Supplementary Materials

Glycyrrhizic acid activates chicken macrophages and enhances their *Salmonella*-killing capacity in vitro

Bai-kui WANG[1], Yu-long MAO[1], Li GONG[1], Xin XU[1], Shou-qun JIANG[2], Yi-bing WANG[1], Wei-fen LI[†1]

[1]*Key Laboratory of Animal Molecular Nutrition of Education of Ministry, Key Laboratory of Animal Feed and Nutrition of Zhejiang Province, College of Animal Sciences, Zhejiang University, Hangzhou 310058, China*

[2]*Institute of Animal Science, Guangdong Academy of Agricultural Sciences, State Key Laboratory of Livestock and Poultry Breeding,*

Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Guangdong Public Laboratory of Animal Breeding and Nutrition, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China

[†]E-mail: wfli@zju.edu.cn

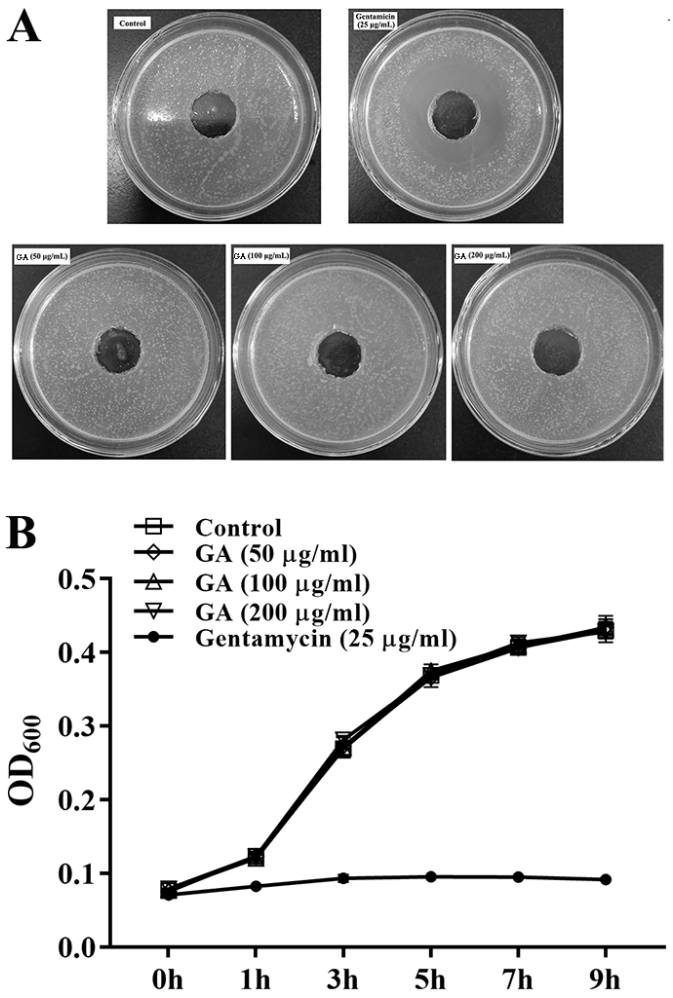

Fig. S1 In vitro antibacterial activity of glycyrrhizic acid against *Salmonella* Typhimurium

(A) Radial diffusion assay, ST was incorporated into Luria-Bcrtani (LB) agarose medium (2×10^5 CFU/ml). PBS, gentamycin (25 μg/ml) and GA (50, 100, 200 μg/ml) were added into each well (8 mm diameter), and the plates were incubated at 37 °C for 18 h. The antibacterial activity was measured by the inhibition zone. (B) Bacterial growth kinetics, ST was grown to mid-exponential phase and resuspended in LB medium (2×10^7 CFU/ml), and then incubated with PBS, gentamycin (25 μg/ml) and GA (50, 100, 200 μg/ml). The cultures were incubated at 37 °C with shaking (180 r/min) for 9 h. The OD_{600} of cultures was recorded at indicated time points (0 h, 1 h, 3 h, 5 h, 7 h and 9 h) using SpectraMax M5 (MD). Data are mean±standard deviation for three independent experiments. OD: optical density

