

**Table S1a.** Genomic sequences of *Trichoderma* spp. that were retrieved from the National Center for Biotechnology Information (NCBI) database for their use in the phylogenetic analyses carried out in the present work.

| Species                                     | GenBank accession number | Reference                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|---------------------------------------------|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Trichoderma asperellum</i> CBS 433.97    | GCF_003025105.1          | Druzhinina, I.S., Chenthamara, K., Zhang, J., Atanasova, L., Yang, D., Miao, Y., Rahimi, M.J., Grujic, M., Cai, F., Pourmehdi, S., Salim, K.A., Pretzer, C., Kopchinskiy, A.G., Henrissat, B., Kuo, A., Hundley, H., Wang, M., Aerts, A., Salamov, A., Lipzen, A., LaButti, K., Barry, K., Grigoriev, I.V., Shen, Q., Kubicek, C.P. 2018. Massive lateral transfer of genes encoding plant cell wall-degrading enzymes to the mycoparasitic fungus <i>Trichoderma</i> from its plant-associated hosts. <i>PLoS Genet.</i> 14: e1007322. <a href="http://doi.org/10.1371/journal.pgen.1007322">http://doi.org/10.1371/journal.pgen.1007322</a> . |
| <i>Trichoderma citrinoviride</i> TUCIM 6016 | GCF_003025115.1          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <i>Trichoderma guizhouense</i> NJAU 4742    | GCA_002022785.1          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <i>Trichoderma harzianum</i> CBS 226.95     | GCF_003025095.1          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <i>Trichoderma atroviride</i> IMI 206040    | GCF_000171015.1          | Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, et al. 2011. Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of <i>Trichoderma</i> . <i>Genome Biol.</i> 12(4): R40. <a href="http://doi.org/10.1186/gb-2011-12-4-r40">http://doi.org/10.1186/gb-2011-12-4-r40</a>                                                                                                                                                                                                                                                                                                                                 |
| <i>Trichoderma virens</i> Gv29-8            | GCF_000170995.1          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <i>Trichoderma hamatum</i> GD12             | GCA_000331835.2          | Studholme DJ, Harris B, Le Cocq K, Winsbury R, Perera V, Ryder L, Ward JL, Beale MH, Thornton CR, Grant M. 2013. Investigating the beneficial traits of <i>Trichoderma hamatum</i> GD12 for sustainable agriculture-insights from genomics. <i>Front. Plant Sci.</i> 4: 258. <a href="http://doi.org/10.3389/fpls.2013.00258">http://doi.org/10.3389/fpls.2013.00258</a> .                                                                                                                                                                                                                                                                      |
| <i>Trichoderma arundinaceum</i> IBT 40837   | GCA_003012105.1          | Proctor, R.H., McCormick, S.P., Kim, H.S., Cardoza, R.E., Stanley, A.M., Lindo, L., Kelly, A., Brown, D.W., Lee, T., Vaughan, M.M., Alexander, N.J., Busman, M., Gutiérrez, S. 2018. Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. <i>PLoS Pathog.</i> 14(4): e1006946. <a href="http://doi.org/10.1371/journal.ppat.1006946">http://doi.org/10.1371/journal.ppat.1006946</a>                                                                                                                                                                                |
| <i>Trichoderma brevicompactum</i> IBT 40841 | GCA_003012085.1          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <i>Trichoderma atrobrunneum</i> ITEM 908    | GCA_003439915.1          | Fanelli, F., Liuzzi, V.C., Logrieco, A.F., Altomare, C. 2018. Genomic characterization of <i>Trichoderma atrobrunneum</i> ( <i>T. harzianum</i> species complex) ITEM 908: insight into the genetic endowment of a multi-target biocontrol strain. <i>BMC Genomics.</i> 19: 662. <a href="http://doi.org/10.1186/s12864-018-5049-3">http://doi.org/10.1186/s12864-018-5049-3</a>                                                                                                                                                                                                                                                                |
| <i>Trichoderma koningii</i> JCM 1883        | GCA_001950475.1          | Manabe, R., Endoh, R., Uzuhashi, S., Okada, G., Takashima, M., Ohkuma, M. 2016. <i>Trichoderma koningii</i> strain JCM 1883, whole genome shotgun sequencing project. Direct submission                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <i>Trichoderma koningiopsis</i> POS7        | GCA_002246995.1          | Castrillo, M.L., Bich, G.A., Modenutti, C.P., Turjanski, A.G., Zapata, P.D., Villalba, L. 2017. First whole-genome shotgun sequence of a promising cellulase secretor, <i>Trichoderma koningiopsis</i> strain POS7. <i>Genome Announc.</i> 5: e00823-17. <a href="http://doi.org/10.1128/genomeA.00823-17">http://doi.org/10.1128/genomeA.00823-17</a>                                                                                                                                                                                                                                                                                          |
| <i>Trichoderma lentiforme</i> CFAM-422      | GCA_011066345.1          | Steindorff, A.S., Formighieri, E.F., Midorikawa, G.E.O., Tamietti, M.S., Ramos, E.Z., Silva, A.S., Bon, E.P.S., Mendes, T.D., Damaso, M.C.T., Favaro, L.C.L. 2018. <i>Trichoderma lentiforme</i> strain CFAM-422, whole genome shotgun sequence project. Direct submission.                                                                                                                                                                                                                                                                                                                                                                     |

