

# Antibacterial mechanism of chitosan-gentamicin and its effect on the intestinal flora of *Litopenaeus vannamei* infected with *Vibrio parahaemolyticus*

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## 1. Tables

**Table S1.** Inhibition rate and IC<sub>50</sub> of *V. parahaemolyticus* dealt with CS, GT and CS-GT ( $\bar{x} \pm SD$ , n=3)

Sample	Inhibition rate(%, 20 $\mu$ g/mL)	IC <sub>50</sub> ( $\mu$ g/mL)
CS	12.36 $\pm$ 4.10 <sup>c</sup>	125.62 $\pm$ 4.35 <sup>a</sup>
CS-GT	77.72 $\pm$ 5.31 <sup>b</sup>	18.72 $\pm$ 3.17 <sup>b</sup>
GT	97.38 $\pm$ 3.20 <sup>a</sup>	7.89 $\pm$ 5.08 <sup>c</sup>

Note: a,b,c indicate statistically significant variations in the same column,  $p < 0.01$ .

**Table S2.** The increase multiple of OD<sub>260</sub> and K<sup>+</sup>, increase multiple of conductivity dealt with CS, GT and CS-GT at 12 h ( $\bar{x} \pm SD$ , n=3)

Sample	OD <sub>260</sub> increase multiple	K <sup>+</sup> increase multiple	Conductivity increase rate (%)
CK	/	/	/
CS	5.80 $\pm$ 0.21 <sup>c</sup>	24.41 $\pm$ 4.30 <sup>b</sup>	8.53 $\pm$ 0.05 <sup>b</sup>
GT	11.56 $\pm$ 0.33 <sup>a</sup>	43.70 $\pm$ 4.23 <sup>a</sup>	17.71 $\pm$ 0.05 <sup>a</sup>
CS-GT	8.62 $\pm$ 0.22 <sup>b</sup>	39.52 $\pm$ 5.01 <sup>a</sup>	15.35 $\pm$ 0.04 <sup>a</sup>

Note: “/” means no data provided; a,b,c indicate statistically significant variations in the same column,  $p < 0.05$ .

**Table S3.** Composition and relative abundance of gut microbial communities at phylum level ( $\bar{x} \pm SD$ , n=3)

Group	Tenericutes (%)	Proteobacteria (%)	Firmicutes (%)	Bacteroidetes (%)
CK	4.41 $\pm$ 0.12 <sup>d</sup>	34.57 $\pm$ 1.53 <sup>b</sup>	16.33 $\pm$ 3.46 <sup>b</sup>	34.95 $\pm$ 1.26 <sup>a</sup>
Only-infected group	3.32 $\pm$ 0.18 <sup>d</sup>	59.99 $\pm$ 1.65 <sup>a</sup>	21.41 $\pm$ 6.96 <sup>b</sup>	13.65 $\pm$ 4.72 <sup>c</sup>
CS-GT-10	18.68 $\pm$ 5.15 <sup>c</sup>	54.65 $\pm$ 1.02 <sup>a</sup>	5.78 $\pm$ 0.32 <sup>c</sup>	5.86 $\pm$ 0.64 <sup>d</sup>
CS-GT-50	58.25 $\pm$ 0.27 <sup>b</sup>	4.03 $\pm$ 0.07 <sup>c</sup>	11.12 $\pm$ 7.72 <sup>b</sup>	16.01 $\pm$ 5.31 <sup>c</sup>
CS-GT-100	79.05 $\pm$ 1.22 <sup>a</sup>	7.95 $\pm$ 0.35 <sup>c</sup>	3.76 $\pm$ 0.35 <sup>c</sup>	6.78 $\pm$ 0.59 <sup>d</sup>
GT-10	1.68 $\pm$ 0.23 <sup>d</sup>	5.41 $\pm$ 0.35 <sup>c</sup>	37.37 $\pm$ 1.09 <sup>a</sup>	36.52 $\pm$ 1.72 <sup>a</sup>
CS-250	23.75 $\pm$ 7.31 <sup>c</sup>	34.54 $\pm$ 1.23 <sup>b</sup>	34.87 $\pm$ 4.41 <sup>a</sup>	25.26 $\pm$ 2.34 <sup>b</sup>

Note: a,b,c indicate statistically significant variations in the same column,  $p < 0.05$

**Table S4.** Relative intensities of the fitted C1s peak of CS, OCS, GT, P-CS-GT and CS-GT.

Sample	Position (eV)	Relative intensity (%)	Possible element state
CS	285.2	50.73%	C-C/C-H
	286.7	30.16%	C-N
	288.3	13.17%	C-O

	289.2	5.64%	C=O
OCS	285.0	56.27%	C-C/C-H
	286.7	20.53%	C-N
	288.4	10.56%	C-O
	289.2	12.64%	C=O
GT	285.2	50.33%	C-C/C-H
	286.7	36.73%	C-N
	288.4	12.94%	C-O
P-CS-GT	285.0	38.10%	C-C/C-H
	286.7	27.58%	C-N
	287.5	19.16%	C=N
	288.4	10.74%	C-O
	289.3	4.42%	C=O
CS-GT	285.7	42.42%	C-C/C-H
	286.7	29.48%	C-N
	287.5	10.08%	C=N
	288.5	12.24%	C-O
	289.5	5.77%	C=O

