

Supplementary Information

# The Multilateral Efficacy of Chitosan and *Trichoderma* on Sugar Beet

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## Appendix A: Supplementary tables

Supplementary Table S1: RT-PCR primers for *T. atroviride* used in this study

pID (JGI) <sup>1</sup>	gene	primer name	Sequence (5' - 3')	number of cycles	source
28913	<i>cda1</i>	Ta_cda1_RT	TCCTCCATGCAGGACTCTGA	30	this study
		Ta_cda1_RTa	GCTTGGAGCAGCAGTTTTTGT		this study
147996	<i>cda2</i>	Ta_cda2_RT	CAAAGTCATTGCCCTGTCGC	30	this study
		Ta_cda2_RTa	AGACGTCAGTCCGTGA		this study
78914	<i>cda3</i>	Ta_cda3_RT	CACTCGTCCGTCATAGAGGC	30	this study
		Ta_cda3_RTa	CCACAGCGTCTTCCAATTGC		this study
291124	<i>cda4</i>	Ta_cda4_RT	ACCACCAAAAGGGAGTGTG	30	this study
		Ta_cda4_RTa	ACCGGCCATTGGAGGATTTC		this study
292288	<i>cda5</i>	Ta_cda5_RT	CAACTCCTCGACGTGTTGGA	30	this study
		Ta_cda5_RTa	GTGCAAGTAGCTGTCGGTCT		this study
142446	<i>cda6</i>	Ta_cda6_RT	CTGCCAGGCCTACTATCTGC	30	this study
		Ta_cda6_RTa	TCATGCGTCCTTTCGTAGCA		this study
54365	<i>cho1</i>	Ta_cho1_RT	GCTCCAACAACTCTCGGGA	30	this study
		Ta_cho1_RTa	TTATCGCCGCTCAGACCATC		this study
80199	<i>cho2</i>	Ta_cho2_RT	GCGACATTCCTCAATGCCATC	30	this study
		Ta_cho2_RTa	GCTGTTCTCAAAATCCGCCG		this study
145108	<i>cho3</i>	Ta_cho3_RT	TATGGCGAGACAGCGTTCAA	30	this study
		Ta_cho3_RTa	CCGCTTGAGGTTCCAAGACT		this study
197013	<i>cho4</i>	Ta_cho4_RT	GCGGTCTTCCAAATGCCATT	30	this study
		Ta_cho4_RTa	CAGGCTGGTAACGGGTTGAT		this study
216890	<i>cho5</i>	Ta_cho5_RT	ACCATCACCAGCTACAAGGC	30	this study
		Ta_cho5_RTa	ATGCAGGAGGAGGAGGAGAG		this study
16857	<i>cho6</i>	Ta_cho6_RT	GTAGCAGACCTGGCTGGAAG	30	this study
		Ta_cho6_RTa	GAAGCACCAGCACAATGTCC		this study
131598	<i>chi18-5</i>	Ta_chi18-5_RT	GTCTCTGGAGATGCCTACGC	30	this study
		Ta_chi18-5_RTa	AGTCGAGAACTTGCCAAGG		this study
136120	<i>nag1</i>	Ta_nag1_RT	GAGCGATGTCCTACAGCCTC	30	this study
		Ta_nag1_RTa	GGGCCAGATGATGTTGTCCA		this study
299956	<i>gfa1</i>	Ta_gfa1_RT	TCATCGAGGGTCTTGGAAC	30	this study
		Ta_gfa1_RTa	TCGACAGTGACAGACTTGGC		this study

302952	<i>epl1</i>	Ta_epl1_RTa	CTTGCTCTCTTCACCGCCGC	27	[1]
		Ta_epl1_RTa	CCGTTGGTCAGGGCATTTCAT		[1]
300828	<i>tef1</i>	Ta_tef1_RT2s	CGACATTGCCCTCTGGAAGT	20	this study
		Ta_tef1_RT2a	ATCCTGAAGGGGAAGACGGA		this study

*cda*...predicted chitin deacetylase, *cho*...predicted chitosanase, *chi18-5*... endochitinase, *nag1*... N-acetylglucosaminidase, *gfa1*... predicted glucosamine-fructose-6-phosphate aminotransferase, *epl1*...ceratoplatenin, *tef1*... translation elongation factor as housekeeping gene <sup>1</sup><https://mycocosm.jgi.doe.gov/Triat2/Triat2.home.html> .