|                                              |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|----------------------------------------------|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Trichoderma oligosporum</i> CGMCC 3.17527 | GCA_015266385.1 | Wang, C., Zeng, Z., Zhuang, w. 2020. Comparative molecular evolution of chitinases in Ascomycota with emphasis on mycoparasitism lifestyle. Direct submission.                                                                                                                                                                                                                                                                                         |
| <i>Trichoderma parareesei</i> CBS125925      | GCA_001050175.1 | Yang, D., Pomraning, K., Kopchinskiy, A., Karimi-Aghcheh, R., Atanasova, L., Chenthamara, K., Baker, S.E., Zhang, R., Shen, Q., Freitag, M., Kubicek, C.P., Druzhinina, I.S. 2017. Genome sequence and annotation of <i>Trichoderma parareesei</i> , the ancestor of the cellulase producer <i>Trichoderma reesei</i> . <i>Genome Announc.</i> 20153(4). <a href="http://doi.org/10.1128/genomeA.00885-15">http://doi.org/10.1128/genomeA.00885-15</a> |
| <i>Trichoderma reesei</i> QM6a               | GCF_000167675.1 | Li, W.C., Huang, C.H., Chen, C.L., Chuang, Y.C., Tung, S.Y., Wang, T.F. 2017. <i>Trichoderma reesei</i> complete genome sequence, repeat-induced point mutation, and partitioning of CAZyme gene clusters. <i>Biotechnol Biofuels.</i> 10: 170. <a href="http://doi.org/10.1186/s13068-017-0825-x">http://doi.org/10.1186/s13068-017-0825-x</a>                                                                                                        |

**Table S1b.** Genomic sequences retrieved from the National Center for Biotechnology Information (NCBI) database of non-*Trichoderma* species for their use in the phylogenetic analyses carried out in the present work.

| Species                                       | GenBank accession number | Reference                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------------------------------|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Fusarium longipes</i> NRRL 20695           | PXOG00000000             | Proctor, R.H., McCormick, S.P., Kim, H.S., Cardoza, R.E., Stanley, A.M., Lindo, L., Kelly, A., Brown, D.W., Lee, T., Vaughan, M.M., Alexander, N.J., Busman, M., Gutiérrez, S. 2018. Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. <i>PLoS Pathog.</i> 14 (4): e1006946. <a href="http://doi.org/10.1371/journal.ppat.1006946">http://doi.org/10.1371/journal.ppat.1006946</a> |
| <i>Fusarium fasciculatum</i> NRRL 13405       | PXXK00000000             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>Fusarium sporotrichioides</i> NRRL 3299    | PXOF00000000             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>Microcyclospora tardicrescens</i> HJS 1936 | PXOE00000000             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>Myrothecium roridum</i> NRRL 2183          | PXOD00000000             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>Spicellum ovalisporum</i> DAOM 186447      | PXOC00000000             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>Trichothecium roseum</i> DAOM 195227       | PXNY00000000             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>Aspergillus hancockii</i>                  | GCA_001696595.2          | Pitt, I.P., Lange, L., Lacey, A.E., Vuong, D., Midgley, D.J., Greenfield, P., Bradbury, M.I., Lacey, E., Busk, P.K., Pilgaard, B., Chooi, Y.-H., Piggott, A.M. 2017. <i>Aspergillus hancockii</i> sp. nov., a biosynthetically talented fungus endemic to southeastern Australia soils. <i>PLoS ONE</i> 12: 30170254. <a href="https://doi.org/10.1371/journal.pone.0170254">https://doi.org/10.1371/journal.pone.0170254</a>                                     |
| <i>Beauveria bassiana</i> ARSEF 2860          | ADAH00000000             | Xiao, G., Ying, S.-H., Zheng, P., Wang, A.-L., Zhang, S., Xie, S.-Q., Shang, Y., Leger, R.J., Zhao, G.-P., Wang, C., Geng, M.-G. 2012. Genomic perspectives on the evolution of fungal entomopathogenicity in <i>Beauveria bassiana</i> . <i>Sci. Rep.</i> 2: 483. <a href="http://doi.org/10.1038/srep00483">http://doi.org/10.1038/srep00483</a> .                                                                                                              |
| <i>Cordyceps confragosa</i> UM487             | GCA_001653215.1          | Fine-scale spatial genetic structure of a fungal parasite of coffee scale insects: Unpublished – Jackson, D., Zemenick, K.A., Malloure, B., Quandt, C.A., James, T.Y.                                                                                                                                                                                                                                                                                             |
| <i>Fusarium graminearum</i> PH-1              | AACM00000000             | Walkowiak, S., Rowland, O., Rodrigue, N., Subramaniam, R. 2016. Whole genome sequencing and comparative genomics of closely related <i>Fusarium</i> Head Blight fungi: <i>Fusarium graminearum</i> , <i>F. meridionale</i> and <i>F. asiaticum</i> . <i>BMC Genomics.</i> 17: 1014. <a href="http://doi.org/10.1186/s12864-016-3371-1">http://doi.org/10.1186/s12864-016-3371-1</a>                                                                               |
| <i>Stachybotrys chartarum</i> 40293           | ASEQ00000000             | Semeiks, J., Borek, D., Otwinowski, Z., Grishin, N.V. 2014. Comparative genome sequencing reveals chemotype-specific gene clusters in the toxigenic black mold <i>Stachybotrys</i> . 15: 590. <a href="https://doi.org/10.1186/1471-2164-15-590">https://doi.org/10.1186/1471-2164-15-590</a>                                                                                                                                                                     |
| <i>Stachybotrys chlorohalonata</i> IBT 40285  | APWP00000000             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

