Supplementary Material

Journal: Applied Microbiology and Biotechnology

The identification of key gene highlights macrocyclic ring's role in trichothecene toxicity

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Table S1. Oligonucleotides used in the present work

Name		**Sequence 5´-3´**
Construction of pΔtri24 to delete *Paramyrothecium roridum TRI24* gene		
PRORI_TRI24_3R_F_KpnI (1,000 bp)		ggtaccAGAGAGTAAGCTTAGCCG
PRORI_TRI24_3R_R_SmaI		cccgggGAACTTCCATCAAGCAGG
PRORI_TRI24_5R_F_SmaI (1,028 bp)		cccgggTGAATTGGTGGCATGGAG
PRORI_TRI24_5R_R_BamHI		ggatccTGATTCTTGGGGACACCA
Construction of pΔtri24 to delete *Parayrothecium roridum TRI25* gene		
PRORI_TRI25_5R_F_BamHI (1,009 bp)		ggatccTGGCGTAGAATCGATGTC
PRORI_TRI25_5R_R_SmaI		cccgggCTCAATTGCCCACGAACA
PRORI_TRI25_3R_F_SmaI (979 bp)		cccgggGACGAAGAGGAATCCATG
PRORI_TRI25_3R_R_KpnI		ggtaccAGTTATGGCTTGACGGAG
Oligonucleotides to amplify an internal fragment to *P. roridum TRI24* gene		
PRORI_TRI24_IF_Ct (997 bp)		GGACAAGCTTCTCCATCA
PRORI_TRI24_IF_Nt		CTGGGAGCGACATATTCT
Oligonucleotides to amplify an internal fragment to *P. roridum TRI25* gene		
PRORI_TRI25_IF_Nt (987 bp)		CCTTGCCTTTACAGAGCT
PRORI_TRI25_IF_Ct		GGCTCACCAACGATATAC
Oligonucleotides to amplify *P. roridum TRI24* gene for its overexpression		
PRORI_TRI24_ATG		TCTTCCACCGTTCAGACT
PRORI_TRI24_Ct_end		CTATAGTTTTGCCTGGGA
Oligonucleotides to amplify *P. roridum TRI25* gene for its overexpression		
PRORI_TRI25_ATG		TCTTCCACCGTTCAGACT
PRORI_TRI25_Ct_end		CTATAGTTTTGCCTGGGA
Amplification of 1,398 bp and 1,288 bp fragments corresponding to the 5´ and 3´ extremes, respectively, of the recombination cassettes designed for *TRI24* gene deletion		
PRORI_TRI24_5R_rr (1,398 bp)		GGCTATAAATCACGGACC
TtrpC-d		GTAACCATGCATGGTTGC
PgpdA-d (1,288 bp)		ATCATCCACTGCACCTCA
PRORI_T24_3R_ff		TTCAGCTGTGGCTTGACT
Amplification of 1,370 bp and 1,274 bp fragments corresponding to the 5´ and 3´ extremes, respectively, of the recombination cassettes designed for *TRI25* gene deletion		
PRORI_TRI25_5R_ff (1,370 bp)		AATACCAGGCTGTCCTTG
PRORI_TRI25_3R_rr (1,274 bp)		ACTGCTGTTCACGGTGTT
Amplification of the 1,658 bp fragment corresponding to *TRI24* gene expressed in *Trichoderma arundinaceum*		
Tcbh2*		CTGGCAACAATCCATCAG
*This oligonucleotide was used in combination with PRORI_TRI24_ATG for this study		
Oligos for qPCR analysis	**Sequence 5´-3´**	**Efficiency (%)**
P. roridum actin	AGGAGAAGCTCTGCTACGTC	99.1
	GCCAATGGTGATGACCTGAC	
P. roridum TRI5	GTGCTTTATGCACGGCTACA	93.1
	GCGGAACTTGATTGCATCCT	
P. roridum TRI4	GTGTCAACGCGAGAGAAGTC	88.6
	GGTCCTTGTTCGTCTTTCGG	
P. roridum TRI22	TGCTTGAGCTCTTCACTCCA	92.7
	TCGTTGAGACCTTGAGGCTT	
P. roridum TRI3	CATCTCTACTGGGACGGCAT	95.4
	GTGTGGAAGTTGGACAGCTC	
P. roridum TRI17	ATCAGTGCGGACTCATTTGC	98.1

	CAGGACAACCTGTCCAGACT	
P. roridum TRI18	CCCGCACGTTCAGTACAAAT	90.2
	GTCGCCCGCACAGAAATAAT	
P. roridum TRI24	CCACCACAGATCCTCAACCT	91.2
	GGCTTGAGTTTCGGATCACC	
P. roridum TRI25	AGAGGAACCAATGGAGCGAA	83.6
	GCTGCGCAGTACGAGAATAG	
P. roridum TRI6	TCGACACTGTCTCACACTCC	85.1
	TTTCGTTGAGGAGGTTTCGC	
P. roridum TRI10	GCATTGGTCGACCTCATCAC	88.5
	GCATCAGGGAGCTGTTTCAG	
P. roridum g3	GTAGCCTCTCGAAGCCTCTT	93.3
	CTGCGCTGGTTTCCGAAATA	
P. roridum g4	TCTTCTTAGCCCAACAGCCA	81.7
	CACAATCTGATGCAGCCACA	
P. roridum g5	AAGGAGGGTTTGACGCTGTA	89.0
	CCACTCGGATGTAGCAGGAT	
P. roridum g6	AAGACCGCAACGATGTGAAG	82.6
	GTAGCTTTGCTCCTCGTGTG	

Table S2. Blastp analysis of *P. roridum* Tri24-aminoacid sequence against the UniProtKB/Swiss-Prot database at the NCBI.

Description	Specific name	Total Score	Query Cover	E value	Per. Ident	Accesion
Loline biosynthesis cluster 1 transcription factor lolU1 [*Epichloë uncinata*]	*Epichloe uncinata*	317	94%	8e-102	36.14%	Q5MNI2.1
Loline biosynthesis cluster 2 transcription factor lolU2 [*Epichloë uncinata*]	*Epichloe uncinata*	307	94%	9e-98	35.32%	Q5MNH4.1
Satratoxin biosynthesis SC2 cluster transcription factor SAT15	*Stachybotrys chartarum*	239	28%	4e-76	75.51%	A0A084API5.1
Probable acetyltransferase tazD [*Aspergillus terreus*]	*Aspergillus terreus*	91.7	94%	1e-18	22.54%	Q0CS99.1
Azaphilone biosynthesis cluster protein M [*Aspergillus terreus* NIH2624]	*Aspergillus terreus*	81.6	58%	2e-15	26.73%	Q0CSA0.1

Table S3. Blastp analysis of *P. roridum* Tri25-aminoacid sequence against the NCBI non-redundant database.

Description	Specific name	Total Score	Query Cover	E value	Per. Ident	Accesion
Putative *O*-acetyltransferase SAT14	*Stachybotrys chartarum*	699	99%	0.0	73.03%	A0A084API4.1
N^5-Hydroxyornithine:cis-anhydromevalonyl coenzyme A-N^5-transacylase SidF	*Aspergillus fumigatus*	389	91%	7e-131	45.28%	Q4WF55.1
Hydroxyornithine transacylase SID3	*Histoplasma capsulatum*	379	92%	2e-127	46.37%	B2KWI2.1
Acyltransferase fer5	*Ustilago maydis*	191	70%	1e-54	33.94%	Q4PEN1.1
Putative lysine *N*-acyltransferase C17G9.06c [*Schizosaccharomyces pombe* 972h-]	*Schizosaccharomyces pombe*	188	42%	1e-54	46.63%	Q9UUE3.1

Table S4. RNA-seq analysis: genes showing the highest fold change (fc) values in the Δ*tri24* mutant (ΔT24-72) versus the wild-type strain (PR-72)* in RNAs extracted from 72 h grown mycelia.

Gene ID	blast higher hit	e-value	ΔT24-72/ PR-72.fc
PRORI_g7025	Zinc--binding dehydrogenase family	0E0	25.625
PRORI.910495	Tri17	0E0	25.333
PRORI.g10494	Tri6	0E0	25.685
PRORI.g5700	Hypotetical- Snoal-like polyketide cyclase	1.14E-117	27.272
PRORI.g8225	ZEB2-regulate ABC transporter	0E0	28.938
PRORI.g6853	Polyketide synthase	0E0	31.049
PRORI.g7029	FAD/NAD(P)-binding domain-containing protein	0E0	32.370
PRORI.g10474	Ent-Kaurene oxidase	1.42E-175	32.681
PRORI.g11262	Cytochrome P450	0E0	32.175
PRORI.g345	AMP-binding enzyme	0E0	35.925
PRORI.g10477	Norsolorinic acid reductase B	0E0	36.708
PRORI.g11065	Bifunctional 4-hydroxyphenylacetate degradation	4.03E-171	46.967
PRORI.g4922	Non-ribosomal peptide synthetase	0E0	55.252
PRORI.g7095	6-hydroxy-D-nicotine oxidase	0E0	56.512
PRORI.g11263	FAD binding domain-containing protein	2.09E-162	58.011
PRORI.g11352	Hypothetical protein VHEMI07173	7.19E-9	69.735
PRORI.g1180	Aldo/keto reductase	0E0	70.509
PRORI.g10504	Tri14	0E0	77.016
PRORI.g10492	Tri3	0E0	83.023
PRORI.g10478	Short-chain dehydrogenase/reductase-like protein	0E0	92.883
PRORI.g7027	2og-Fe(II) oxygenase	1.38E-189	93.783
PRORI.g11265	Tri25	0E0	110.550
PRORI.g6898	Tryptophan dimethylallyltransferase 1	2.25E-166	119.785
PRORI.g4783	Polyketide synthase	0E0	124.756
PRORI.g6861	Extracellular dihydrogeodin oxidase/laccase, putative	0E0	130.855
PRORI.g6896	Integral membrane protein PTH11	6.49E-176	151.205
PRORI.g7423	Hypothetical protein	9.73E-118	168.487
PRORI.g4788	FAD binding domain-containing protein	0E0	177.993
PRORI.g6860	Hypothetical protein	2.85E-129	245.553
PRORI.g8840	Cytochrome P450 3A17	0E0	254.214
PRORI.g6858	Hypothetical protein	1.16E-65	263.200
PRORI.g6859	Dienelactone hydrolase	6.07E-131	265.561
PRORI.g6857	FAD binding domain protein	0E0	268.035
PRORI.g10491	Tri4	0E0	291.403
PRORI.g10496	Tri18	0E0	311.581
PRORI.g11170	Alpha/beta hydrolase fold-3	8.54E-124	382.609
PRORI.g10503	Tri12	0E0	387.278
PRORI.g10502	Tri5	0E0	601.321
PRORI.g11264	Long-chain acyl synthetase	0E0	619.821
PRORI.g10505	Tri9	-	3272.449

*Green shadow rows pointed to *TRI* genes. Shadow in colors other than green correspond to genes belonging to other secondary metabolite biosynthetic gene clusters, as deduced from antiSMASH software. White shadow corresponds to genes putatively not assigned to any secondary metabolism cluster.

Table S5. Blastp analysis of the ORFs located in the *P. roridum TRI* genomic region that were found to be up-regulated in the Δ*tri24* mutant when compared versus de wild-type strain, in 72 h grown mycelia.