Supplementary Table S2: RT-qPCR primers for *B. vulgaris* used in this study

GenBank identifier/ reference sequence <sup>1</sup>	gene	primer name	Sequence (5' - 3')	source
GQ375163.1	DMRL	qRT_Bvulg_DMRLf	TCCGCCTCCTCTGGAATACT	this study
		qRT_Bvulg_DMRLr	CGCTGCCTCAGAACCCTTAT	this study
X75946.1	GLU	qRT_Bvulg_GLUf	CAACTTACCTTCCGAGGAAG	[2]
		qRT_Bvulg_GLUr	CTCTAACCCTTGGAGGGTC	this study
S66038.1	SE2	qRT_Bvulg_SE2f	ATTGTCATATACTGGGGCC	[2]
		qRT_Bvulg_SE2r	TTGTAGCAGGGTCACAGTGC	this study
BI073261.1	GST	qRT_Bvulg_GSTf	GCTTTGGATTTGTGGACATTGC	this study
		qRT_Bvulg_GSTr	TCGGACACTCTGCCTCTACA	this study
BQ585675.1	PAL	qRT_Bvulg_PALf	GAAGATCGGAGCCTTCGAGG	this study
		qRT_Bvulg_PALr	TTACCACTCTCGTAGGCAGC	this study
BQ593253.1	AAT	qRT_Bvulg_AATf	GCAAGACACCCACGACTTCT	this study
		qRT_Bvulg_AATr	AGCAAACGCCTTGGAAGGC	this study
XM_010676890.2	PR-3	qRT_Bvulg_PR-3f	CACTGGAGGATGGGCTACTG	this study
		qRT_Bvulg_PR-3r	CCACCCTCCAACGATGACAT	this study
XM_010679634.2	GAPDH	qRT_Bvulg_GAPDHf	CATCAAGGCGGAATCAGAAGG	[3]
		qRT_Bvulg_GAPD Hr	ACGAGCTTTGCGAAGTGGTC	[3]
XM_010671243.2	TUB1	qRT_Bvulg_TUBf	CTATGCATCTTCACTTTGAAACAGTTTT	[4]
		qRT_Bvulg_TUBr	CTAGAAGAGGCTGACGAGAAAGAAG	[4]
XM_010673056.2	ACT7	qRT_Bvulg_ACT7f	GGCAAACAGGGAAAAGATGA	this study
		qRT_Bvulg_ACT7r	GGCTCACACCATCACCAGAA	this study

GLU... predicted glucanase, SE2/PR-3... predicted chitinases, AAT... predicted aspartate aminotransferase, PAL... predicted phenylalanine ammonia lyase, GST... predicted glutathione-S-transferase, DMRL... predicted 6,7-dimethyl-8-ribityl lumazine synthase; housekeeping genes: GAPDH... predicted glyceraldehyde-3-phosphate dehydrogenase, TUB1... predicted tubulin, ACT7... predicted actin.

<sup>1</sup><https://www.ncbi.nlm.nih.gov/nuccore/>

Supplementary Table S3: Expression analysis of indicator genes in *B. vulgaris* corresponding to Figure 4D.

gene #	0.1% HMW CHSN		<i>T. atroviride</i> spores	
	t1	t2	t1	t2
GLU	0.387 <sup>a</sup>	0.553	0.206 <sup>b</sup>	0.577
SE2	0.922	0.926	0.411 <sup>b</sup>	0.873
PR-3	1.742 <sup>a</sup>	1.328	1.985 <sup>b</sup>	1.785 <sup>b</sup>
AAT	1.422 <sup>a</sup>	0.991	1.081	1.261
PAL	1.385 <sup>b</sup>	1.574 <sup>b</sup>	1.093	1.157
GST	1.209 <sup>b</sup>	1.966 <sup>c</sup>	0.964	0.992
DMRL	1.264 <sup>b</sup>	0.861	1.054	0.823