**Table S2.** Housekeeping genes used for the phylogenetic and evolutive analyses

| Gene        | <i>T. arundinaceum</i><br>Gene Model | Predicted Protein Product                                           |
|-------------|--------------------------------------|---------------------------------------------------------------------|
| <i>cpr1</i> | TARUN_9366                           | Cytochrome P450 Reductase                                           |
| <i>dpa1</i> | TARUN_6778                           | DNA Polymerase Alpha Subunit                                        |
| <i>dpd1</i> | TARUN_237                            | DNA Polymerase Delta Subunit                                        |
| <i>erg1</i> | TARUN_9990                           | Squalene Monooxygenase                                              |
| <i>fas1</i> | TARUN_2912                           | Fatty Acid Synthase Alpha Subunit                                   |
| <i>fas2</i> | TARUN_2913                           | Fatty Acid Synthase Beta Subunit                                    |
| <i>his3</i> | TARUN_10324                          | Histone H3                                                          |
| <i>lae1</i> | TARUN_10114                          | Global Regulatory Gene/Chromatin Methyl Transferase                 |
| <i>lcb1</i> | TARUN_6733                           | Sphinganine Palmitoyl Transferase Subunit 1                         |
| <i>lcb2</i> | TARUN_696                            | Sphinganine Palmitoyl Transferase Subunit 2                         |
| <i>mcm7</i> | TARUN_2249                           | DNA Replication Licensing Factor                                    |
| <i>pgk1</i> | TARUN_275                            | Phosphoglycerate Kinase                                             |
| <i>rpb1</i> | TARUN_7976                           | RNA Polymerase II Largest Subunit                                   |
| <i>rpb2</i> | TARUN_7706                           | RNA Polymerase II Subunit 2                                         |
| <i>sph1</i> | TARUN_9560                           | Longevity Assurance Factor/Sphinganine N Acyl Transferase Subunit 1 |
| <i>top1</i> | TARUN_773                            | Topoisomerase                                                       |
| <i>tps1</i> | TARUN_4479                           | Trehalose Phosphate Synthase                                        |
| <i>tsr1</i> | TARUN_1474                           | Ribosomal Biogenesis Protein                                        |
| <i>tub1</i> | TARUN_4212                           | Tubulin Alpha Subunit                                               |
| <i>ubt1</i> | TARUN_8065                           | Ubiquitin Thiolesterase                                             |

Note. Genes in red color are those used to construct the phylogenetic tree including *Trichoderma* and other fungi belonging to the order Hypocreales that were previously shown to have a *tri5* copy in their genomes (Proctor et al., 2018).

Proctor, R.H., McCormick, S.P., Kim, H.-S., Cardoza, R.E., Stanley, A.M., Lindo, L., Kelly, A., Brown, D.W., Lee, T., Vaughan, M.M., Alexander, N.J., Busman, M., Gutiérrez, S. (2018). Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. *PLoS Pathog.* 14(4)e1006946. doi: 10.1371/journal.ppat.1006946