SeqName	Description	Length	E value
P. roridum g11260	3-Oxoacyl-[acyl-carrier-protein] reductase FabG	266	9.7E-178
P. roridum g11261	Fungal specific transcription factor	140	2.14E-28
P. roridum g11262	Cytochrome P450	514	0E0
P. roridum g11263	FAD binding domain-containing protein	389	2.09E-162
P. roridum g11264	Long-chain acyl synthetase	575	0E0

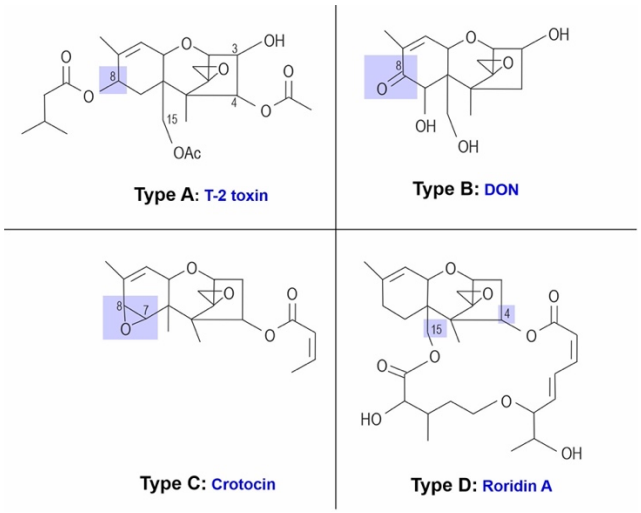


Figure S1. Structural classification of trichothecenes according to Ueno et al. (1977, 1984). Numbering of the relevant carbons is indicated at the compounds used to describe each of the four types of trichothecenes. Type D trichothecene is synonymous with macrocyclic trichothecenes. Carbons corresponding to the substituents characteristic for each type are shaded in blue.

Ueno Y (1977) Mode of action of trichothecenes. Pure Appl Chem 49: 1737-1745. doi: 10.1351/pac197749111737.

Ueno Y (1984) Toxicological features of T-2 toxin and related trichothecenes. Fundam Appl Toxicol 4: S124–S132. doi: 10.1016/0272-0590(84)90144-1.

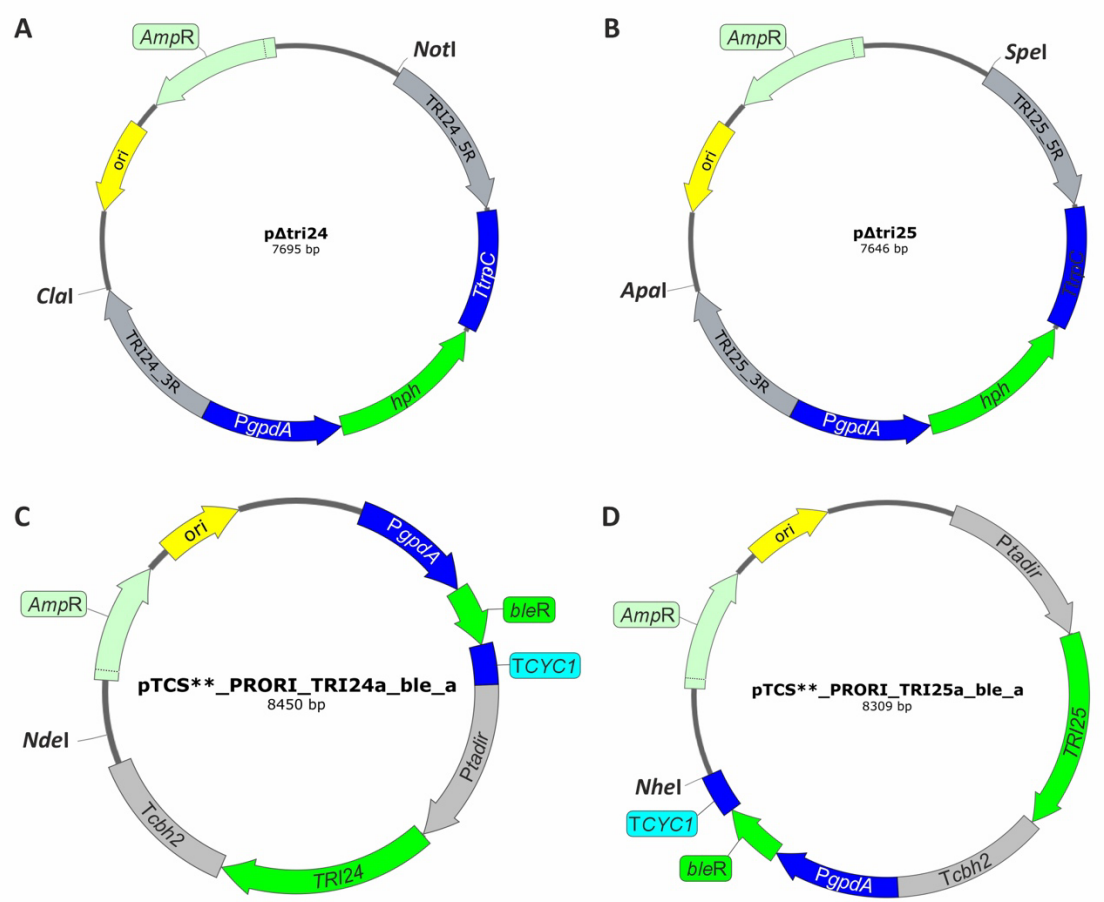


Figure S2. **A**. Scheme of pΔtri24 plasmid. TRI24_5R and TRI24_3R are the regions located at the 5´ and 3´ sides of *P. roridum TRI24* gene. P*gpdA* - promoter region of the *Aspergillus nidulans* glyceraldehyde-3-phosphate dehydrogenase; T*trpC* - *A. nidulans trpC* terminator region; *hph* - *E. coli* hygromycin B resistance gene. **B**. Scheme of pΔtri25 plasmid. TRI25_5R and TRI25_3R are the regions located at the 5´ and 3´ sides of *P. roridum TRI25* gene**. C, D**. Map of plasmids pTCS**_PRORI_TRI24a_ble_a (**C**) and pTCS**_PRORI_TRI25a_ble_a (**D**), designed to express *P. roridum TRI24* and *P. roridum TRI25* genes, respectively. *TRI24* and *TRI25* correspond to *P. roridum TRI24* and *TRI25* genes, respectively. *ble*R- phleomycin/bleomycin resistance gene from *Streptoalloteichus hindustanus*. P*tadir* indicates the promoter region of the *Trichoderma harzianum tadir* gene; and T*cbh2* indicates the transcriptional terminator of the *T. reesei* cellobiohydrolase 2 encoding gene.

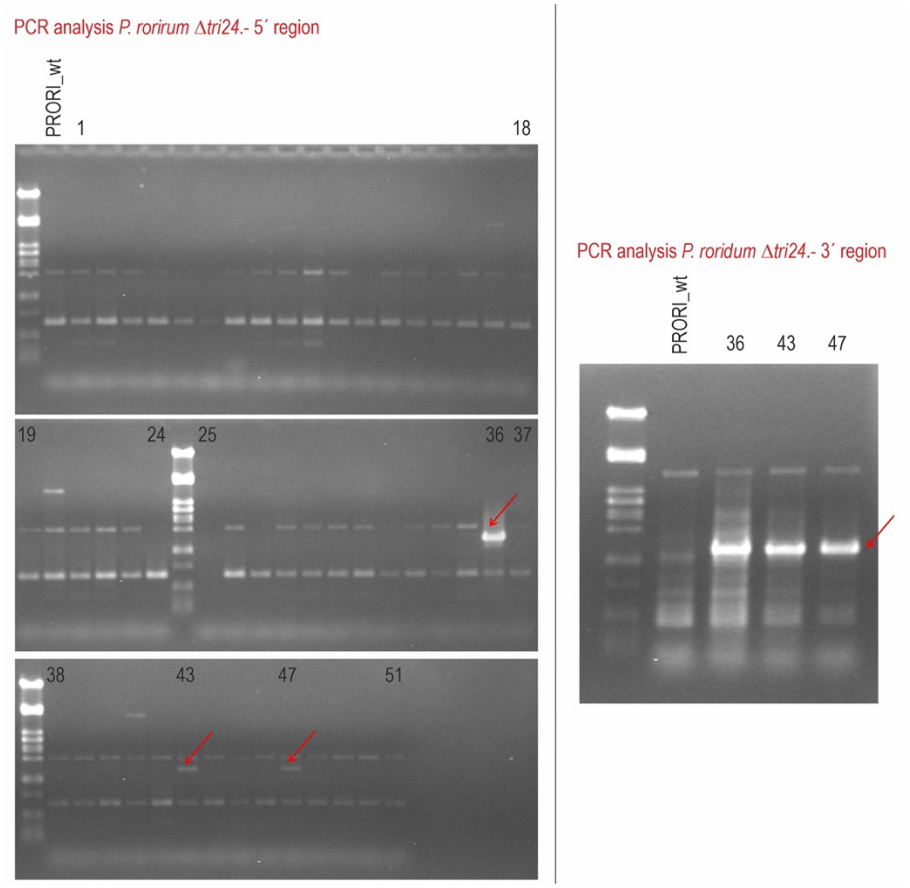


Figure S3. PCR analysis of transformants isolated by transformation of *P. roridum* wild type with the pΔtri24 plasmid using oligonucleotides designed to detect the 5´ and 3´ constructs generated as a result of the double recombination process. Note that the PCR fragments from transformants 36, 43 and 47 were sequenced to confirm that the recombination process occurred as designed. Red arrows point to the bands with the expected size (1,398 bp in the 5´ region- **left panel**, and 1,288 for the 3´ region- **right panel**). PRORI_wt= *P. roridum* wild-type strain.

