

## Molecular characterization, expression and antibacterial function of interleukin-17 in the common Chinese cuttlefish (Sepiella japonica)

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**Abstract:** Interleukin-17 (IL-17) cytokine family plays critical roles in both acute and chronic inflammatory responses in vertebrates, while few studies have been reported so far in Cephalopoda. In this study, twenty IL-17 transcripts obtained from Sepiella japonica were divided into eight groups (designated as Sj IL-17-1 to Sj IL-17-8). Multiple alignments and phylogenetic analysis showed that Sj IL-17-5, Sj IL-17-6 and Sj IL-17-8 have low homology with the other five Sj IL-17s. Eight Sj IL-17 mRNAs were ubiquitously expressed in ten examined tissues, with dominant expression in the hemolymph. qRT-PCR data showed that the mRNA expression levels of Sj IL-17-2, Sj IL-17-3, Sj IL-17-6, and Sj IL-17-8 were significantly up-regulated in infected cuttlefishes, and Sj IL-17-2, Sj IL-17-6, Sj IL-17-7, and Sj IL-17-8 mRNAs were significantly up-regulated after bath infection of Vibrio harveyi, suggesting that certain Sj IL-17s were involved in the immune response of S. japonica against V. harveyi infection. In addition, the experiment of antibacterial activity of Sj IL-17-4 and Sj IL-17-7 showed that the bacterial growth rate decreased gradually with the increase of protein concentration, indicating that both Sj IL17-4 and Sj IL17-7 possessed significant function of killing bacteria. This study aims to understand the involvement of Sj IL-17 genes in immune responses of cuttlefish against bacterial infections.

### Twenty transcripts are divided into eight groups Sj IL-17 transcript 15 Sj\_IL-17 transcript 17 Sj IL-17 transcript 12 Sj IL-17 transcript 16 Group Sj\_IL-17 transcript 13 Sj IL-17 transcript 18 i IL-17 transcript 14 Sj IL-17 transcript 4 Sj IL-17 transcript 6 Sj IL-17 transcript Sj\_IL-17 transcript 7 Sj\_IL-17 transcript 8 Sj\_IL-17 transcript 1 Group 1 Sj\_IL-17 transcript 10 Group 5 Sj\_IL-17 transcript 19 | Group 8 - Sj IL-17 transcript 20 Sj\_IL-17 transcript 11 | Group 6 Figure 1. Phylogenetic analysis and multiple alignment of Sj\_IL-17 transcripts. (A) Phylogenetic analysis of Sj IL-17 transcripts defined eight groups. Twenty transcripts from S. japonica were aligned and constructed the neighbor-joining tree using MEGA 6. (B) Phylogenetic tree constructed based on deduced amino acid sequences of mollusks and vertebrate IL-17 genes using MEGA 6.06. A bootstrap analysis is performed using 1000 replicates to test the relative support for particular clades. (C) Multiple alignment of the deduced amino acid sequences of Sj IL-17 genes with those of other vertebrates. The amino acid sequences of these genes were predicted using MEGA 6. ClustalW program in MEGA 6 and BoxShade were used for multiple sequence alignments. Similar amino acid residues are marked as grey shadow and identical residues as black shadow. Absent amino acids are indicated by dashes (-). The seven highly conserved cysteine residues are highlighted in red. $\rightarrow$ : $\beta$ -sheet. Cysteine residues potentially forming disulfide bonds are linked by black lines. SJ: Sepiella japonica; CG: Crassostrea gigas; LG: Lottia gigantea; HS: Homo sapiens. All eight genes have the highest expression level in the hemolymph

### A possible link between Sj\_IL-17s and immune response against infection Figure 4. The changes of mRNA expression of Sj IL-17 genes in healthy and infected cuttlefish. (A) The main phenotype of sick S. japonica. (B) Histological sections of gill and liver from healthy and infected cuttlefish by H&E staining. (C) The mRNA expressions of eight Sj IL-17 genes were examined in the white body, gill, and skin after pathogen infection by qRT-PCR. The $\beta$ -actin and EF- $I\gamma$ genes were employed as double internal controls (n=3). Significant differences between healthy and infected cuttlefish are indicated by asterisks (\*: P < 0.05, \*\*: P < 0.01).

Distinct antibacterial activities of Sj IL-17-4 and Sj IL-17-7 proteins

# E. coli C V. parahaemolyticus V. harveyi 0 0.5 1 10 50 100 150 200 Concentration of protein (µg/mL

Figure 5. Antimicrobial activies of Sj IL-17-4 and Sj IL-17-7. (A-D) Antibacterial activity of Sj IL-17-4 recombinant protein against Gram-negative bacteria (Escherichia coli, Vibrio harveyi, Vibrio parahaemolyticus), and also Gram-positive bacteria (Staphylococcus aureus). The coincubation solutions with Sj IL-17-4 protein concentrations of 0, 10, 200 μg/mL were diluted at :1000 and then streaked onto plates, followed by incubation at an appropriate temperature for 18 h. (E-H) Antibacterial activity of Sj IL-17-7 protein against Gramnegative, -positive bacteria. The co-incubation solutions with Sj IL-17-7 protein concentrations of 0, 20, 600 µg/mL were diluted at 1:1000 and then streaked onto plates, followed by incubation at an appropriate temperature for 18 h. All the experiments were performed in triplicate, and results were shown as mean ±

### Si IL-17s might exist functional diversification in immune response

0.05).