**Table S3.** Oligonucleotides used

| <i>Species</i>                                                                                               | Oligo name     | Sequence 5´-3´           |                           |
|--------------------------------------------------------------------------------------------------------------|----------------|--------------------------|---------------------------|
| <b>Oligonucleotides to construct plasmid pΔtri5</b>                                                          |                |                          |                           |
| <i>T. arundinaceum</i>                                                                                       | tri5_5rF_BamHI | GGATCCCTATTGAACCGTGGCAAA |                           |
|                                                                                                              | tri5_5Rr_SmaI  | CCCGGGGCTCAACCATGATGATGA |                           |
|                                                                                                              | tri5_3rF_SmaI  | CCCGGGGCTTATTCGAGGCTATG  |                           |
|                                                                                                              | tri5_3rR_SalI  | GTCGACAGGCTCTGGAATAGCATA |                           |
| <b>Oligonucleotides to analyze <i>T. arundinaceum</i> tri5 deleted mutants</b>                               |                |                          |                           |
| <i>T. arundinaceum</i>                                                                                       | T5_5rr         | CTGTGATGCAGTAAGGCT       |                           |
|                                                                                                              | T5_3rr         | GCATGTGATACGCATGCA       |                           |
|                                                                                                              | PgpdA-d        | ATCATCCACTGCACCTCA       |                           |
|                                                                                                              | TrpC-d         | GTAACCATGCATGGTTGC       |                           |
| <b>Oligonucleotides to amplify tri5 gene from non-trichothecene producers<br/><i>Trichoderma</i> species</b> |                |                          |                           |
| <i>T. arundinaceum</i>                                                                                       | TARUN_T5_5     | GTTGAGCTCAACGACGTC       |                           |
|                                                                                                              | TARUN_T5_3     | CTACTGGGCGACGGAGGA       |                           |
| <i>T. stromaticum</i>                                                                                        | TSTRO_T5_5     | GTTGACCTCAACAGCATT       |                           |
|                                                                                                              | TSTRO_T5_3     | TTAGCCATCCCTCTCTTG       |                           |
| <i>T. cf. fertile</i>                                                                                        | TFERT_T5_5     | GTTGAGCTCAACAACGCT       |                           |
|                                                                                                              | TFERT_T5_3     | TCATTGTTCCAGCCTCTT       |                           |
| <i>T. polysporum</i>                                                                                         | TPOLY_T5_5     | GTTGAGCTTAATGATATC       |                           |
|                                                                                                              | TPOLY_T5_3     | TCACGCAAAGGATAAGAT       |                           |
| <i>T. decipiens</i>                                                                                          | TDECI_T5_5     | GATGGGATCAATATTGTC       |                           |
|                                                                                                              | TDECI_T5_3     | TCAGGGTTTCCCAGCAAG       |                           |
| <i>T. gamsii</i> T065                                                                                        | TGAMS_T5_5     | GTTGAGCTCAATGATGTT       |                           |
|                                                                                                              | TGAMS_T5_3     | TCACCACTCAACAGACGT       |                           |
| <i>T. balearicum</i>                                                                                         | TBALE_T5_5     | CCTGAGCTCAATGACGTC       |                           |
|                                                                                                              | TBALE_T5_3     | TCAGTACTGAGCAACAGA       |                           |
| <b>Oligonucleotides for qPCR analysis</b>                                                                    |                |                          | <b>PCR efficiency (%)</b> |
| <b>tri5 gene</b>                                                                                             |                |                          |                           |
| <i>T. arundinaceum</i>                                                                                       | TARUN_tri5_F   | TGTGTCGGCTCTTCAATCTG     | 91.3                      |
|                                                                                                              | TARUN_tri5_R   | GCTTATCGAGAGCCTGATCG     |                           |
| <i>T. stromaticum</i>                                                                                        | TSTRO_T5_L1    | GCAGTTCCTCGAACTACCT      | 103.5                     |
|                                                                                                              | TSTRO_T5_R1    | GCTCGGCAGCTTTTCAATAA     |                           |
| <i>T. cf. fertile</i>                                                                                        | TFERT_T5_L1    | TGTCGTGTCTCCAAGGAAGT     | 108.0                     |
|                                                                                                              | TFERT_T5_R1    | CGTACCAGGTCGCCATACTA     |                           |
| <i>T. polysporum</i>                                                                                         | TPOLY_T5_L1    | GCGTTTATTCTGTTGCCAT      | 107.9                     |
|                                                                                                              | TPOLY_T5_R1    | CCATGGTATCAGCAGGCTCT     |                           |
| <i>T. decipiens</i>                                                                                          | TDECI_T5_L1    | CTGGCTGCTGCTGTTAAGAC     | 107.8                     |
|                                                                                                              | TDECI_T5_R1    | GCGGCTGGCATTTAGAAGG      |                           |
| <i>T. gamsii</i> T065                                                                                        | TGAMS_T5_L1    | AAAGTGCCTCCTAAGCGTCT     | 106.3                     |
|                                                                                                              | TGAMS_T5_R1    | GTATCGGCAGGCTCTTCTCT     |                           |
| <i>T. balearicum</i>                                                                                         | TBALE_T5_L1    | CGCTAGTGACTATCCCGGTT     | 110.6                     |
|                                                                                                              | TBALE_T5_R1    | GAGCAATGGCTGTGGTGATT     |                           |
| <b>actin gene</b>                                                                                            |                |                          |                           |