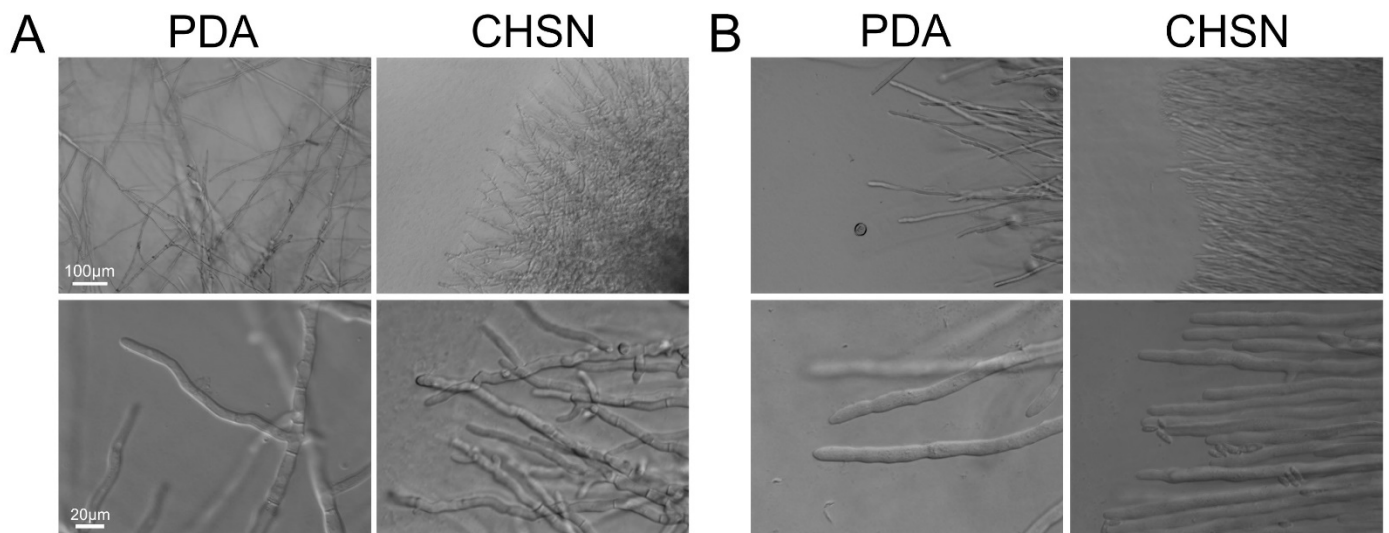
Statistical significance indicated with: a,  $p < 0.001$ ; b,  $p < 0.01$ ; c,  $p < 0.05$

Supplementary Table S4: Expression analysis of indicator genes in *B. vulgaris* seedlings corresponding to Figure 5.