## 2. Figures

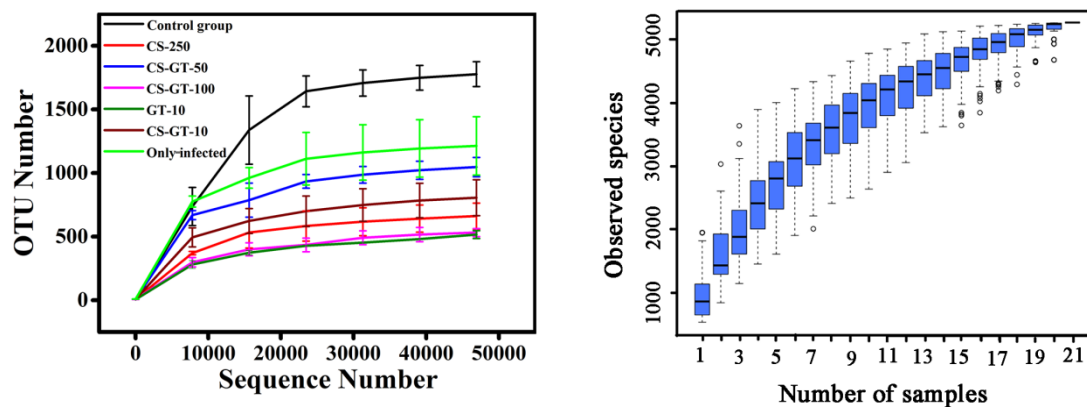
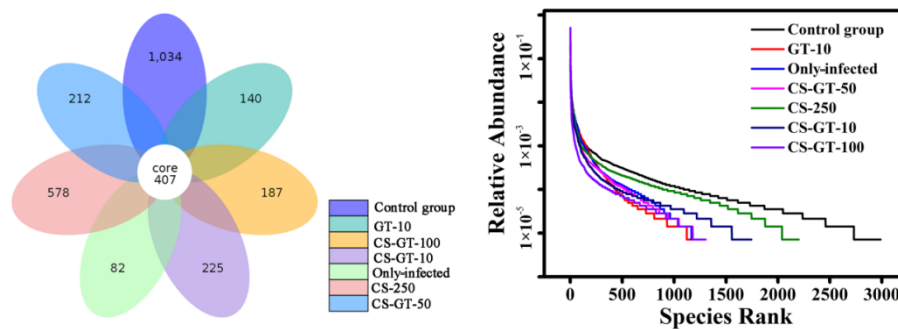
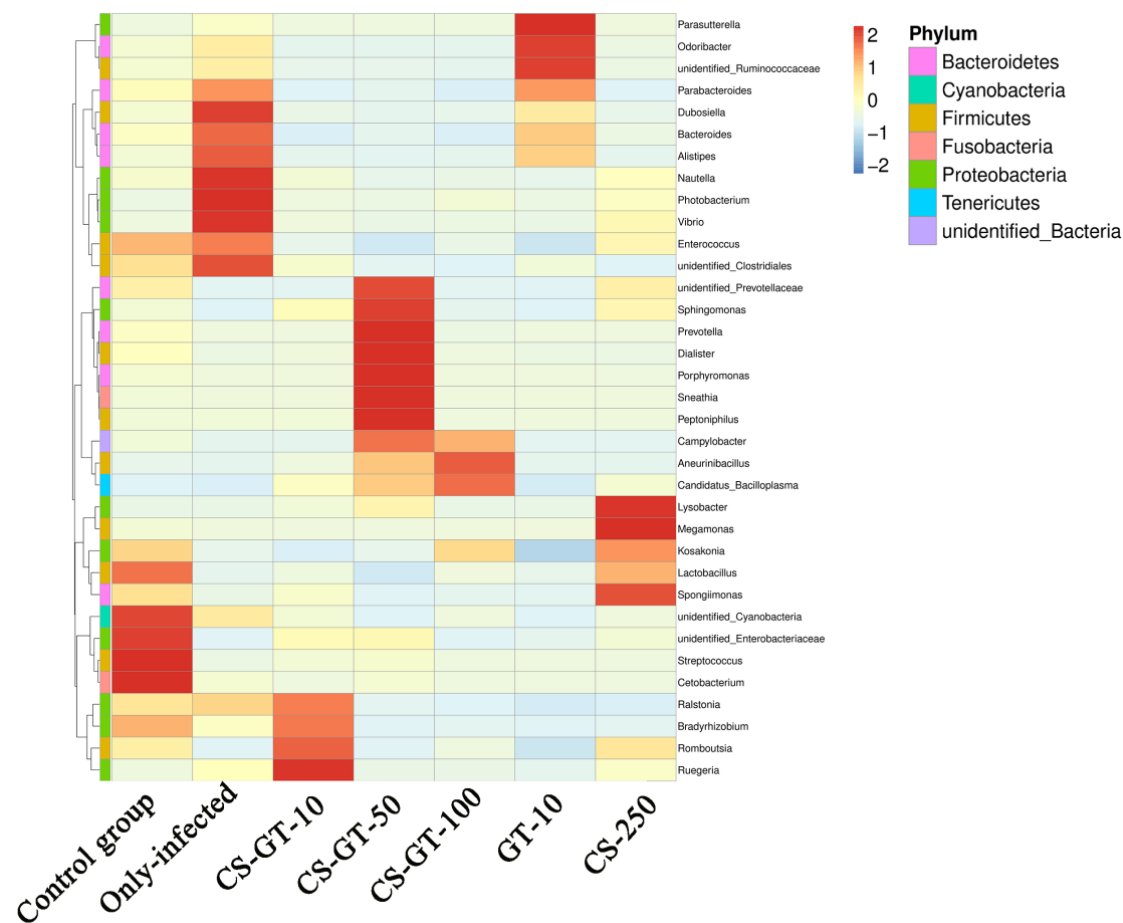