|                        |                |                      |       |
|------------------------|----------------|----------------------|-------|
| <i>T. arundinaceum</i> | TARUN_actin_F  | ACTGGGACGACATGGAGAAG | 92.1  |
|                        | TARUN_actin_R  | GGCCTGGATGGAGACATAGA |       |
| <i>T. stromaticum</i>  | TSTRO_actin_L1 | TGAGCGTGGTTACACCTTCT | 106.6 |
|                        | TSTRO_actin_R1 | GCCAATGGTGATGACCTGAC |       |
| <i>T. cf. fertile</i>  | TFERT_actin_L1 | AGGAGAAGCTCTGCTACGTC | 94.9  |
|                        | TFERT_actin_R1 | AAGGACAGAAGGCTGGAACA |       |
| <i>T. polysporum</i>   | TPOLY_actin_L1 | AGGAGAAGCTCTGCTACGTC | 105.9 |
|                        | TPOLY_actin_R1 | AAGGACAGAAGGCTGGAACA |       |
| <i>T. decipiens</i>    | TDECI_actin_L1 | TCCAGGCTGTTCTGTCTCTG | 108.0 |
|                        | TDECI_actin_R1 | CGACCAGCCATATCAACACG |       |
| <i>T. gamsii</i> T065  | TGAMS_actin_L1 | AGGAGAAGCTCTGCTACGTC | 106.8 |
|                        | TGAMS_actin_R1 | ACCGCTCTCAAGACCAAGAA |       |
| <i>T. balearicum</i>   | TBALE_actin_L1 | TGACCCAGATCGTCTTCGAG | 107.4 |
|                        | TBALE_actin_R1 | GTCAACACGAGCAATAGCGT |       |

**Table S4a.** GenBank database (National Center for Biotechnology Information) accession numbers of housekeeping gene sequences used for the phylogenetic studies, which were retrieved from the genome sequences generated as part of the current study.

| <i>Species</i>            | Gene/GenBank accession number |             |             |             |             |             |             |             |             |             |
|---------------------------|-------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                           | <i>cpr1</i>                   | <i>dpa1</i> | <i>dpd1</i> | <i>erg1</i> | <i>fas1</i> | <i>fas2</i> | <i>his3</i> | <i>lae1</i> | <i>lcb1</i> | <i>lcb2</i> |
| <i>T. albolutescens</i>   | OK513278                      | OK626232    | OL335883    | OK659798    | OK659817    | OK659836    | OL101211    | OL101268    | OL101230    | OL101249    |
| <i>T. aurantioeffusum</i> | OK513279                      | OK626251    | OL335884    | OK659799    | OK659818    | OK659837    | OL101212    | OL101269    | OL101231    | OL101250    |
| <i>T. balearicum</i>      | OK513280                      | OK626233    | OL335885    | OK659800    | OK659819    | OK659838    | OL101213    | OL101270    | OL101232    | OL101251    |
| <i>T. calamagrostidis</i> | OK392595                      | OK626234    | OL335886    | OK659801    | OK659820    | OK659839    | OL101214    | OL101271    | OL101233    | OL101252    |
| <i>T. crystalligenum</i>  | OK513281                      | OK626235    | OL335887    | OK659802    | OK659821    | OK659840    | OL101215    | OL101272    | OL101234    | OL101253    |
| <i>T. decipiens</i>       | OK513282                      | OK626236    | OL335888    | OK659803    | OK659822    | OK659841    | OL101216    | OL101273    | OL101235    | OL101254    |
| <i>T. deliquescens</i>    | OK513283                      | OK626237    | OL335889    | OK659804    | OK659823    | OK659842    | OL101217    | OL101274    | OL101236    | OL101255    |
| <i>T. cf. fertile</i>     | OK513284                      | OK626238    | OL335890    | OK659805    | OK659824    | OK659843    | OL101218    | OL101275    | OL101237    | OL101256    |
| <i>T. gamsii</i> T065     | OK513285                      | OK626239    | OL335891    | OK659806    | OK659825    | OK659844    | OL101219    | OL101276    | OL101238    | OL101257    |
| <i>T. margaretense</i>    | OK513286                      | OK626240    | OL335892    | OK659807    | OK659826    | OK659845    | OL101220    | OL101277    | OL101239    | OL101258    |
| <i>T. oligosporum</i>     | OK513277                      | OK626241    | OL355103    | OL343670    | OL343671    | OL343672    | OL343673    | OL343674    | OL343675    | OL343676    |
| <i>T. polysporum</i>      | OK513287                      | OK626242    | OL335893    | OK659808    | OK659827    | OK659846    | OL101221    | OL101278    | OL101240    | OL101259    |
| <i>T. protrudens</i>      | OK513288                      | OK626243    | OL335894    | OK659809    | OK659828    | OK659847    | OL101222    | OL101279    | OL101241    | OL101260    |
| <i>T. psychrophillum</i>  | OK513289                      | OK626244    | OL335895    | OK659810    | OK659829    | OK659848    | OL101223    | OL101280    | OL101242    | OL101261    |
| <i>T. rhododendri</i>     | OK513290                      | OK626245    | OL335896    | OK659811    | OK659830    | OK659849    | OL101224    | OL101281    | OL101243    | OL101262    |
| <i>T. rodmanii</i>        | OK513291                      | OK626246    | OL335897    | OK659812    | OK659831    | OK659850    | OL101225    | OL101282    | OL101244    | OL101263    |
| <i>T. rubi</i>            | OK513292                      | OK626247    | OL335898    | OK659813    | OK659832    | OK659851    | OL101226    | OL101283    | OL101245    | OL101264    |
| <i>T. stromaticum</i>     | OK513294                      | OK626248    | OL335899    | OK659814    | OK659833    | OK659852    | OL101227    | OL101284    | OL101246    | OL101265    |
| <i>T. taxi</i>            | OK413293                      | OK626249    | OL335900    | OK659815    | OK659834    | OK659853    | OL101228    | OL101285    | OL101247    | OL101266    |
| <i>T. turrialbense</i>    | OK513295                      | OK626250    | OL335901    | OK659816    | OK659835    | OK659854    | OL101229    | OL101286    | OL101248    | OL101267    |

