

The Gpr1-regulated Sur7 family protein Sfp2 is required for hyphal growth and cell wall stability in the mycoparasite *Trichoderma atroviride*

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Supplementary Data

Supplementary Table 1: Differently regulated genes

Supplementary Table 2: FunCat categories assigned to differently regulated genes

Supplementary Table 3: Oligonucleotides used

Supplementary Table 4: Primers for gene expression analysis

Supplementary Table 5: RT-qPCR quantification of selected genes

Supplementary Figure 1: Genomic environment of the *gpr1* gene

Supplementary Figure 2: Semi-quantitative expression analysis of chitinase genes

Table S1: List of *T. atroviride* genes whose transcription is significantly differently regulated comparing the responses of the *gpr1*-silenced mutant *gpr1* sil-8 and the WT to *R. solani*. The robustness of the combined evidence from multiple analyses is indicated by the aggregate rank (aRank; the lower the rank the more robust evidence is provided by the data). aLogFC (WT) and aLogFC (*gpr1*-si) show the average log fold-changes of the respective strains' response to the prey; aLogFC (WT-*gpr1*-si) represents the aLogFC of the *gpr1*-si mutants' response subtracted from the WT response (for details see the Methods section). Filtered models correspond to the protein IDs of the filtered models in the JGI DOE *T. atroviride* genome database.

Please see file "Supplementary Tables S1 to S5.xls"

Table S2: List of FunCat categories assigned to transcripts that were significantly differently regulated comparing the responses of the *gpr1* sil-8 mutant and the WT to *R. solani*. The most robustly identified categories with the lowest aggregate rank (aRank) are listed first. Aggregate enrichment score (r) values close to 1 and 0 annotate overrepresented and underrepresented categories, respectively. Aggregate direction (aDir) values close to 1 or -1 show good agreement across analysis variants with over- or underrepresentation of categories, whereas values close or equal to 0 mark poor or no agreement across the analyses, respectively. Aggregate significance (aSig) represents the percentage of analyses that inferred an under- or overrepresentation of categories with a probability larger than 90%. The yellow highlighted FunCat codes mark the highest level annotation categories. The functional categories tested correspond to the functional classification catalogue (FunCat) version 2.1 (<http://mips.helmholtz-muenchen.de/funcatDB/>) slightly adapted to version 2.0 as listed in Ruepp et al.⁶¹.

Please see file "Supplementary Tables S1 to S5.xls"

Table S3: Oligonucleotides used for cloning, genotypic verification, sequencing and generation of transformation cassettes.

Please see file "Supplementary Tables S1 to S5.xls"

Table S4: qPCR and RT-PCR primers for gene expression analysis used in this study.

Gene	Tm [°C]	Primers (5'→ 3')		PE
		fw	rev	
<i>sar1</i>	60	CTCGACAATGCCGGAAGACC	TTGCCAAGGATGACAAAGGGG	1
<i>tef1</i>	58.5	TACTATGTCACCGTCATTG	CAGCGATAATCAGGATA	1
<i>actin</i>	60	GCACGGAATCGCTCGTTGC	TTCTCCACCACCGCCAAGC	1
<i>ech42</i>	60	CGCAACTTCCAGCCTCAGAACC	TCAATACCATCGAAACCCAGTCC	1
<i>chs1</i>	57	ACTCGGACCTCAATGGAA	GGCGGCTATGAAGTAACG	0.9
<i>chs2</i>	57	TCCGACCATTCAATCTTC	CCAGACTTGTAATCAGGAT	1
<i>tac2</i>	55	GCCCTCGTGCTCCATCAG	GGTCTCGTAGTTGCCGGG	1
<i>tac6</i>	55	CGGGACTTATGGTTTGGGCGG	CGAACGGTCCAGATGCGGG	1
<i>hsp70</i>	58	GGTGCTACAGTCAAGTAA	CCATAGGGAAAGAAACAAAG	1
<i>tef2</i>	58	AAGAAGTGGACCAAGAAC	TCATAACAGCAGAGAAGAT	1
<i>gh3</i>	58	TATGAGAGCATCCTATCC	AACACTAATGTAGCCTTC	1
<i>coA</i>	60	ATGTCGTTATTGCTTCTT	CATTAAATGTGGAGGGTTA	1
<i>sur4</i>	55.7	TACACTTACTACTACTCTG	AATGTATGTGATGAAGGA	0.96

Table S5: Real Time PCR quantification of selected genes for verification of microarray data.