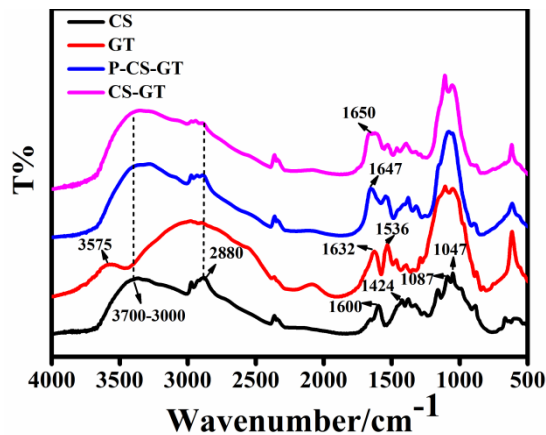
Figure S1. Rarefaction curves and species accumulation box diagram of intestine samples ( $\bar{x} \pm SD$ ,  $n = 3$ )



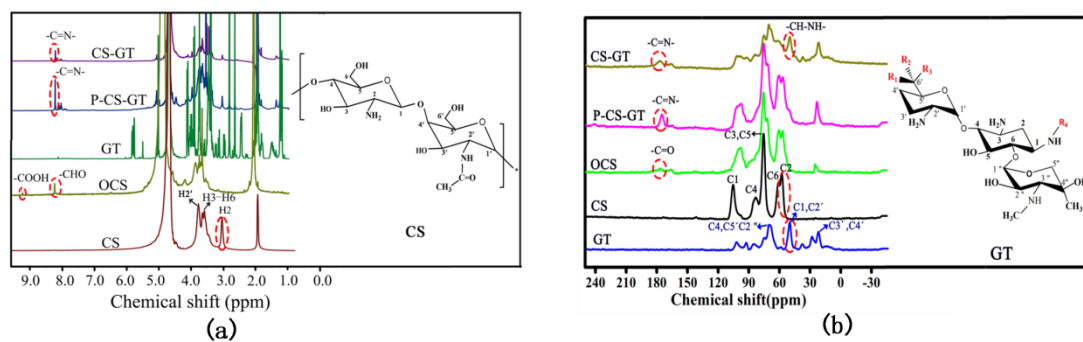
**Figure S2.** Venn diagram of operational taxonomic unit (OTU) numbers and rank abundance curve ( $\bar{x} \pm$  SD, n=3)



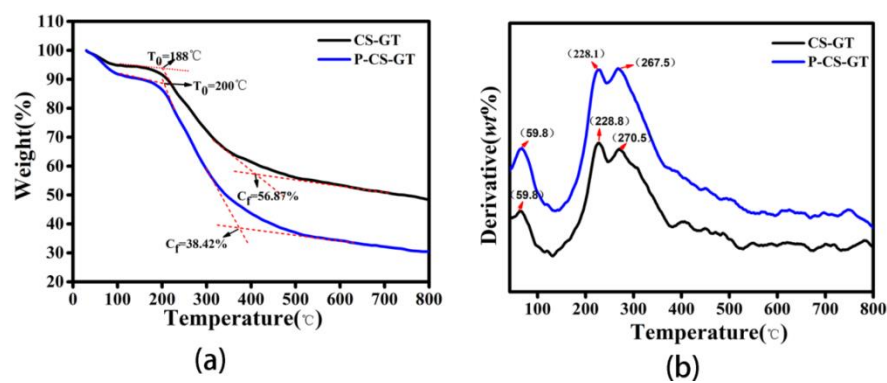
**Figure S3.** Heatmap of the species abundance clustering in the top 35 at the genus level ( $\bar{x} \pm$  SD, n=3)



**Figure S4.** The FTIR spectrum of CS, GT, P-CS-GT (C=N) and CS-GT (C-N).



**Figure S5.** The  $^1\text{H}$ -NMR (a) and solid-state  $^{13}\text{C}$ -NMR spectrum of CS, OCS, GT, P-CS-GT and CS-GT.



**Figure S6.** The TG curves (a) and DTG curves (b) of P-CS-GT and CS-GT.