| <i>Species</i>            | Gene/GeneBank accession number |             |             |             |             |             |             |             |             |             |
|---------------------------|--------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                           | <i>mcm7</i>                    | <i>pgk1</i> | <i>rpb1</i> | <i>rpb2</i> | <i>sph1</i> | <i>top1</i> | <i>tps1</i> | <i>tsr1</i> | <i>tub1</i> | <i>ubt1</i> |
| <i>T. albolutescens</i>   | OK813947                       | OK813928    | OK813909    | OK813890    | OK656756    | OK656737    | OK656718    | OK631850    | OK587421    | OK587402    |
| <i>T. aurantioeffusum</i> | OK813948                       | OK813929    | OK813910    | OK813891    | OK656757    | OK656738    | OK656719    | OK631851    | OK587422    | OK587403    |
| <i>T. balearicum</i>      | OK813949                       | OK813930    | OK813911    | OK813892    | OK656758    | OK656739    | OK656720    | OK631852    | OK587423    | OK587404    |
| <i>T. calamagrostidis</i> | OK813950                       | OK813931    | OK813912    | OK813893    | OK656759    | OK656740    | OK656721    | OK631853    | OK587424    | OK587405    |
| <i>T. crystalligenum</i>  | OK813951                       | OK813932    | OK813913    | OK813894    | OK656760    | OK656741    | OK656722    | OK631854    | OK587425    | OK587406    |
| <i>T. decipiens</i>       | OK813952                       | OK813933    | OK813914    | OK813895    | OK656761    | OK656742    | OK656723    | OK631855    | OK587426    | OK587407    |

|                          |          |          |          |          |          |          |          |          |          |          |
|--------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| <i>T. deliquescens</i>   | OK813953 | OK813934 | OK813915 | OK813896 | OK656762 | OK656743 | OK656724 | OK631856 | OK587427 | OK587408 |
| <i>T. cf. fertile</i>    | OK813954 | OK813935 | OK813916 | OK813897 | OK656763 | OK656744 | OK656725 | OK631857 | OK587428 | OK587409 |
| <i>T. gamsii</i> T065    | OK813955 | OK813936 | OK813917 | OK813898 | OK656764 | OK656745 | OK656726 | OK631858 | OK587429 | OK587410 |
| <i>T. margaretense</i>   | OK813956 | OK813937 | OK813918 | OK813899 | OK656765 | OK656746 | OK656727 | OK631859 | OK587430 | OK587411 |
| <i>T. oligosporum</i>    | OL335905 | OL335906 | OL335907 | OL335908 | OL335902 | OL335903 | OL335904 | OK631849 | OK587440 | OK504622 |
| <i>T. polysporum</i>     | OK813957 | OK813938 | OK813919 | OK813900 | OK656766 | OK656747 | OK656728 | OK631860 | OK587431 | OK587412 |
| <i>T. protrudens</i>     | OK813958 | OK813939 | OK813920 | OK813901 | OK656767 | OK656748 | OK656729 | OK631861 | OK587432 | OK587413 |
| <i>T. psychrophillum</i> | OK813959 | OK813940 | OK813921 | OK813902 | OK656768 | OK656749 | OK656730 | OK631862 | OK587433 | OK587414 |
| <i>T. rhododendri</i>    | OK813960 | OK813941 | OK813922 | OK813903 | OK656769 | OK656750 | OK656731 | OK631863 | OK587434 | OK587415 |
| <i>T. rodmanii</i>       | OK813961 | OK813942 | OK813923 | OK813904 | OK656770 | OK656751 | OK656732 | OK631864 | OK587435 | OK587416 |
| <i>T. rubi</i>           | OK813962 | OK813943 | OK813924 | OK813905 | OK656771 | OK656752 | OK656733 | OK631865 | OK587436 | OK587417 |
| <i>T. stromaticum</i>    | OK813963 | OK813944 | OK813925 | OK813906 | OK656772 | OK656753 | OK656734 | OK631866 | OK587437 | OK587418 |
| <i>T. taxi</i>           | OK813964 | OK813945 | OK813926 | OK813907 | OK656773 | OK656754 | OK656735 | OK631867 | OK587438 | OK587419 |
| <i>T. turrialbense</i>   | OK813965 | OK813946 | OK813927 | OK813908 | OK656774 | OK656755 | OK656736 | OK631868 | OK587439 | OK587420 |



