## **SUPPLEMENTARY DATA**

High molecular / low acetylated chitosans reduce adhesion of *Campylobacter jejuni* to host cells by blocking JlpA

Vanessa Kreling<sup>1</sup>, Franco H. Falcone<sup>2</sup>, Fabian Herrmann<sup>1</sup>, Leon Kemper<sup>1</sup>, Daniel Amiteye<sup>1</sup>, Stefan Cord-Landwehr<sup>3</sup>, Corinna Kehrenberg<sup>4</sup>, Bruno M. Moerschbacher<sup>3</sup> and Andreas Hensel<sup>1\*</sup>

<sup>1</sup> University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Corrensstraße 48, 48149 Münster, Germany.

ORCID: 0000-0003-0886-8384

<sup>2</sup> Institute of Parasitology, Justus Liebig University Giessen, Schubertstraße 81, 35392 Giessen, Germany

<sup>3</sup> University of Münster, Institute of Plant Biology and Biotechnology, Schlossplatz 8, 48143 Münster, Germany. ORCID 0000-0003-1915-1936 (SCL), 0000-0001-6067-3205 (BMM)

<sup>4</sup> Institute of Veterinary Food Science, Justus Liebig University Giessen, Frankfurter Straße 92, 35392 Giessen, Germany

## \* Correspondence:

Andreas Hensel, Institute of Pharmaceutical Biology and Phytochemistry, University of Münster, Corrensstraße 48, 48149 Münster, Germany

Phone: +49 251 8333380, e-mail: ahensel@uni-muenster.de

**Table S1:** Primers for amplification of the *C. jejuni* genes *flpA* and *jlpA*, for the cold shock expression plasmid pCOLD I, and for multiplex PCR for identification of *Campylobacter*.

**Table 2:** Primers for amplification of the *C. jejuni* genes *flpA* and *jlpA*, for the cold shock expression plasmid pCOLD I, and for multiplex PCR for identification of *Campylobacter*.

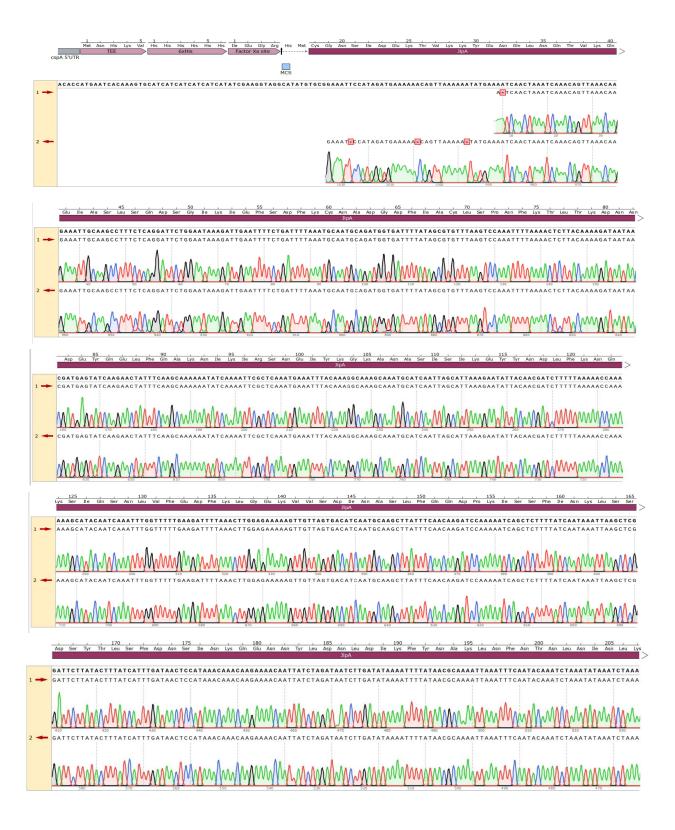
	Sequence (5'-3')		
Amplicon: flpA in pCOI	LD I		
flpA_NdeI-FOR	TATATCATATGAGCTTGCCAAAGGTTGAAAG		
flpA_Xbal-Rev	ATATATCTAGAGACTGAGCCGCCTTAACTTTG		
Amplicon: pCOLD I (se	equencing primers)		
pCOLDI FOR	ACGCCATATCGCCGAAAGG		
pCOLDI REV	GGCAGGGATCTTAGATTCTG		
Amplicon: jlpA in pCO	OLD I		
jlpA_NdeI-FOR	CCCGGGCATATGTGCGGAAATTCCATAG		
jlpA_PstI-REV	ATATATCTGCAGAAATGACGCTCCGCCCA		
16S rDNA	GGGAGCAGCAGTRRGGAAT		
	TGACGGCGTGRGTACAAG		
asp Aspartokinase	GGTATGATTTCTACAAGCGAG		
	ATAAAAGACTATCGTCGCGTG		
hin Hinnuricasa	GACTTCGTGCAGATATGGATGCTT		
hipO Hippuricase	GCTATAACTATCCGAAGAAGCCATCA		