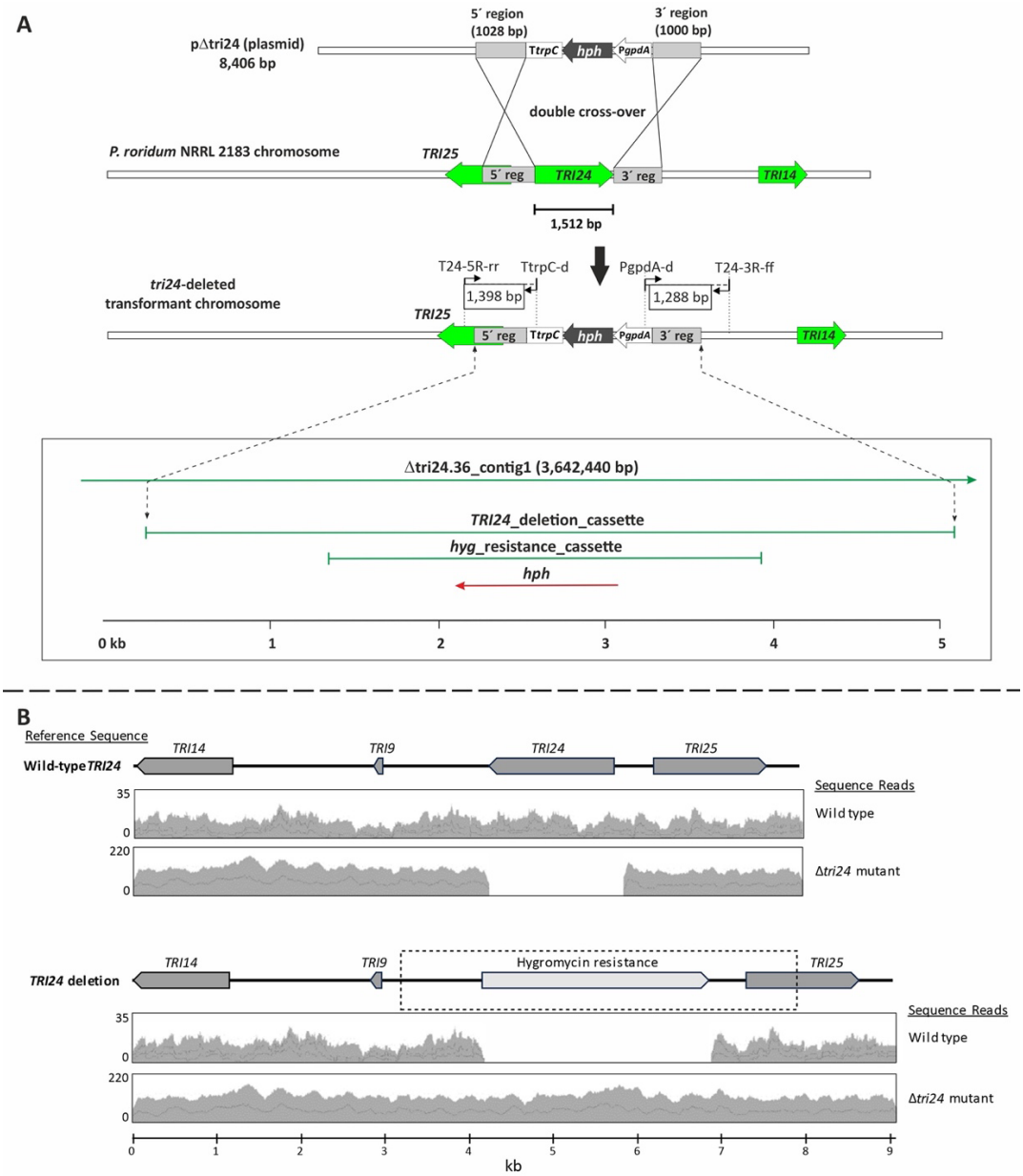


Figure S4. **A**. Strategy used for *TRI24* gene deletion using a double cross-over procedure. **B**. ***TRI24* deletion analysis:** Confirmation of *TRI24* deletion in the Δ*tri24* mutant of *P. roridum* by *in silico* mapping analysis of whole genome sequence reads to two reference sequences. The reference sequences are shown at the top of the upper and lower panels, and mapped reads are depicted as jagged and undulating gray areas within the two rectangles below each reference sequence. Reference sequences: **Wild-type *TRI24*** 8 kb segment in the *TRI24* region of the wild-type progenitor strain of *P. roridum* (**upper panel**); and ***TRI24* deletion** - predicted 9.1 kb segment resulting from deletion of *TRI24* (**lower panel**) using the deletion plasmid pΔtri24. Within the reference sequences, genes are indicated by arrows that point in the direction of transcription. The rectangle outlined with broken lines and surrounding part of reference sequence ***TRI24* deletion** indicates the segment homologous to the deletion cassette in plasmid pΔtri24. Genome sequence reads used in the analysis were from the wild-type (Wild type) and Δ*tri24* mutant (Δ*tri24* mutant) strains of *P. roridum*. Numbers to the left of rectangles with mapped reads indicate minimum and maximum read coverage. The marked differences maximum coverage values resulted from different methods used to generate sequence data for the two strains. The mapping analysis was done using the Map Reads to Reference function in QIAGEN CLC Genomics Workbench version 24 (https://digitalinsights.qiagen.com). hyg= Hygromycin.

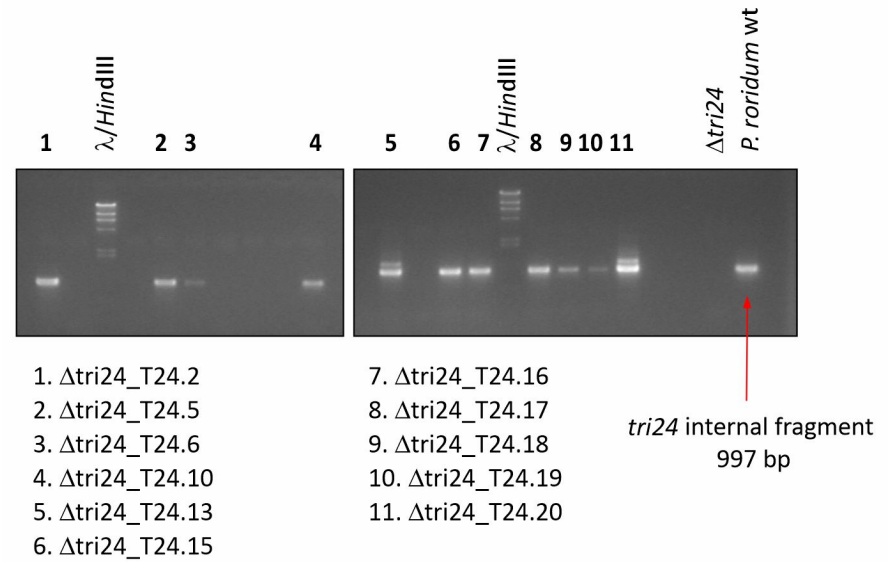


Figure S5. Analysis by PCR of 19 isolates obtained after transformation of the Δ*tri24* mutant with pTCS**_PORI_TRI24a_blea. The expected 997 bp fragment has been indicated by a red arrow. Lanes indicated with a number in the upper part correspond to the transformants that gave the expected amplification band. *P. roridum* wt = *P. roridum* wild-type strain.

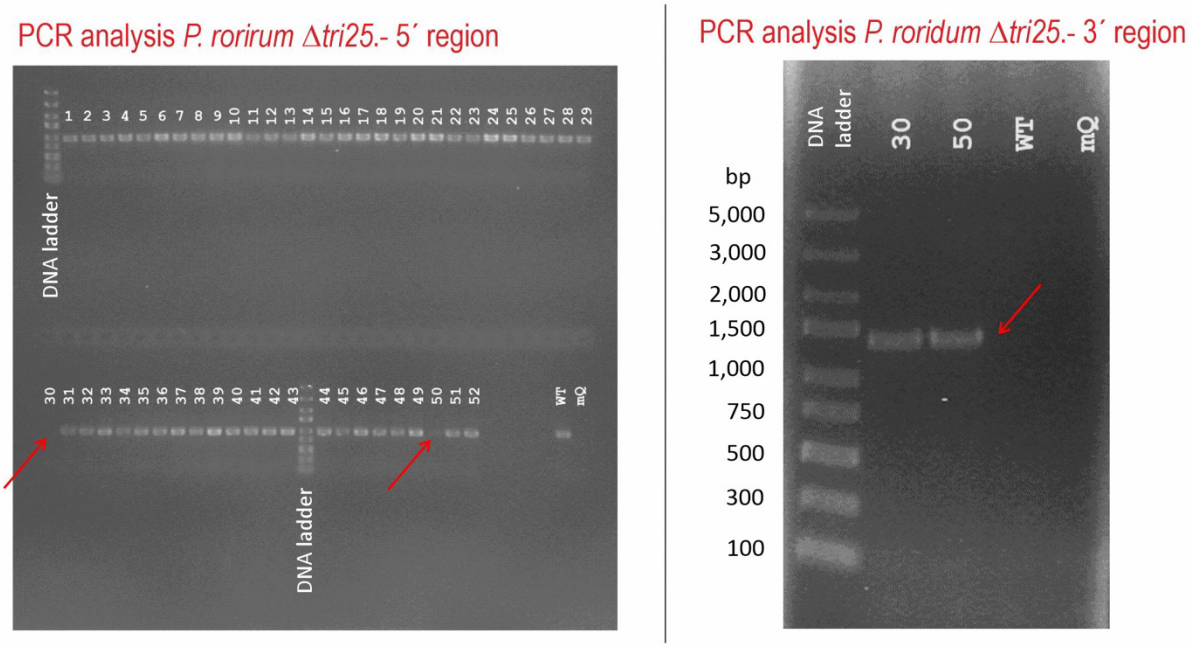


Figure S6. **Left panel**. PCR analysis of transformants isolated by transformation of *P. roridum* NRRL 2183 with the pΔtri25 plasmid using oligonucleotides designed to detect an internal fragment of *TRI25* gene (987 bp). Transformants #30 and #50 lack this band. **Right panel**. PCR using oligonucleotides designed to detect the 5´ region (1,370 bp) generated as result of the double cross-over process. Note that the PCR fragment observed for transformants #30, and #50 (right panel) were sequenced to confirm that the recombination process occurred as designed. Red arrows point to the bands with the expected size, or the lack of it in the left panel. DNA ladder= O´GeneRuler Express DNA Ladder (Thermo Scientific), WT and mQ.- control reactions using genomic DNA of the *P. roridum* wild-type strain, or water in the PCR reactions.

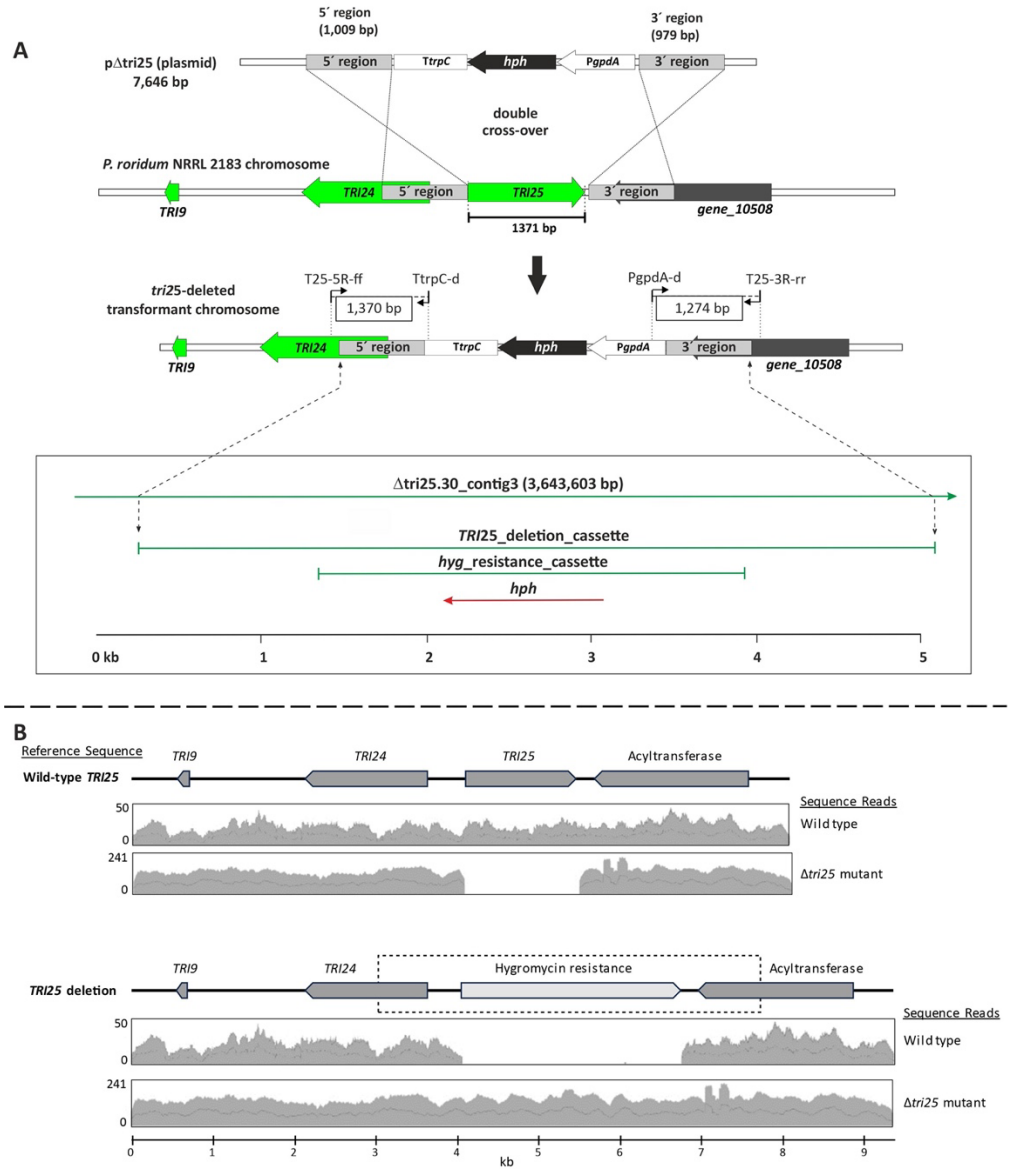


Figure S7. **A**. Strategy used for *TRI25* gene deletion using a double cross-over procedure. **B**. ***TRI25* deletion analysis:** Confirmation of *TRI25* deletion in the Δ*tri25* mutant of *P. roridum* by *in silico* mapping analysis of whole genome sequence reads to two reference sequences. Reference sequences are shown at the top of the upper and lower panels, and mapped reads are depicted as jagged and undulating gray areas within the two rectangles below each reference sequence. Reference sequences: **Wild-type *TRI25*** - 8 kb segment from the *TRI25* region in the wild-type progenitor strain of *P. roridum* (**upper panel**); and ***TRI25* deletion** - predicted 9.5 kb segment resulting from deletion of *TRI25* (**lower panel**) using deletion plasmid pΔtri25. In the reference sequences, genes are indicated by arrows that point in the direction of transcription. The rectangle outlined with broken lines and surrounding part of reference sequence ***TRI25* deletion** indicates the segment homologous to the deletion cassette in plasmid pΔtri25. Genome sequence reads used in the analysis were from the wild-type (Wild type) and Δ*tri25* mutant (Δ*tri25* mutant) strains of *P. roridum*. Numbers to the left of rectangles with mapped reads indicate minimum and maximum read coverage. The marked difference in maximum coverage values resulted from different methods used to generate sequence data for the two strains. The mapping analysis was done using the Map Reads to Reference function in QIAGEN CLC Genomics Workbench version 24 (https://digitalinsights.qiagen.com). hyg= Hygromycin