Figure 2. Tissues expression levels of *IL-17* mRNAs in

S. japonica. (A-H) The mRNA expressions of eight

Sj IL-17 genes were detected in the 10 tissues

(hemolymph, brain, kidney, liver, gonad, gut, gill, skin,

white body and optic lobe) from healthy S. japonica by

qRT-PCR. The  $\beta$ -actin and EF- $I\gamma$  genes were employed

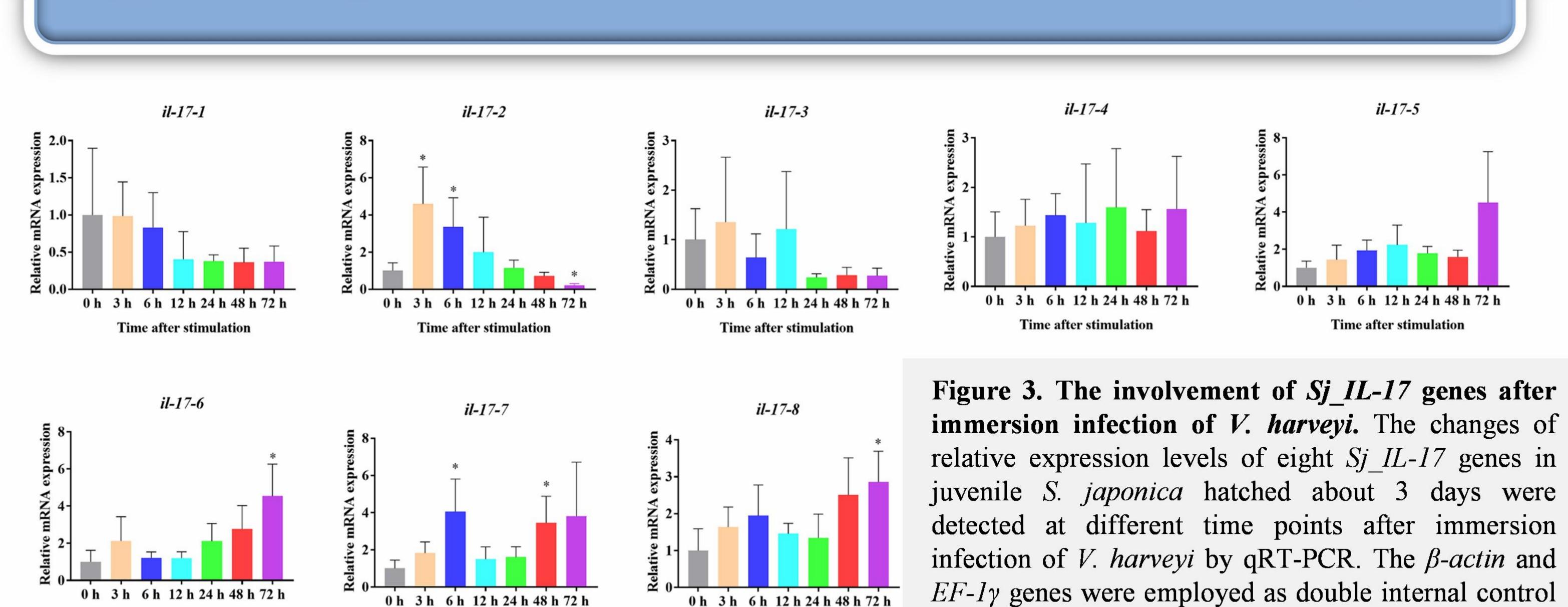
as double internal control (n=4). Different letters above

the bars indicate significant differences among/between

the means (One-way ANOVA and Duncan test, P <

(n=3). Significant differences between healthy and

infected cuttlefish are indicated by asterisks (\*: P <



0 h 3 h 6 h 12 h 24 h 48 h 72 h

Time after stimulation

0.05).

0 h 3 h 6 h 12 h 24 h 48 h 72 h

Time after stimulation

0 h 3 h 6 h 12 h 24 h 48 h 72 h

### Conclusions

Twenty Sj IL-17 transcripts were classified into eight groups (Sj IL-17-1 to Sj IL-17-8). Multiple alignment and phylogenetic analysis of amino acids showed that Sj IL-17-5, Sj IL-17-6 and Sj IL-17-8 have low homology with other five Sj IL-17s. All Sj IL-17 transcripts had high expression levels in the hemolymph, implying that Sj IL-17 maybe mediate the immune response of hemocytes. Five Sj IL-17 (Sj IL-17-2, Sj IL-17-3, Sj IL-17-6, Sj IL-17-7 and Sj\_IL-17-8) genes were notably up-regulated either in sick S. japonica or after V. harveyi immersion infection, indicating that they were involved in the immune response against bacterial infection. These results illustrated that certain Sj IL-17 genes were involved in the immune response against V. harveyi infection. The results of antibacterial activity showed that Sj IL-17-4 and Sj IL-17-7 had strong inhibitory effects on the growth of both Gram-negative and Grampositive bacteria, suggesting that they may play important roles in the process of resisting bacterial infection of individuals. Consequently, further understanding the potential biological functions and mechanisms of Sj IL-17s would help us to uncover their functional diversity in cuttlefish immunity and may provide new strategies for the prevention and control of bacterial disease in cuttlefish culture.