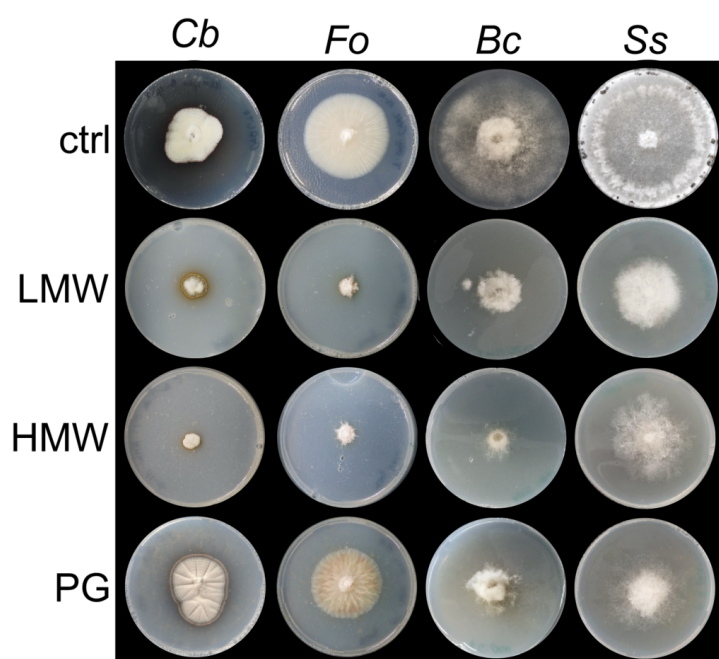
gene #	seeds coated 1% HMW CHSN		seeds coated <i>T. atroviride</i> spores		uncoated phytagel with 0.1% HMW CHSN	
	SHOOT	ROOT	SHOOT	ROOT	SHOOT	ROOT
PR-3	0.732	1.030	2.471 <sup>c</sup>	2.614	3.882 <sup>a</sup>	5.988 <sup>c</sup>
GLU	1.117	0.889	2.953	5.430	1.504	7.753 <sup>b</sup>
SE2	0.854	1.695	3.368 <sup>b</sup>	7.211 <sup>b</sup>	0.340 <sup>c</sup>	4.215 <sup>b</sup>
PAL	0.891	0.963	1.022	1.266	0.544 <sup>c</sup>	1.415
AAT	1.192	1.356	2.420 <sup>a</sup>	2.945 <sup>a</sup>	1.051	2.409 <sup>b</sup>
DMRL	1.317 <sup>a</sup>	1.061	1.931 <sup>a</sup>	1.693 <sup>a</sup>	1.427 <sup>a</sup>	1.390 <sup>b</sup>
GST	1.048	1.349	3.123 <sup>b</sup>	1.998 <sup>b</sup>	0.533 <sup>c</sup>	3.122 <sup>b</sup>

Statistical significance indicated with: a,  $p < 0.001$ ; b,  $p < 0.01$ ; c,  $p < 0.05$

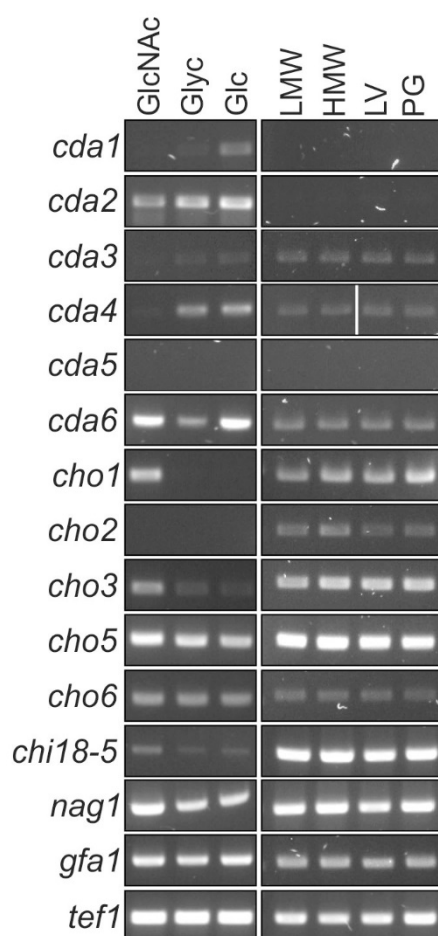
## Appendix B: Supplementary figures



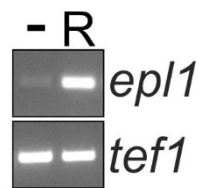
**Supplementary Figure S1: Hyphal agglomeration of *C. beticola* and *F. oxysporum* on chitosan.** Microscopic analysis of *C. beticola* (A) and *F. oxysporum* (B) on PDA or PDA supplemented with 0.6 mg ml<sup>-1</sup> LMW chitosan after growth for 120 h and 48 h, respectively. Scale bars are indicated.