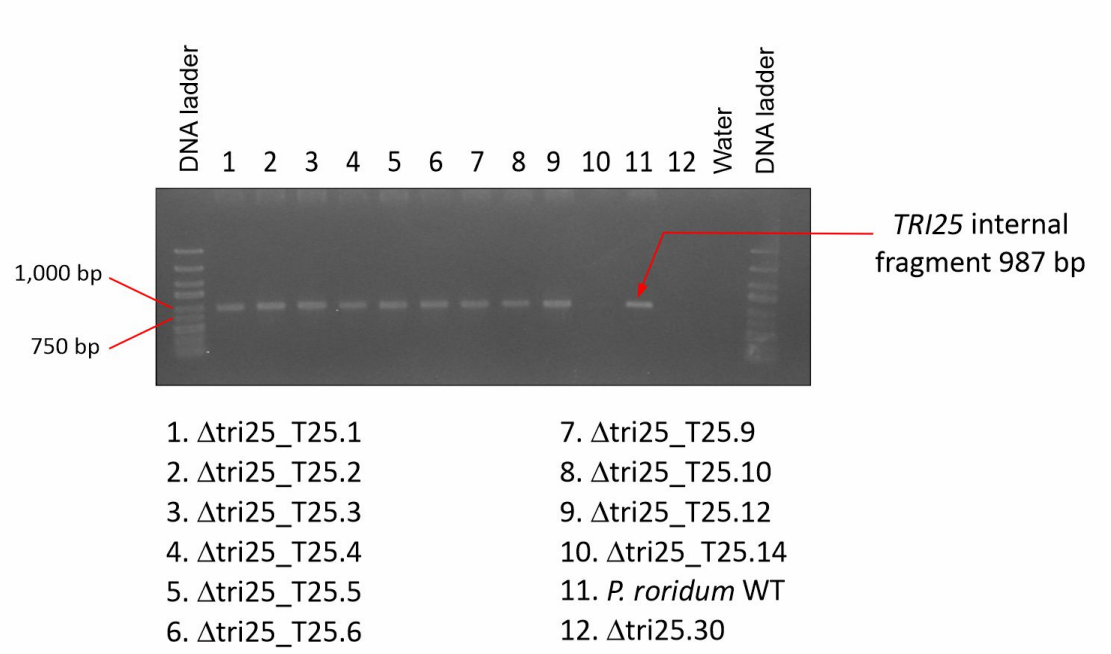


Figure S8. PCR analysis of 10 transformants selected by transformation of the Δ*tri25* mutant with pTCS**_MR_T25a_ble_a.
The expected 987 bp fragment has been indicated by a red arrow at the right of the figure. DNA ladder= O´GeneRuler Express DNA Ladder (Thermo Scientific).

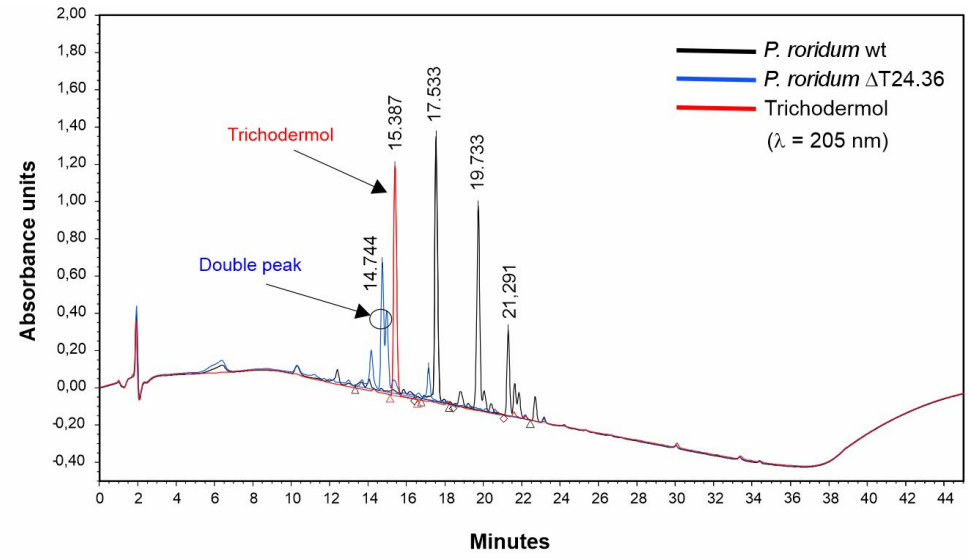


Figure S9. HPLC analysis of broths from *P. roridum* wild type and Δ*tri24* (ΔT24.36) mutant. A standard of trichodermol was included in the study. Note that for this first HPLC analysis a different program was used, e.g., water without the addition of trifluoroacetic acid (90% water/10% acetonitrile) was used, and detection was made at 205 nm. The analysis started with 10% acetonitrile and reaching 100% in 35 min, held for 5 min, and then returned to 10% acetonitrile at 50 min time point.

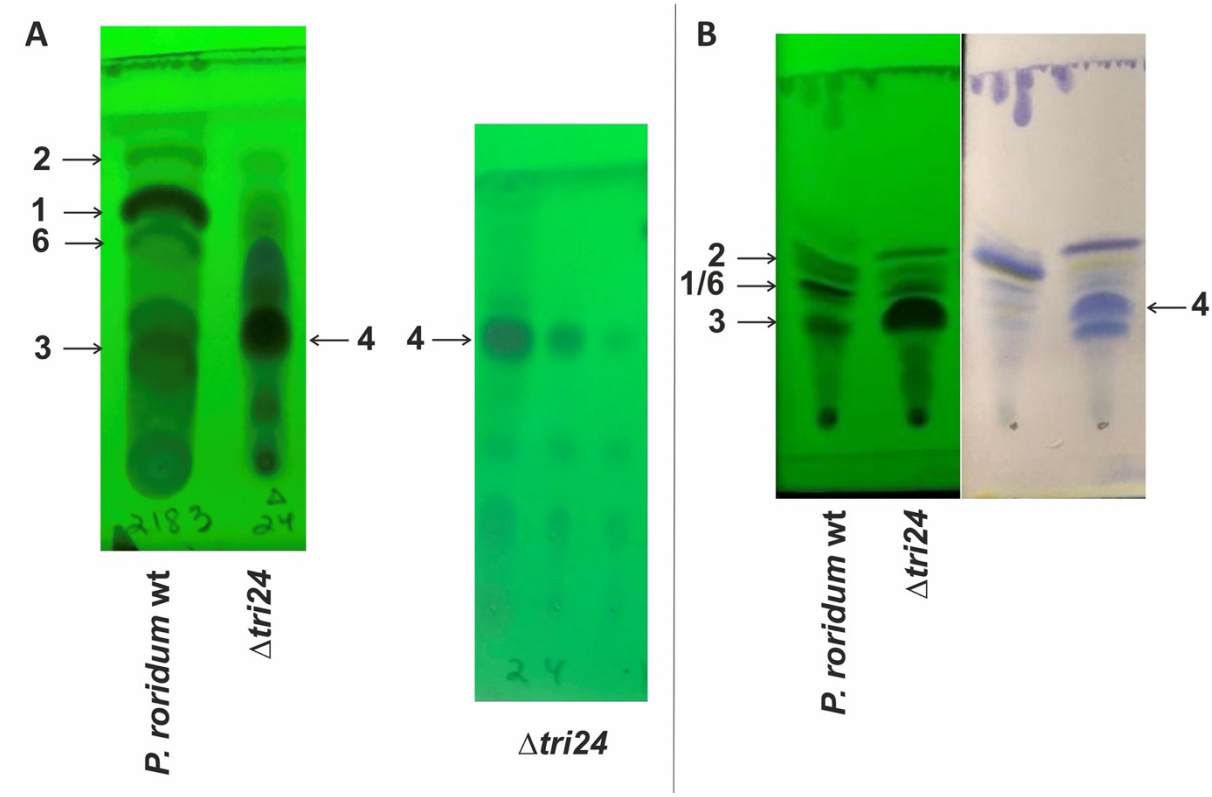


Figure S10. **A**. **Left panel**. Analysis of extracts of cultures of the wild-type stain and the Δ*tri24* mutant using TLC Method 2. **Right panel**. TLC of broths from the Δ*tri24* mutant using three dilutions of the extract and a longer running time. Method 2 was used for growth and TLC analysis (see Materials and Methods section), in which 5% methanol in ethyl acetate was used as mobile phase. Arrows pointed to the main spots, most of them already detected by using Method 1 (**Fig. 4**). **B**. TLC (**left panel**) and NBP/TEPA stain (**right panel**) to visualize trichothecene analogs (blue bands).

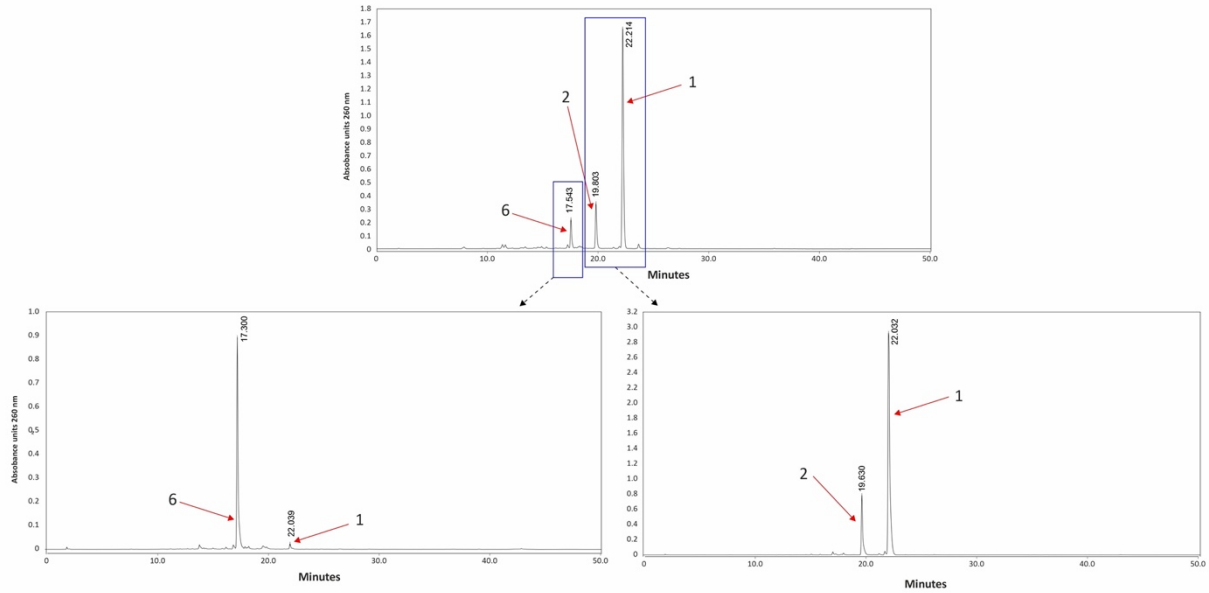


Figure S11. HPLC analysis of spots **1**, **2** and **6** (**lower panels**), after purification from spot **1** (**upper panel**).