**Table S4b.** GenBank database (National Center for Biotechnology Information) accession numbers of sequences generated as part of the current study, corresponding to *tri5*- and *tri5*-related regions.

| <i>Species</i>            | <i>Region*</i>                 | <i>Accession number</i> |
|---------------------------|--------------------------------|-------------------------|
| <i>T. turrialbense</i>    | <i>tri5</i> region             | MZ926819                |
| <i>T. protrudens</i>      | <i>tri5</i> region             | MZ926820                |
| <i>T. margaretense</i>    | <i>tri5</i> region             | MZ926821                |
| <i>T. rodmanii</i>        | <i>tri5</i> region             | MZ968859                |
| <i>T. aurantioeffusum</i> | <i>tri5</i> region             | MZ968994                |
| <i>T. gamsii</i> T065     | dbp3 region**                  | OK037079                |
|                           | <i>tri5</i> region             | OK037075                |
|                           | <i>urea carboxylase</i> region | OK037076                |
| <i>T. polysporum</i>      | dbp3 region**                  | OK094918                |
|                           | <i>tri5</i> region             | OK094919                |
|                           | <i>urea carboxylase</i> region | OK094920                |
| <i>T. rubi</i>            | <i>tri5</i> region             | OK043671                |
| <i>T. taxi</i>            | <i>tri5</i> region             | MZ825154                |
| <i>T. balearicum</i>      | <i>tri5</i> region             | OK037078                |
| <i>T. crystalligenum</i>  | <i>tri5</i> region             | OK094924                |
| <i>T. oligosporum</i>     | <i>tri5</i> region             | OK094926                |
| <i>T. calamagrostidis</i> | <i>tri5</i> region             | MZ869853                |
| <i>T. psychrophilum</i>   | <i>tri5</i> region             | OK094925                |
| <i>T. rhododendri</i>     | <i>tri5</i> region             | OK094923                |
| <i>T. decipiens</i>       | <i>tri5</i> region             | OK043672                |
| <i>T. deliquescens</i>    | dbp3 region                    | OK094921                |
| <i>T. albolutescens</i>   | <i>tri5</i> region             | OK037077                |
| <i>T. cf. fertile</i>     | <i>tri5</i> region             | OK094922                |
| <i>T. stromaticum</i>     | dbp3 region**                  | OK298475                |
|                           | <i>tri5</i> region             | OK298474                |

\*Note that the genomic regions whose sequences were submitted to the GenBank corresponded to those illustrated in Fig. 2.

\*\* dbp3 region- region containing the ATP-dependent RNA helicase encoding gene, corresponding to the genomic region where *tri5* gene is located in those *Trichoderma* trichothecene producer strains (=GR1 in Fig 2).

**Table S5.** RNAseq analysis of expression of genes located in the *tri5* genomic region of *T. arundinaceum*\*.

| Gene number**                   | Blastp highest similarity             | TPM TARUN<br>vs<br>TPM $\Delta tri6$<br>24h | Correspondence<br>with genes in<br>Fig. 2** | TPM<br>TARUN<br>24h | TPM<br>$\Delta tri6$<br>24h |
|---------------------------------|---------------------------------------|---------------------------------------------|---------------------------------------------|---------------------|-----------------------------|
| TARUN_2540                      | ATP-dependent RNA helicase            | 0.88                                        | Red arrow                                   | 23.7                | 26.87                       |
| TARUN_2541                      | oligosaccharyl-lipid flippase         | 0.94                                        | Pink arrow                                  | 29.9                | 31.89                       |
| <b>TARUN_2542 (<i>tri5</i>)</b> | <b>Trichodiene synthase</b>           | <b>53.14</b>                                | <b>Green arrow</b>                          | <b>53.14</b>        | <b>1</b>                    |
| TARUN_2543                      | Cytochrome P450 monooxygenase         | 1.92                                        | Yellow arrow                                | 17.9                | 9.3                         |
| TARUN_2544                      | AraC-type transcriptional regulator   | 1.00                                        | Blue arrow                                  | 1                   | 1                           |
| TARUN_2545                      | Fucose-specific lectin                | 0.24                                        | 5                                           | 2.26                | 9.4                         |
| TARUN_2546                      | Homoserine acetyltransferase          | 4.26                                        | Purple arrow                                | 4.26                | 1                           |
| TARUN_2547                      | WD40-repeat-containing domain protein | 0.80                                        | 4                                           | 105.36              | 132.34                      |
| TARUN_2548                      | Major facilitator superfamily (MFS)   | 0.40                                        | Orange arrow                                | 0.4                 | 1                           |
| TARUN_2549                      | duf1446 domain-containing protein     | 0.64                                        | 3                                           | 1.53                | 2.39                        |
| TARUN_2550                      | Chromo domain-containing protein      | 0.68                                        | 2                                           | 28.96               | 42.48                       |
| TARUN_2551                      | Peptidase m20 domain protein          | 2.57                                        | 1                                           | 8.06                | 3.14                        |
| TARUN_5502***                   | Actin beta gamma 1                    | 0.99                                        | -                                           | 660.77              | 663.62                      |

