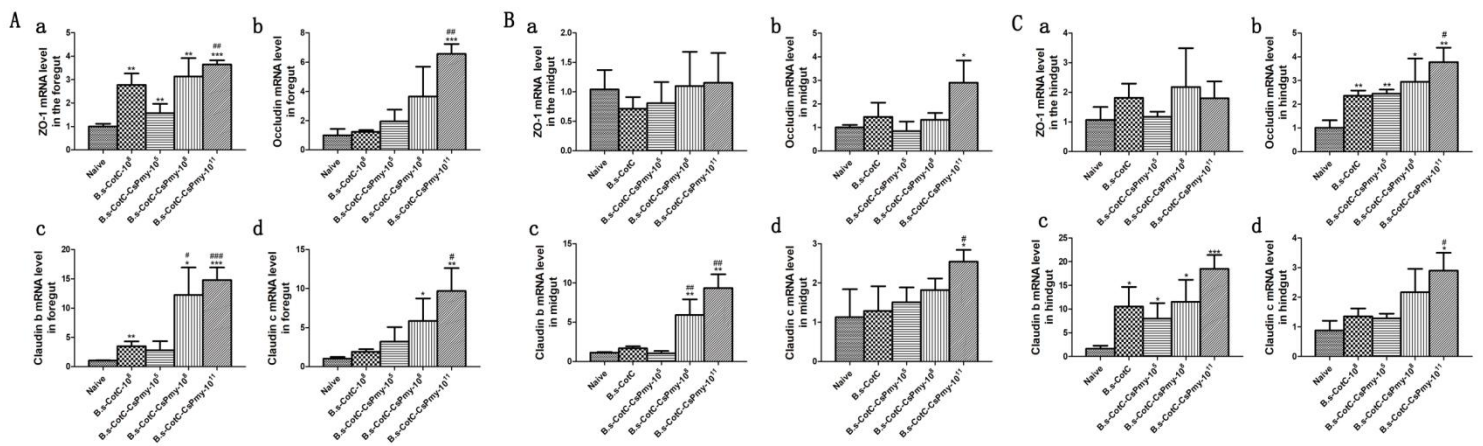


Fig. S2 Transcriptional levels of genes encoding tight junction proteins in different intestinal segments.

The relative transcriptional levels were analyzed by qRT-PCR at 6 weeks after the beginning of the immunization. (A) foregut, (B) midgut, (C) hindgut. (a) ZO-1, (b) occludin, (c) claudin b, (d) claudin c. Naive, basal diet group; B.s-CotC-10⁸: basal diet plus 1×10⁸ CFU/g B.s-CotC spores. B.s-CotC-CsPmy-10⁵, 10⁸, 10¹¹, basal diet plus 10⁵, 10⁸, 10¹¹ CFU/g B.s-CotC-CsPmy spores, respectively. **P* < 0.05, ***P* < 0.01 (compared with naive group); #*P* < 0.05, ##*P* < 0.01 (compared with B.s-CotC-10⁸ group).