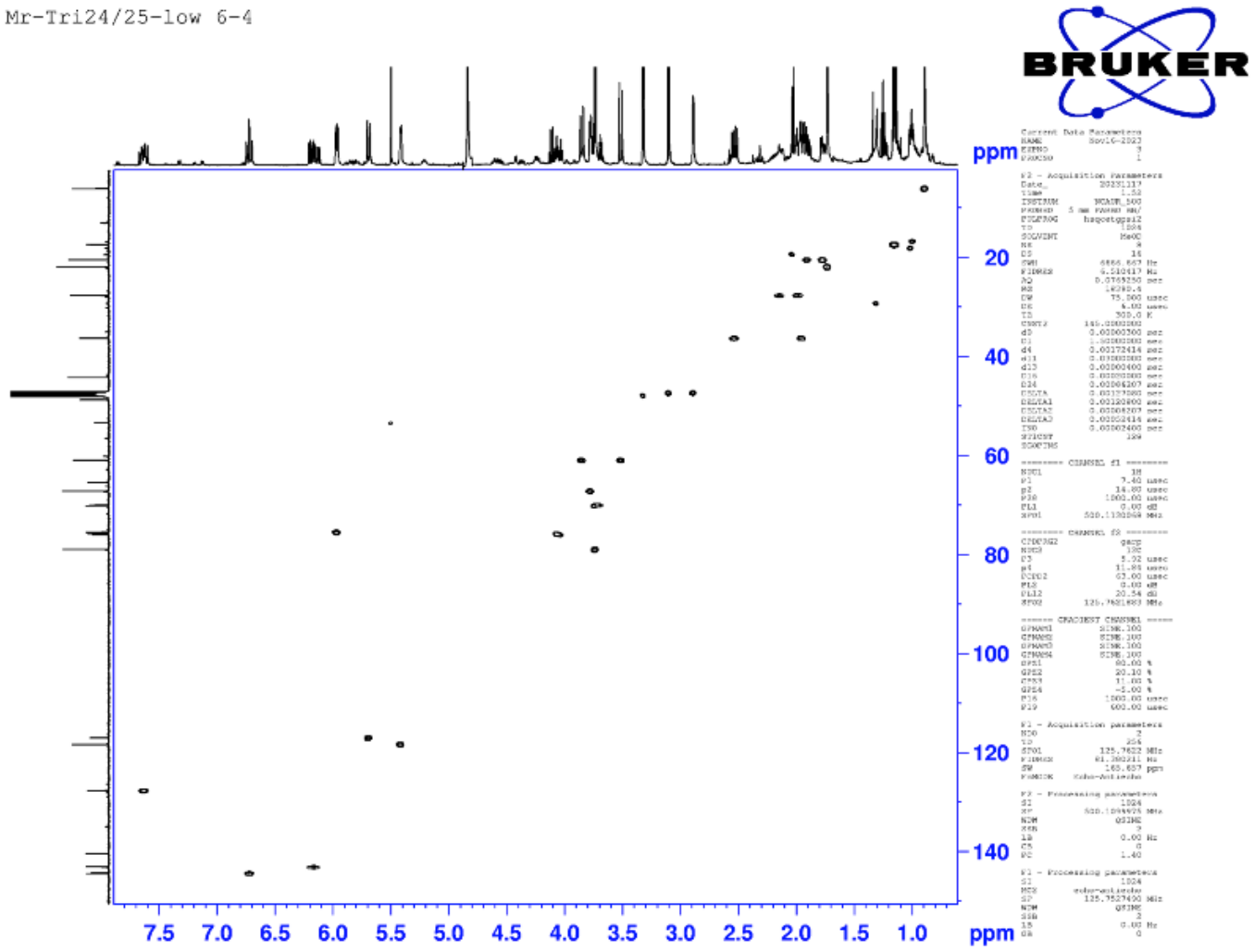
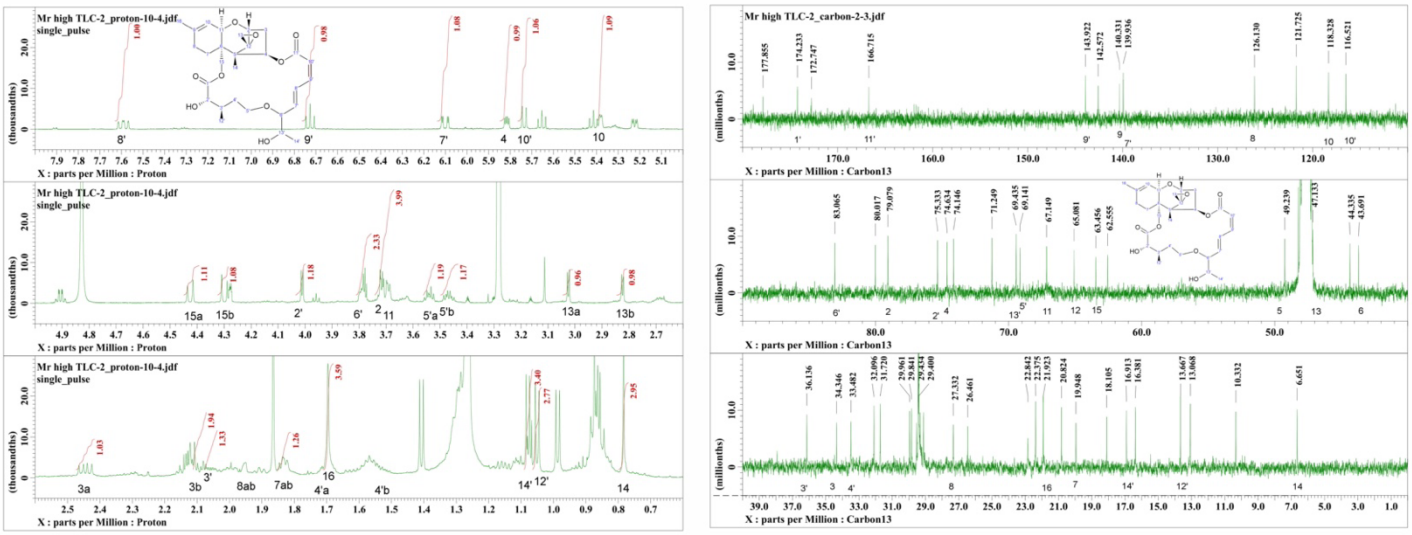


Figure S12. HSQC NMR spectrum of spot **4** (15-Hydroxy trichodermadiene).

Spot 6: Roridin A



Spot 1: Roridin E

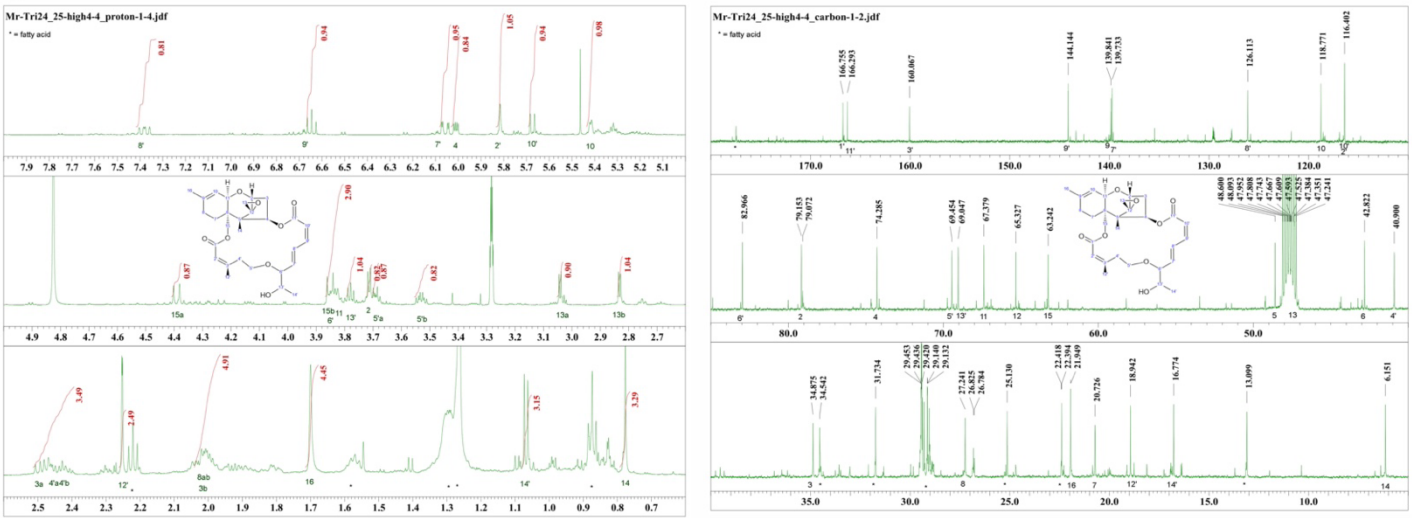


Figure S13. Proton nuclear magnetic resonance (**left panels**), and carbon-13 nuclear magnetic resonance (carbon-13 NMR spectroscopy) (**right panels**) of compound/spot **6**- roridin A (**upper panels**), and **1**- roridin E (**bottom panels**)

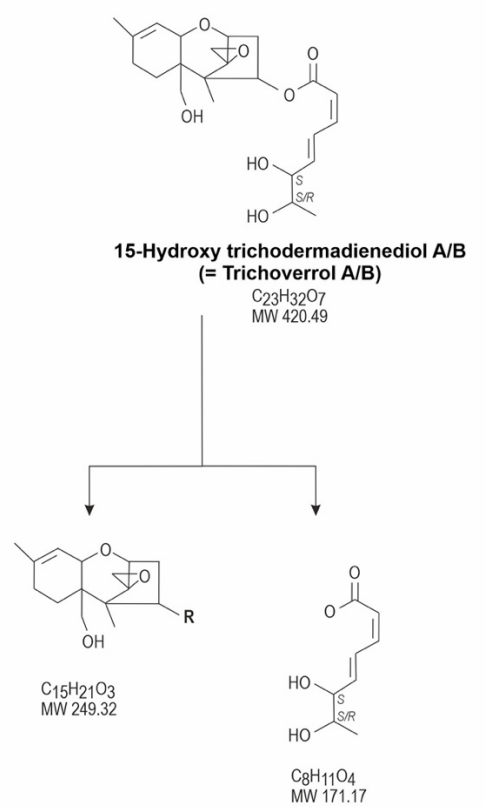


Figure S14. Compounds detected by LC-MS analysis from extracts of culture filtrates of the Δ*tri24* mutant. Molecular formula and molecular weight of each detected compound are included below each chemical structure.

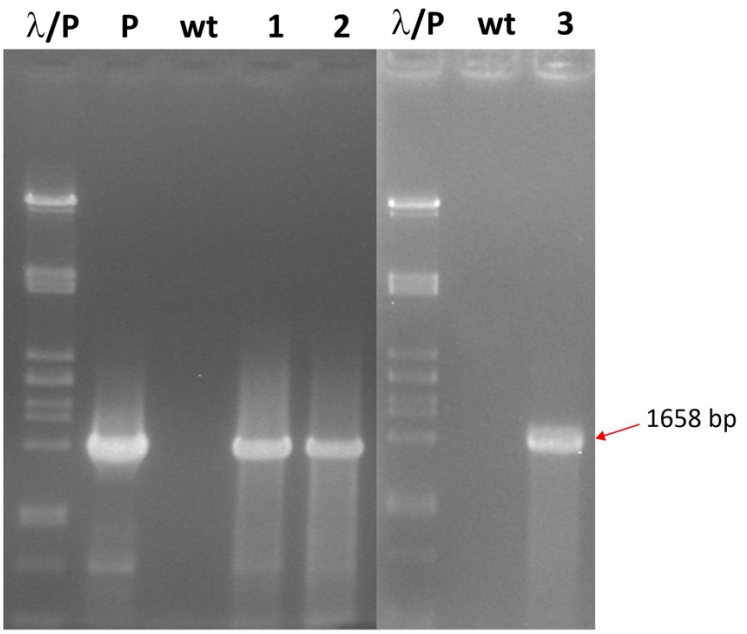


Figure S15. PCR analysis of the three transformants selected by transformation of *Trichoderma arundinaceum* Ta37 with plasmid pTCS**_PRORI_TRI24a_ble_a. The expected 1,658 bp fragment has been indicated by a red arrow at the right of the figure. λ/P= DNA of lambda bacteriophage digested with *Pst*I; P= plasmid; wt= Genomic DNA of the *P. roridum* wild-type strain.

