

## **Chitosan Coupling Makes Microbial Biofilms Susceptible to Antibiotics**

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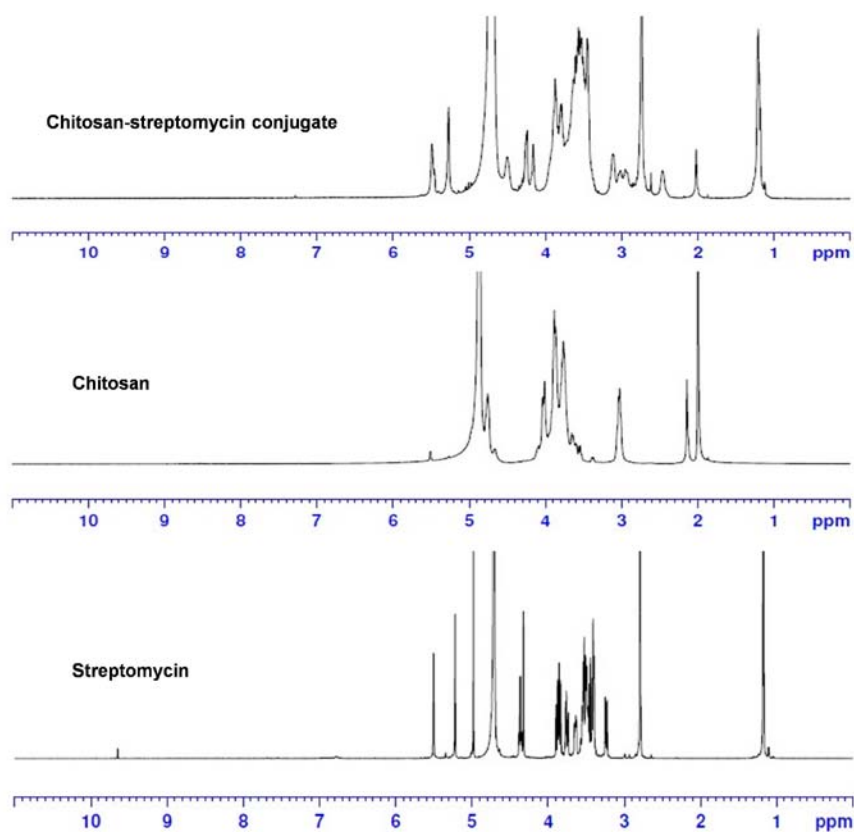
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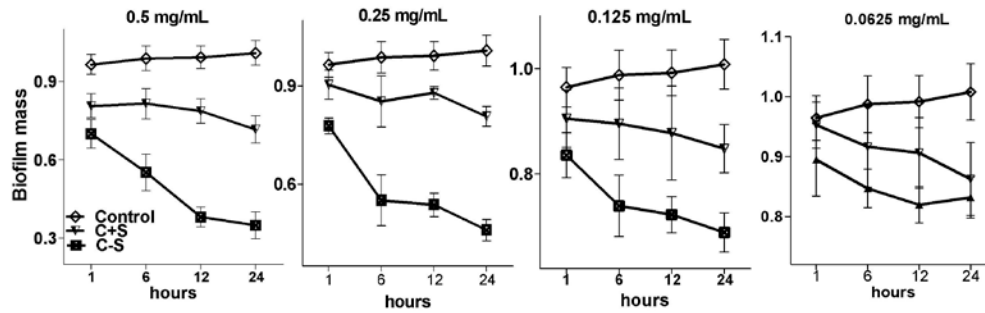
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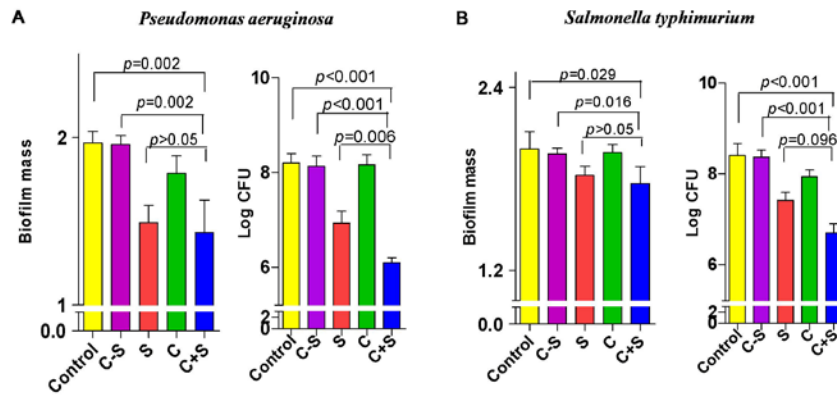
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**Figure S1.** <sup>1</sup>H NMR spectra of chitosan, streptomycin and C-S conjugate. The spectra were recorded at 298 K in deuterium oxide on a Varian VNMRS-500 NMR spectrometer. HOD (4.80 ppm) was used as the chemical-shift reference in NMR experiments.



**Figure S2. Concentration dependence in the anti-biofilm capabilities of C-S conjugate.** *L. monocytogenes* biofilms were exposed to 0.25 mg/mL C-S conjugate (23% streptomycin, ~13 kDa chitosan), equivalent chitosan (C) or streptomycin (S) alone, and the respective mixture (C+S) for 24 h. Biofilms incubated in phosphate-buffered saline were used as control. Biofilm mass (A) and viable cells (B) were quantified. These experiments were performed three times with similar results each time. Error bars represent SD.



**Figure S3. C-S conjugate failed to disrupt preformed biofilms built by Gram-negative organisms.** Biofilms formed by *Pseudomonas aeruginosa* (A) or *Salmonella typhimurium* (B) were exposed to 0.25 mg/mL C-S conjugate (23% streptomycin, ~13 kDa chitosan), equivalent chitosan (C) or streptomycin (S) alone, and the respective mixture (C+S) for 24 h. Biofilms incubated in phosphate-buffered saline were used as control. Biofilm mass and viable cells were quantified. These experiments were performed three times with similar results each time. Error bars represent SD.

Bacteria	MIC ( $\mu\text{g/mL}$ )			
	C-S	C	S	C+S
<i>L. monocytogenes</i>	32	256	8	32
<i>L. innocua</i>	64	1000	8	64
<i>L. welshimeri</i>	32	500	8	32
<i>S. aureus</i>	64	1000	8	64
<i>E. Faecalis</i>	128	2000	32	128
<i>P. Aeruginosa</i>	128	6000	32	128
<i>S.typhimurium</i>	256	8000	64	256

**Table S1. Minimum inhibitory concentrations (MICs) of compounds against planktonic bacteria.** Test compounds including C-S conjugate (23% streptomycin, ~13 kDa chitosan), chitosan (C) or streptomycin (S), and the respective mixture (C+S) were dissolved in TSB broth at an initial concentration of 1024  $\mu\text{g/mL}$  and then serially diluted. The bacteria with a final concentration of  $5 \times 10^5$  CFU/mL in TSB broth per well were inoculated at 37°C for 24 h. These experiments were performed three times with similar results each time.