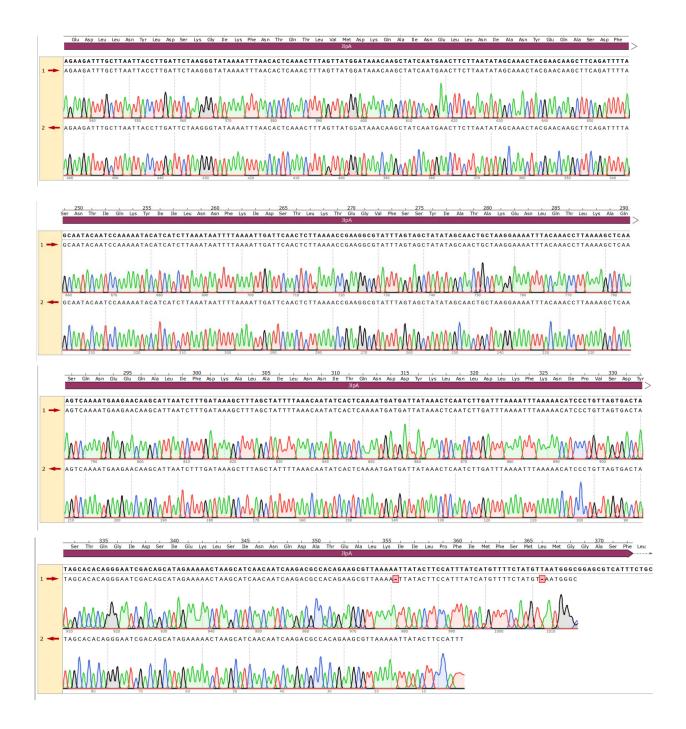
**Table S2:** Relative proliferation [%] of *C. jejuni* after 48 h ( $t_{1/48h}$ ) under chitosan influence and 48 h ( $t_{2/96h}$ ) after changing to test compound-free, unsupplemented media relative to the untreated control UC ( $t_{1/48h}$  value = 100 %). The UC after 96 h ( $t_{2/96h}$  value) had a relative proliferation of 181  $\pm$  17 % (related to the ( $t_{1/48h}$  value = 100 %). Relative values represent the mean  $\pm$  SD from n = 3 independent experiments with n = 4 technical replicates.

Chitosan	Concentration [mg/mL]				
	<i>t</i> [h]	5	2.5	1.25	
		Relative proliferation [%] UC $t_{1/48h}$ = 100%; UC $t_{2/96h}$ = 181 %			
134	t <sub>1/48h</sub>	$0\pm0$	$54 \pm 63$	$74 \pm 40$	
	t <sub>2/96h</sub>	190 ± 75	$176 \pm 68$	176 ± 10	
114	t <sub>1/48h</sub>	26 ± 36	$10 \pm 10$	89 ± 55	
	t <sub>2/96h</sub>	194 ± 14	$100\pm28$	$161 \pm 48$	
652	$t_{1/48h}$	$24 \pm 18$	33 ± 25	87 ± 59	
	t <sub>2/96h</sub>	$123\pm24$	$137\pm27$	161 ± 18	
651	$t_{1/48h}$	6 ± 5	6 ± 8	$177 \pm 123$	
	t <sub>2/96h</sub>	$157 \pm 54$	$119\pm27$	$172\pm70$	
661	$t_{1/48h}$	33 ± 25	$137\pm106$	$156 \pm 91$	
	t <sub>2/96h</sub>	$284 \pm 242$	$273\pm209$	$175 \pm 136$	
90/20	$t_{1/48h}$	10 ± 13	$10 \pm 15$	$181\pm128$	
	t <sub>2/96h</sub>	$174 \pm 45$	$145\pm40$	$197\pm107$	
80/20	t <sub>1/48h</sub>	10 ± 3	7 ± 5	111 ± 76	
	t <sub>2/96h</sub>	177 ± 58	129 ± 42	207 ± 112	
70/20	t <sub>1/48h</sub>	7 ± 6	1 ± 1	123 ± 92	
	t <sub>2/96h</sub>	115 ± 20	120 ± 15	141 ± 50	

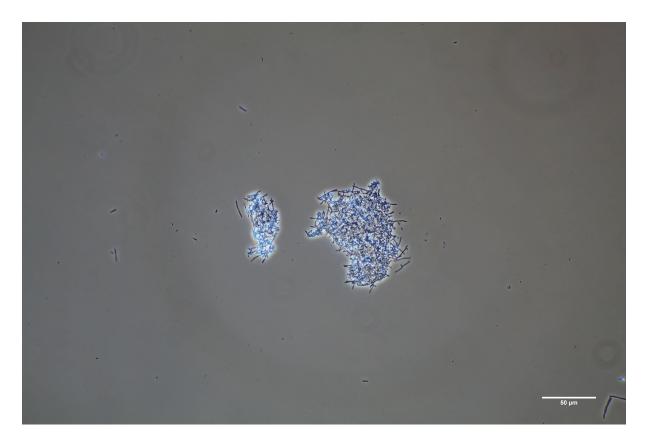


**Figure S1**: Sequence alignment of the *flpA* gene products of the forward and reverse primers with the theoretical *flpA* sequence for cloning into pCOLD using SnapGene. The reference sequence is shown above in bold letters. (1): Sequence of the PCR product with the forward primer. (2) Sequence of the PCR product with the reverse primer. In the figure, the sequenced bases are indicated by the letter code as well as by color: RED/T: thymine; BLUE/C: cytosine; GREEN/A: Adenine; GREY/G: Guanine. In addition, the coded amino acids are listed in this representation.





**Figure S2:** Sequence alignment of the jlpA gene product of the forward and reverse primers with the theoretical jlpA sequence for cloning into pCOLD using SnapGene. Reference sequence is shown above each in thick letters. (1): Sequence of the PCR product with the forward primer. (2) Sequence of the PCR product with the reverse primer. Nucleotides are indicated by the letter code and by color: RED/T: thymine; BLUE/C: cytosine; GREEN/A: adenine; GREY/G: guanine.



**Figure S3:** Chitosan 134 treated *Campylobacter jejuni* after 72 h of incubation leads to strong bacterial agglomeration and elongation of bacteria.