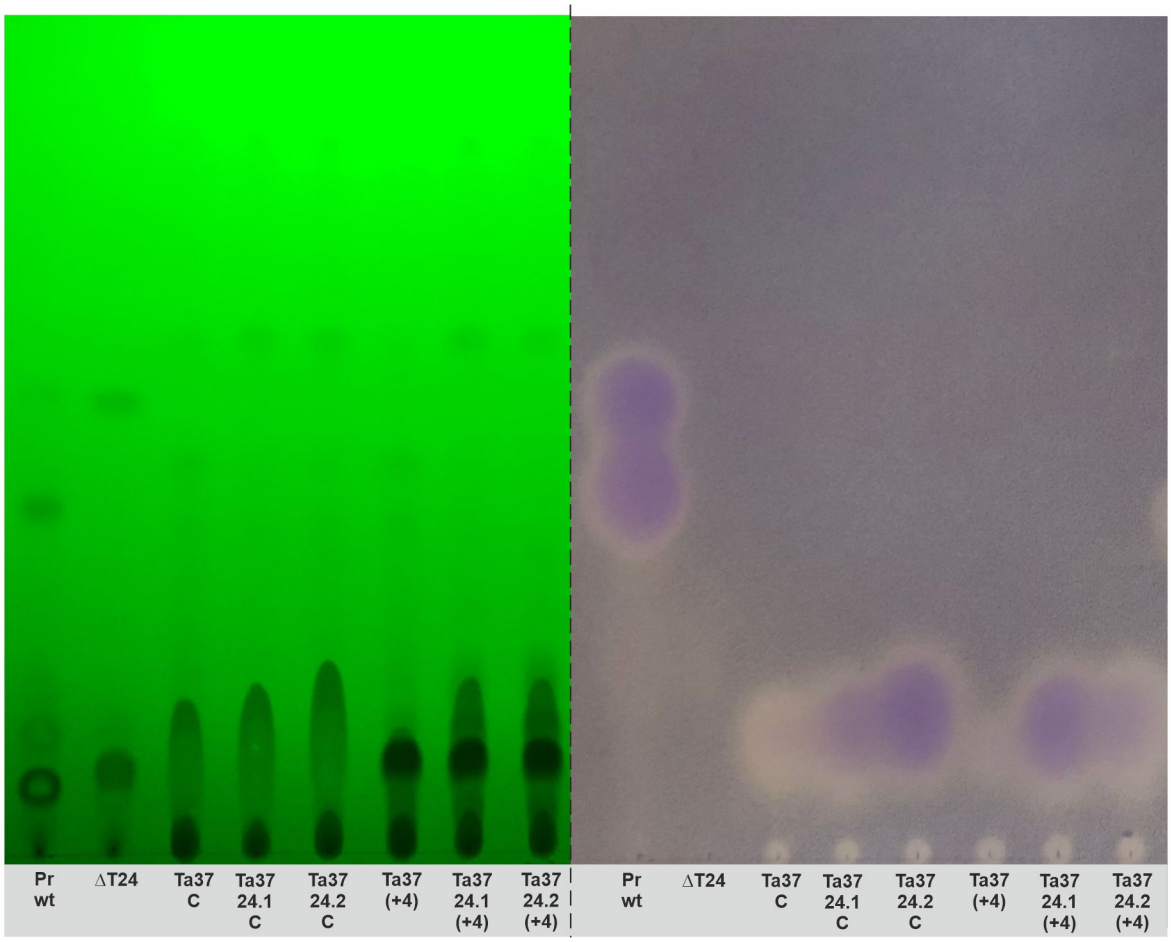


Figure S16. TLC (**left panel**) and Bioautobiogram against *K. marxianus* (**right panel**) of broths from Ta37-C, and transformants Ta37_T24.1-C and Ta37_T24.2-C (C= Control), and from feeding experiments of these three strains with 15-hydroxy trichodermadiene (+ **4**) (1.5 mg/8mL YEPD flask). *P. roridum* control (Pr wt), Δ*tri24* mutant (ΔT24). Note that non feeding and feeding samples were collected from cultures incubated 8 days after feeding onset.

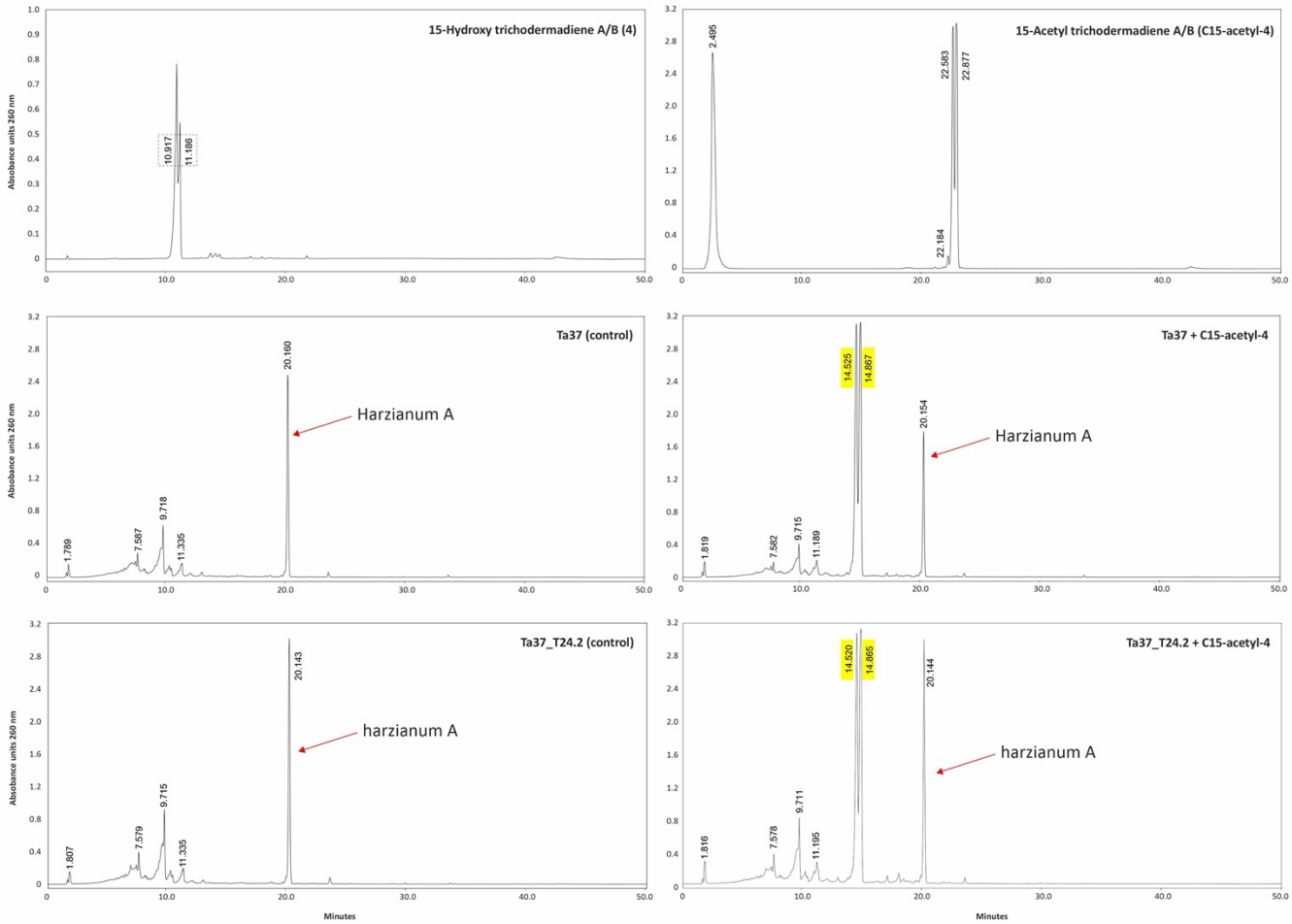


Figure S17. HPLC analysis from feeding cultures made with Ta37 wild type (control) and one transformant of Ta37 expressing *P. roridum TRI24* gene (transformant Ta37_T24.2). Cultures were incubated in YEPD without feeding (**left panels**) and fed with 15-acetyl trichodermadiene A/B (**C15-acetyl 4**) (1.5 mg in 8 mL total volume) (**right panels**). Those peaks appearing in cultures amended with **C15-acetyl 4** are shaded in yellow. Note that these peaks were also detected in the feeding experiment with compound **4** (See **Fig. 20**). Chromatograms of the compound **4** and **C15-acetyl 4** have been included in the top-upper panels for comparative purposes.

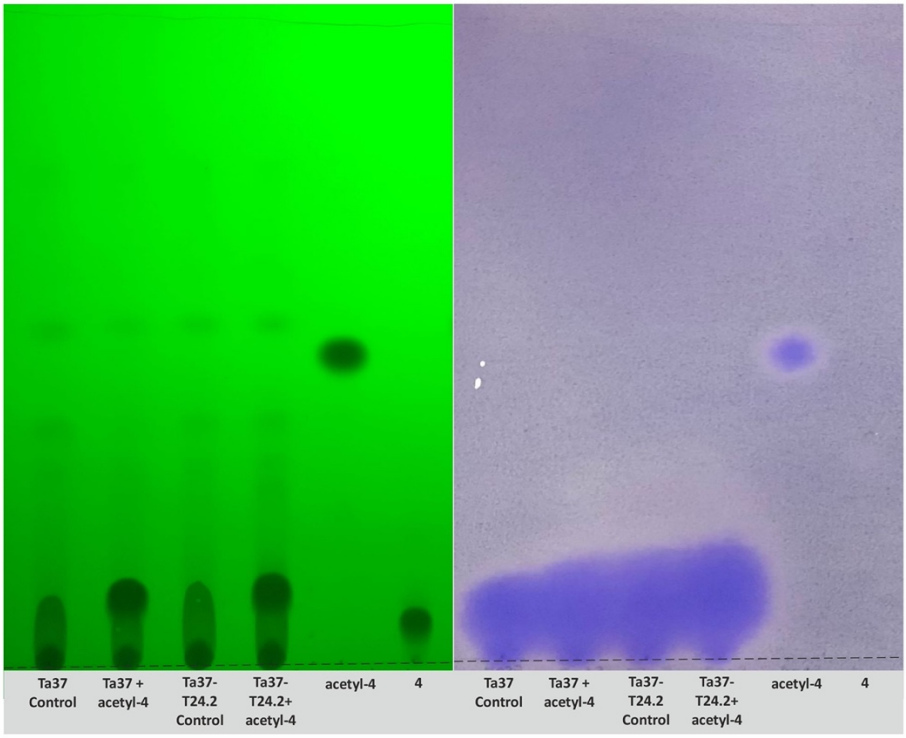


Figure S18. TLC (**left panel**) and Bioautobiogram against *K. marxianus* (**right panel**) of broths from Ta37, and transformant Ta37_T24.2 (Controls) and feeding experiments of these three same strains with 15-acetyl trichodermadiene (**C15-acetyl 4**) (1.5 mg/8 mL YEPD flask). Samples of purified **C15-acetyl 4** (= **acetyl-4**) and **4** compounds were also added for comparative purposes. Note: Feeding samples were from cultures incubated 8 days after feeding onset.