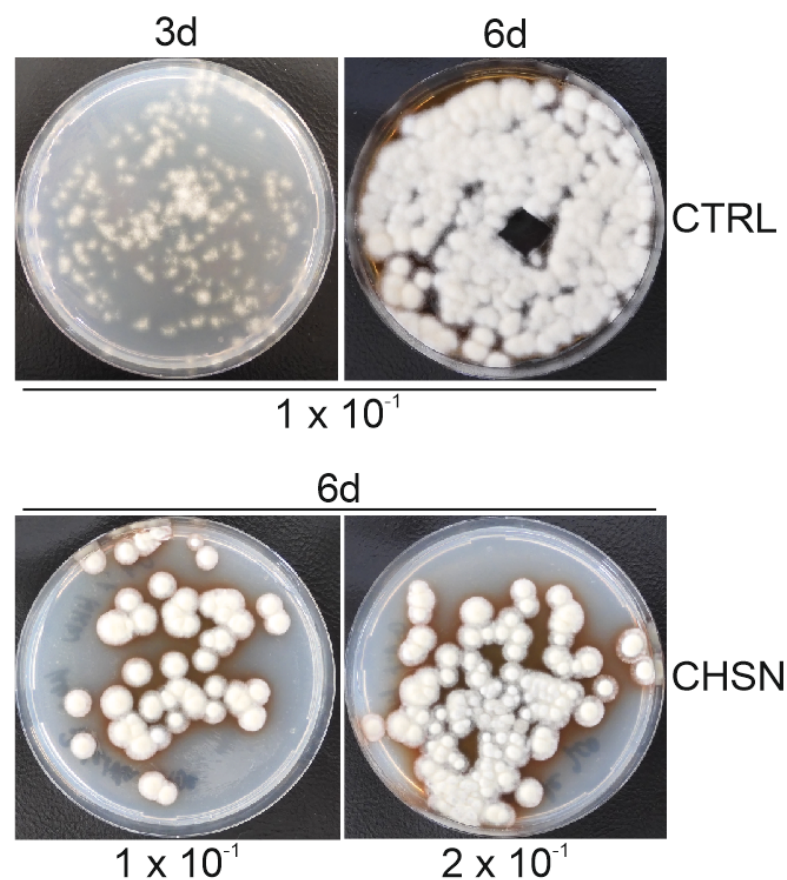
**Supplementary Figure S2: Growth of pathogens on chitosans.** Figure corresponds to Figure 2 in the manuscript, showing growth of the pathogens in absence of confrontation with *T. atroviride*. Strains were cultured on (PDA, ctrl) or supplemented with 2 mg ml<sup>-1</sup> LMW, low molecular weight; HMW, high molecular weight; PG, practical grade chitosan. *Cb*, *C. beticola*; *Fo*, *F. oxysporum*; *Bc*, *B. cinerea*; *Ss*, *S. sclerotiorum*



**Supplementary Figure S3: Differential expression of mycoparasitism related genes in *T. atroviride* in response to growth on chitosan.** RT-PCR was performed from 24 h cultures of *T. atroviride* grown in liquid minimal medium supplemented with 10 mg ml<sup>-1</sup> of the respective carbon source: *N*-acetylglucosamine (GlcNAc), glycerol (Glyc), glucose (Glc), low molecular weigh (LMW), high molecular weight (HMW), low viscous (LV), practical grade (PG) chitosan. The expression of the indicated genes was analysed by use of the RT-PCR primer pairs listed in the Supplementary Table S1.



**Supplementary Figure S4: *T. atroviride* communicates with *Beta vulgaris* when colonizing its roots.** RT-PCR was performed from RNA extracted from *T. atroviride* colonizing 10 day old seedlings from *in vitro* culture and compared to *T. atroviride* cultured on cellophane as surface. The expression of the indicated genes was analysed by use of the RT-PCR primer pairs listed in the Supplementary Table S1.



**Supplementary Figure S5: *C. beticola* colony formation from single spores.** Single spores of *C. beticola* were spread on PDA plates containing  $1 \text{ mg ml}^{-1}$  HMW chitosan (CHSN) and incubated for 6 days (6d) at  $25^\circ\text{C}$  in the dark. PDA plates served as control (CTRL) and were incubated for 3 (3d) and 6 (6d) days. A  $1 \times 10^{-1}$  dilution of the spore solution is shown for the CTRL condition and a  $1 \times 10^{-1}$  and  $2 \times 10^{-1}$  dilution are shown for CHSN.

## References

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