Gene	Gene name	Protein ID	Microarray data [log ₂ (ratio)]		qPCR verification	
					Relative expression	
			WT	<i>gpr1</i> -si	WT	<i>gpr1</i> -si
Putative protein of HSP70 family	<i>hsp70</i>	284614	-2.080	3.780	0.085	1.853
Transcription elongation factor 2	<i>tef2</i>	301275	-4.020	1.350	0.026	1.363
GH3 with Fibronectin type III-like domain	<i>gh3</i>	302027	-3.630	0.980	0.003	14.806
3-hydroxyacyl-CoA dehydrogenase	<i>coA</i>	80898	3.230	0.00006	2.580	0.239
Putative SUR4 fatty acid elongase	<i>sur4</i>	301574	1.890	0.080	4.448	0.127

Figure S1: The genomic environment of the *gpr1* gene in *T. atroviride* (TA), *T. virens* (TV) and *T. reesei* (TR) is conserved and includes *sfp2*, a gene coding for a Sur7 family protein with four transmembrane domains.

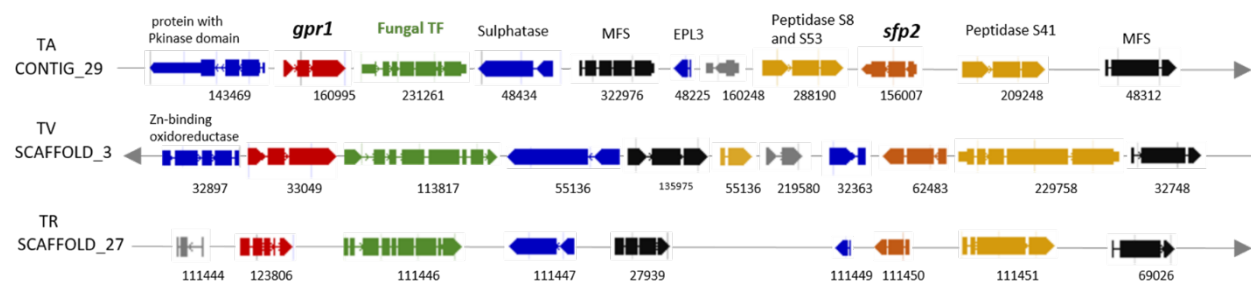
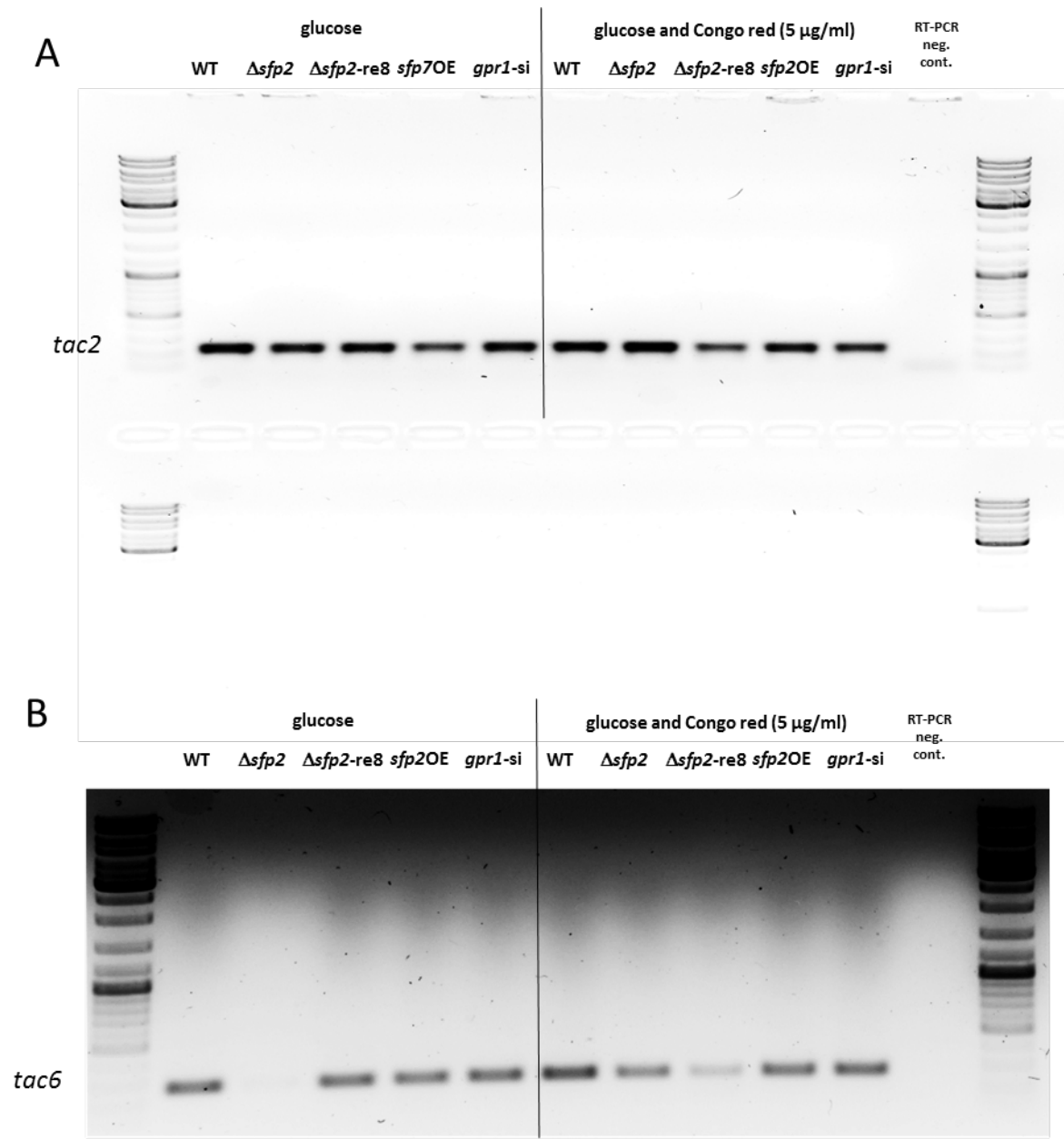
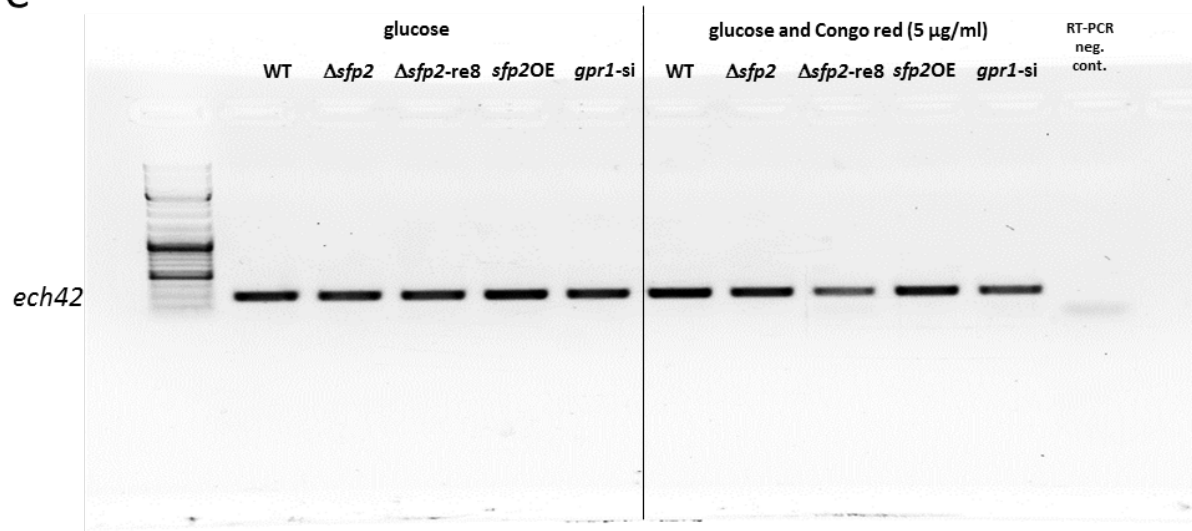


Figure S2: Semi-quantitative expression analysis of chitinase genes (A) *tac2* (Ta217701), (B) *tac6* (Ta214040) and (C) *ech42* (Ta131598) in *T. atroviride* WT, *sfp2* and *gpr1-si* mutants cultivated in liquid PDB medium with and without Congo Red as cell wall stress inducer. (D) The translation elongation factor1–encoding gene *tef1* was used as a control³¹.



C



D