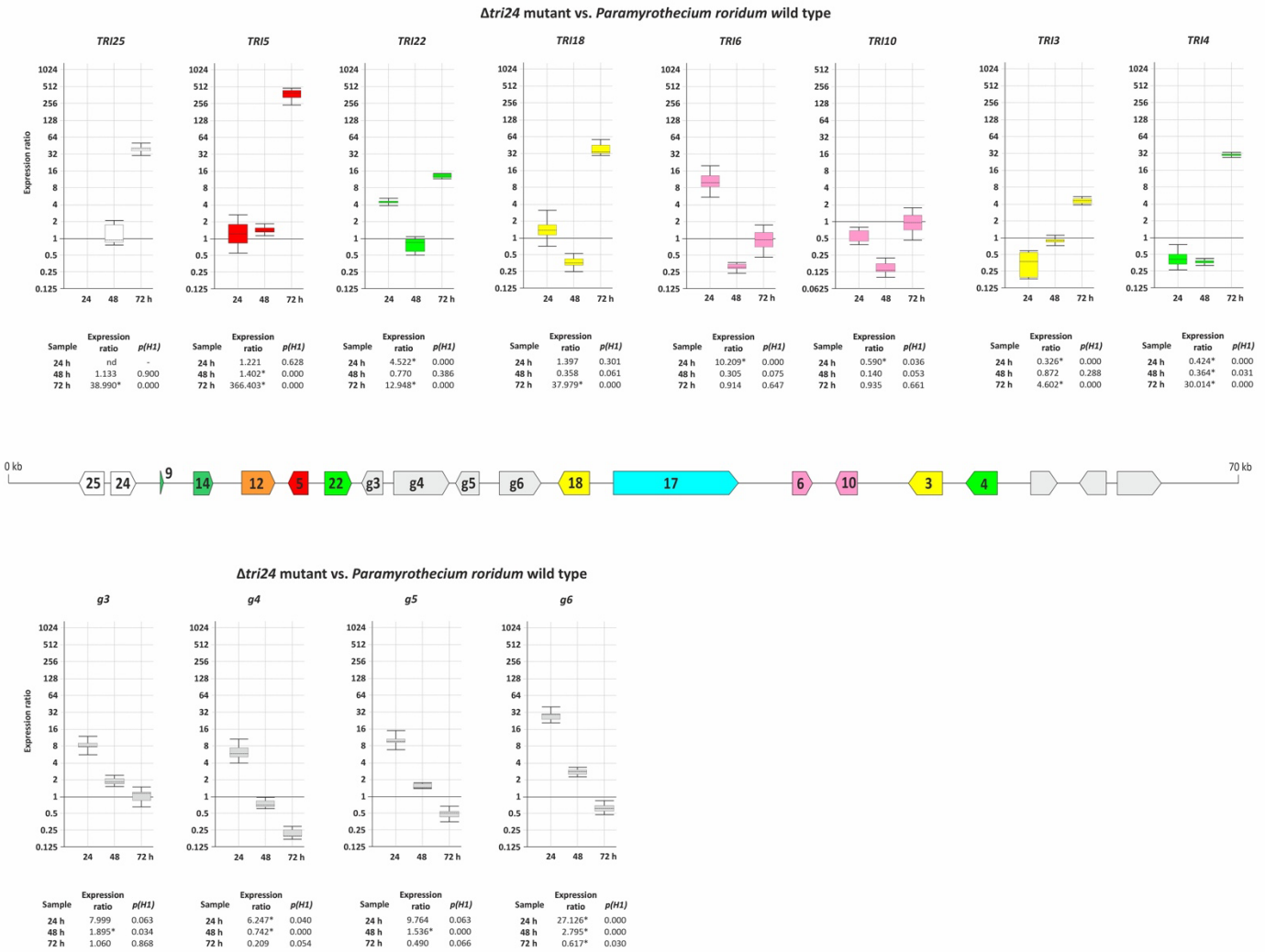
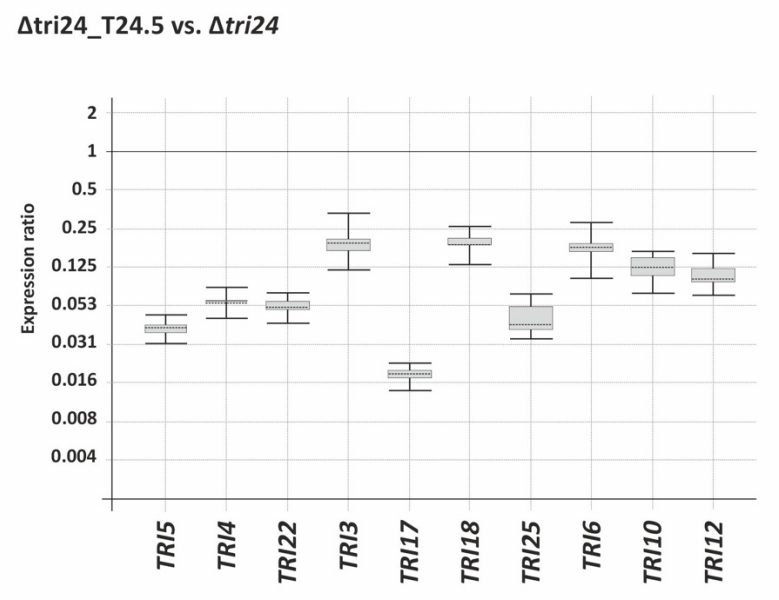
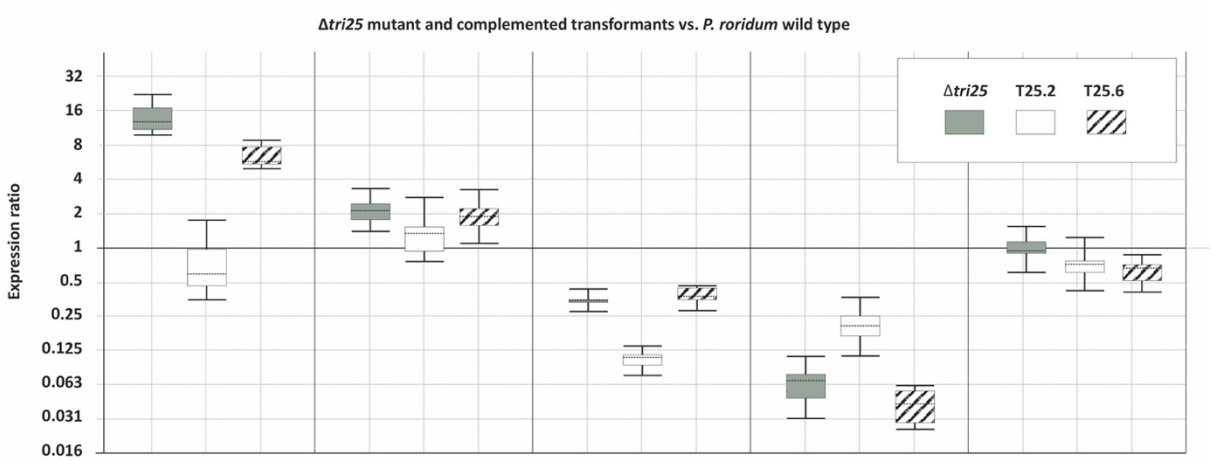


Figure S19. qPCR analysis of expression of genes in the *P. roridum TRI* cluster. Genes are indicated by arrows. Color codes assigned to each gene are described in the legend of Fig. 2. Gene expression values were calculated as ratio of expression in the Δ*tri24* mutant versus the levels observed in the wild-type strain. Numeric values are indicated below the graphic for each gene, and those values statistically significant were indicated by an asterisk.



Gene	Expression ratio	*p(H1)*
TRI5	0.040*	0.000
TRI4	0.063*	0.033
TRI22	0.058*	0.034
TRI3	0.183*	0.020
TRI17	0.017*	0.000
TRI18	0.185*	0.000
TRI25	0.047*	0.000
TRI6	0.166*	0.030
TRI10	0.119*	0.000
TRI12	0.104*	0.047

Figure S20. qPCR analysis of expression of *TRI* genes to determine the effect of complementation of the Δ*tri24* mutant with the plasmid pTCS**_PRORI_TRI24a_ble_a designed to overexpress *P. roridum TRI24* gene. Gene expression values were calculated as ratio of expression in Δtri24_T24.5 complemented transformant versus the levels observed in the Δ*tri24* mutant. Numeric values are indicated below the graphic for each gene, and those values statistically significant were indicated by an asterisk.



Strain	Expression ratio	p(H1)	Expression ratio	p(H1)	Expression ratio	p(H1)	Expression ratio	p(H1)	Expression ratio	p(H1)
Δ*tri25*	13.403*	0.016	2.137*	0.000	0.342*	0.000	0.886*	0.019	0.954	0.893
T25.2	0.646	0.265	0.954	0.562	0.102*	0.000	0.201*	0.000	0.695	0.222
T25.6	10.437*	0.015	1.903	0.065	0.970*	0.000	0.040*	0.000	0.618*	0.026

Figure S21. qPCR analysis of expression of five selected *TRI* genes in the Δ*tri25* mutant and in two complemented transformants, Δtri25_T25.2 (T25.2) and Δtri25_T25.6 (T25.6). Gene expression values were calculated as ratio of expression in those strains versus the levels observed in the wild-type strain. Numeric values are indicated below the graphic for each gene, and those values statistically significant were indicated by an asterisk.

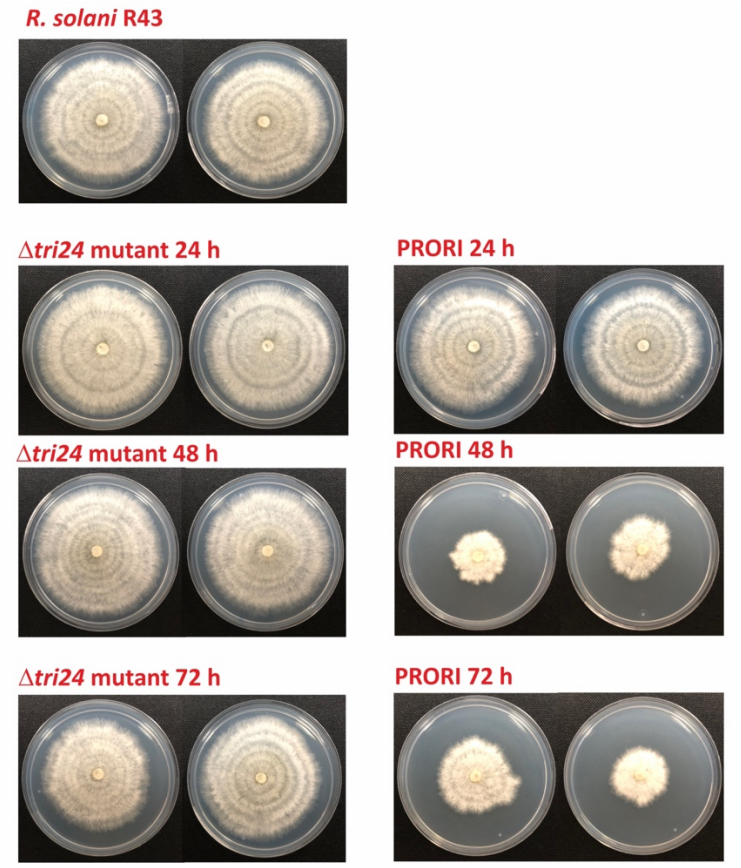


Figure S22. Antifungal assay against *Rhizoctonia solani* of PBD culture filtrates from wild-type *P. roridum* (PRORI) (**right panels**) and the Δ*tri24* mutant (**left panels**) harvested after 24, 48, and 72 h of growth. Photographs were taken 10 days after the pathogen´s plugs were placed in a 7 mm diameter hole made in the center of the plate; cultures filtrates were loaded into the hole after which the plates were incubated at 4 °C for 24 h before placement of the pathogen´s plugs.

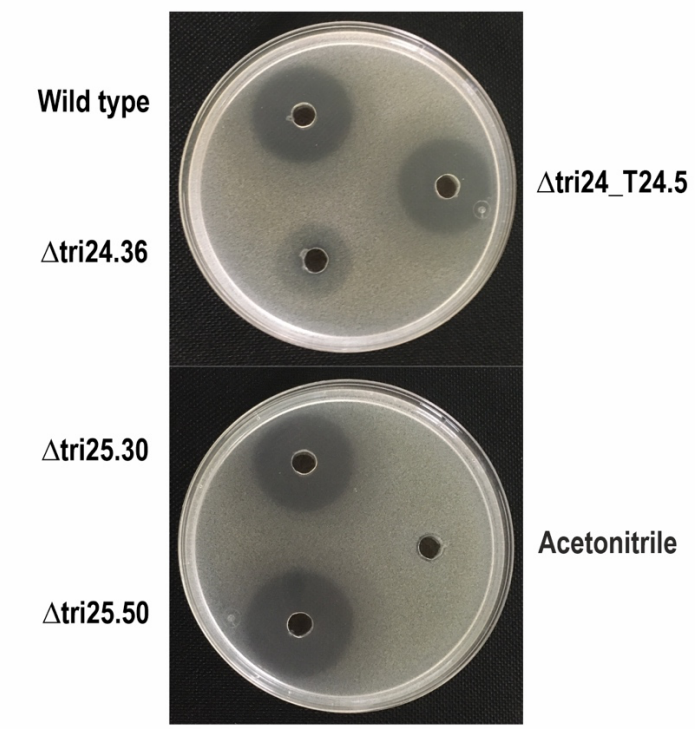


Figure S23. Antibiogram against *K. marxianus* of 72 h PDB broths of wild-type *P. roridum*, the Δtri24.36 mutant, the complemented transformant Δtri24_T24.5, and the Δtri25.30 and Δtri25.50 mutant strains.