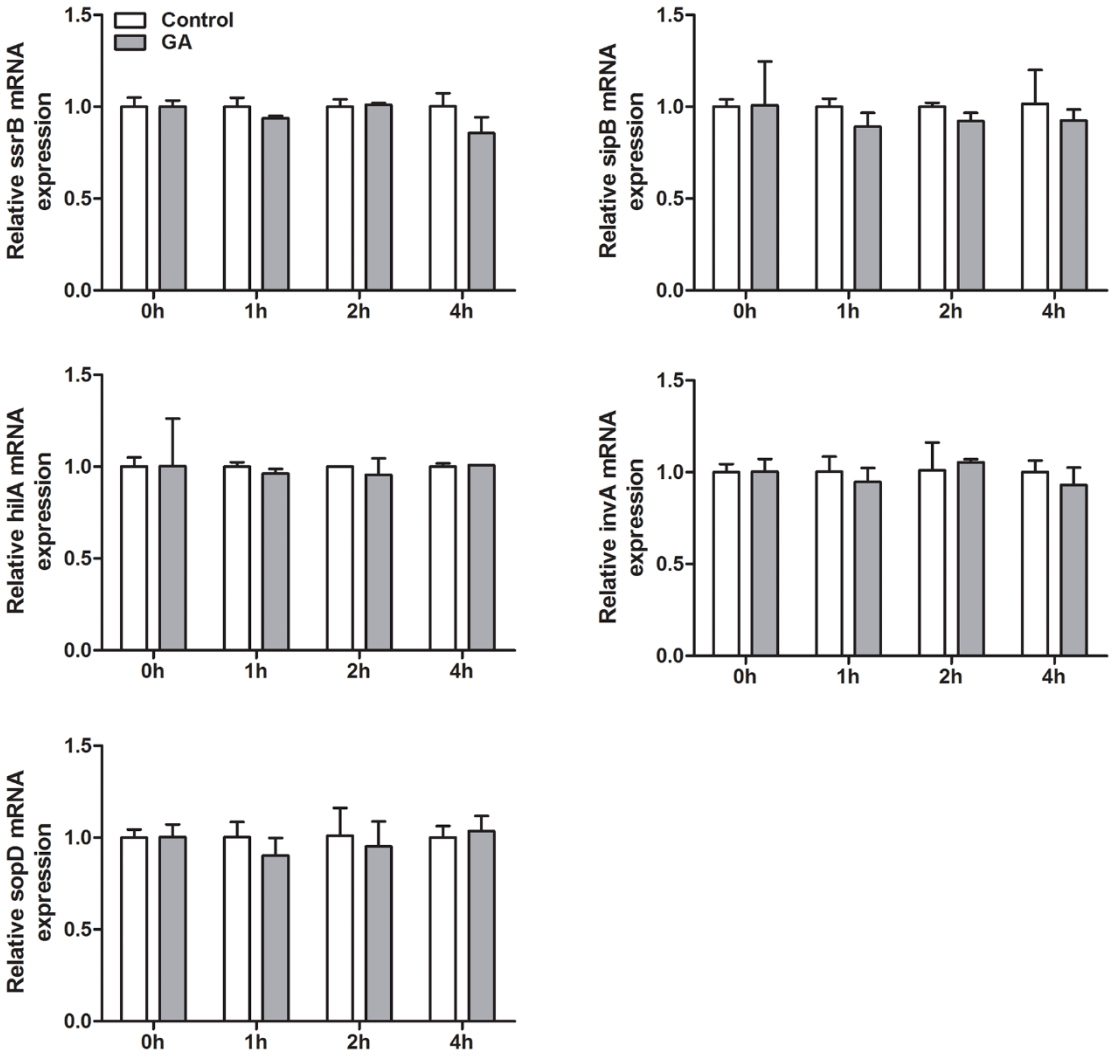

Fig. S2 Effect of glycyrrhizic acid on *Salmonella* Typhimurium virulence gene expression in vitro

ST (1×10^8 CFU/ml) was resuspended in Luria-Bcrtani (LB) medium and incubated with PBS or GA (100 μg/ml) at 37 °C. Total RNA was extracted at 0 h, 1 h, 2 h and 4 h, and then virulence gene (*ssrB*, *sipB*, *hilA*, *invA* and *sopD*) expression was measured by real-time PCR. The primer sequences are listed in Table S1. The relative quantification of genes was determined by changes in expression of transcripts relative to expression in untreated ST. Samples were normalized to the reference gene *16s rRNA*. Data are mean±standard deviation for three independent experiments

Table S1 List of real-time PCR primers

Gene Name	Primers (5'→3')	Product (bp)	Accession number
InvA	F: CATTAACCTTGTGGAGCATATTCG R: CATCCTCAACTTCAGCAGATACC	110	M90846
HilA	F: CGACTCATACATTGGCGATACTT R: CGGCAGTTCTTCGTAATGGT	145	U25352
SipB	F: GTATGGCAGGCGATGATTGA R: ATAAACACTCTTGGCGGTATCC	144	NC_003197
SopD	F: GGACGCTTCTCAGACACAAT R: CGGGACGCATCATCTCATAA	269	AF234265
ssrB	F: ACGAGCCTGACATACTTATCCT R: CGCTAACAGAACTTGCTGACTA	203	Z95891
16s rRNA	F: CGATGTCTACTTGGAGGTTGTG R: CTCTGGAAAGTTCTGTGGATGTC	199	NC_003198

InvA: invasion protein A; *HilA*: hyperinvasive locus A; *sipB*: *salmonella* invasion protein B; *sopD*: *Salmonella* typhi outer protein D; *16s rRNA*: 16s ribosomal RNA