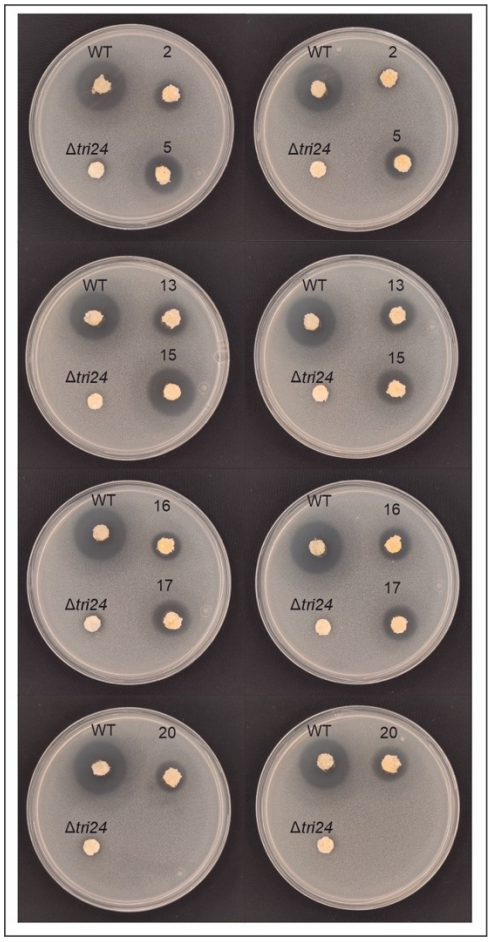


Figure S24. Antibiogram showing antifungal activity against *K. marxianus* of seven transformants obtained from the Δ*tri24* mutant complementation analysis. The transformants were obtained by transformation of Δ*tri24* mutant with plasmid pTCS**_PRORI_TRI24a_blea (Fig. S2) and are labeled as 2, 5, 13, 15, 16, 17 and 20 in the figure. wild-type *P. roridum* (=WT) and the Δ*tri24* mutant (Δ*tri24*) were included as controls in this study. The antibiogram was performed with 7 mm diameter plugs collected from 7 days old CMD_ULE plates.

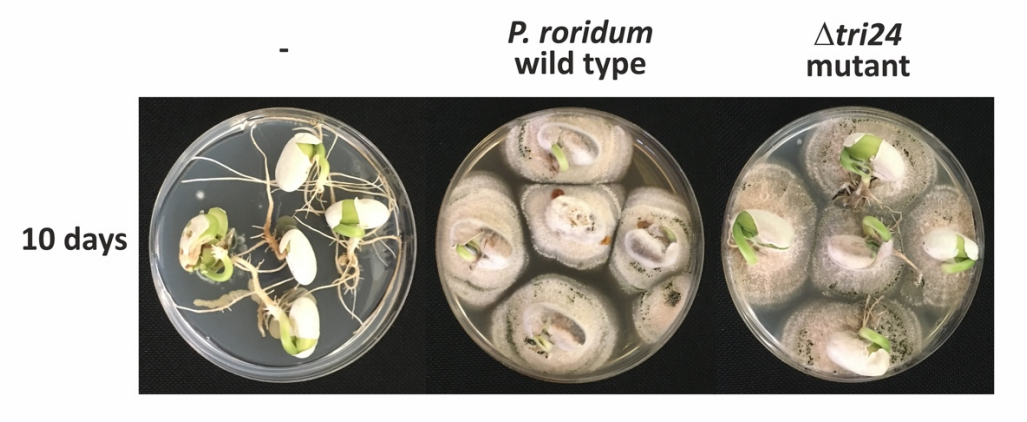


Figure S25. Effect of *TRI24* gene deletion on germination of bean seeds.

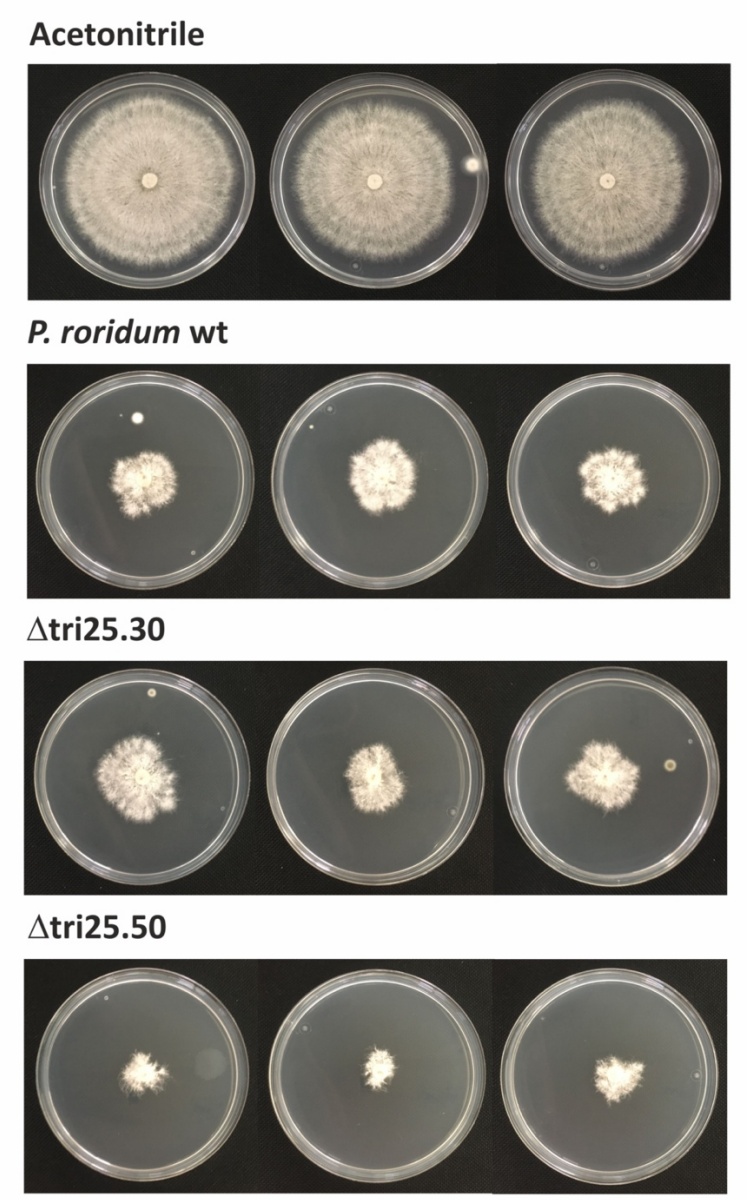


Figure S26. Antifungal effect against *R. solani* of culture PBD broths harvested after 72 h of growth from the *P. roridum* wild type (= wt), and the two Δ*tri25* mutants isolated in the present study (Δtri25.30 and Δtri25.50). Photographs were taken 10 days after the placement of the *R. solani* plugs.

Figure S27. Antibiogram against *K. marxianus* of 9 out of the 20 add-back transformants isolated by transformation of the Δ*tri25* mutant strain with plasmid pTCS**_PRORI_TRI25a_blea. *P. roridum* wild-type strain (= WT) and the Δ*tri25* mutant were used as controls in this study. The antibiogram was performed with 7 mm diameter plugs extracted from 7 days old CMD_ULE plates.

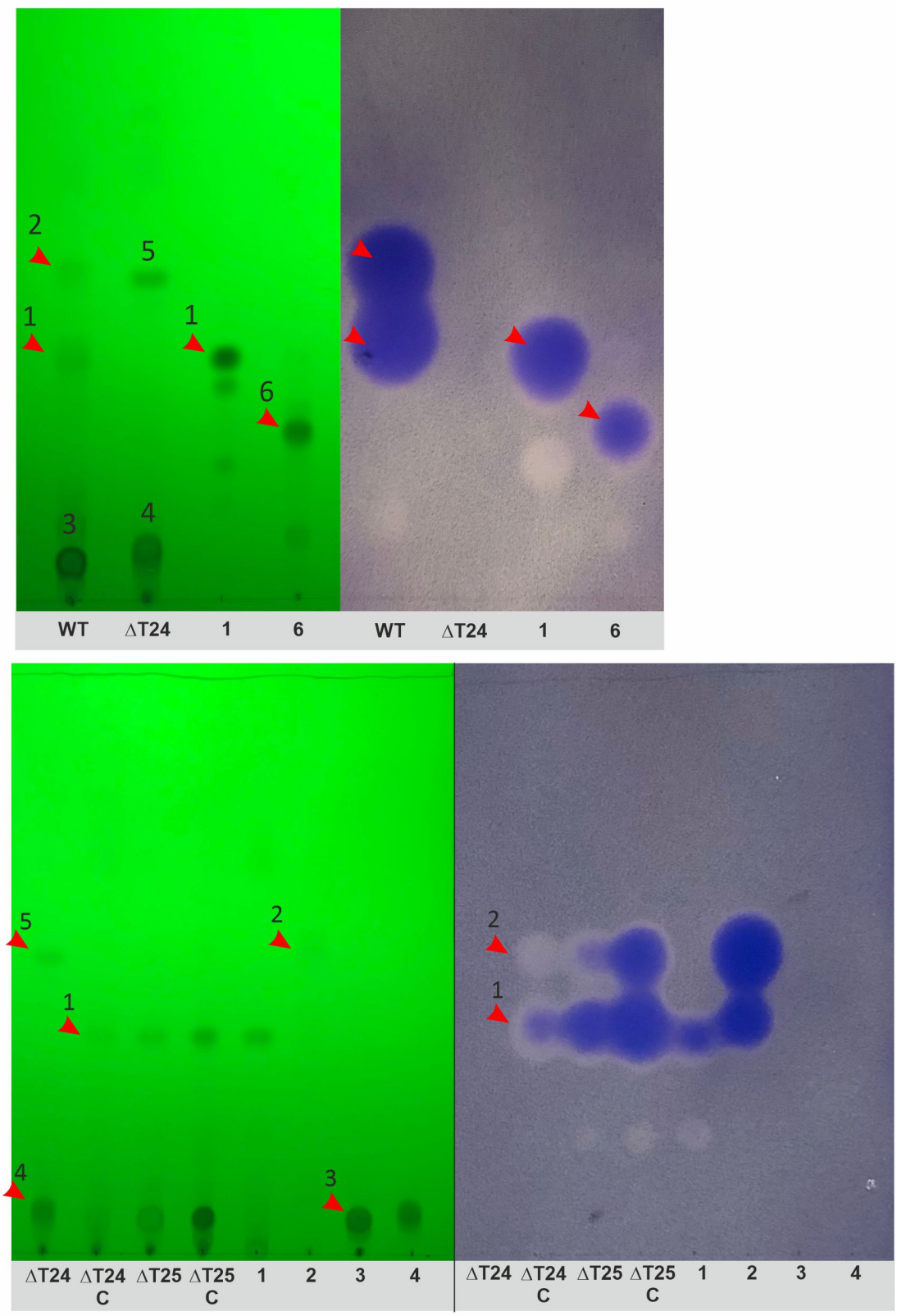


Figure S28. TLC plates (**left panels**) and bioautobiogram against *K. marxianus* (**right panels**) made with purified spots **1**, **2**, **3**, **4**, and **6**, and with extracted broths from the Δ*tri24* mutant (ΔT24), the Δ*tri25* mutant (ΔT25), and their corresponding complemented transformants (C). Some of the main spots were pointed with arrow heads and named according to the manuscript description.