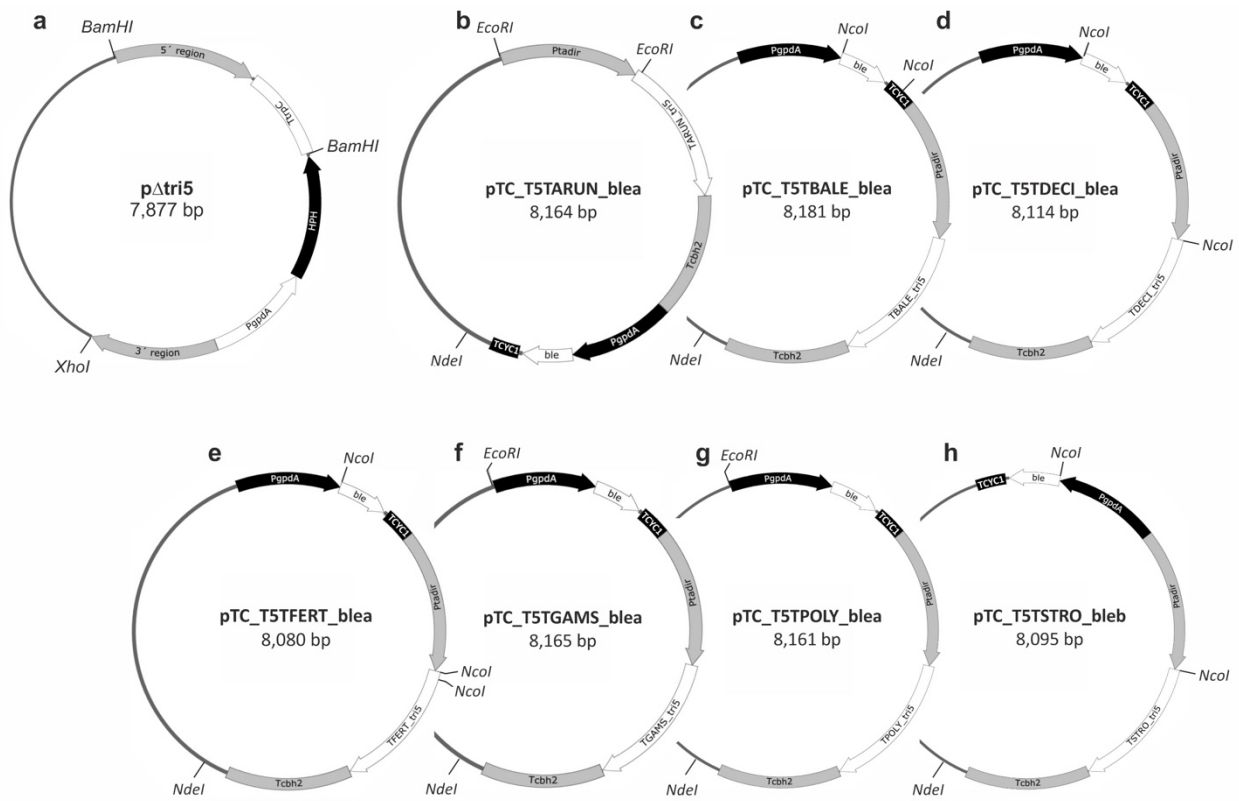
\* Gene expression values were determined by comparison of expression levels found in *T. arundinaceum* wild type (TARUN) versus those found in the  $\Delta tri6.66$ , which is a mutant deleted in the *tri6* gene that encodes a positive regulator of genes involved in trichothecene biosynthesis (Lindo et al., 2018).

\*\*Genes included in this table correspond with those illustrated in Fig. 2 for the *tri5* genomic region of *T. arundinaceum*.

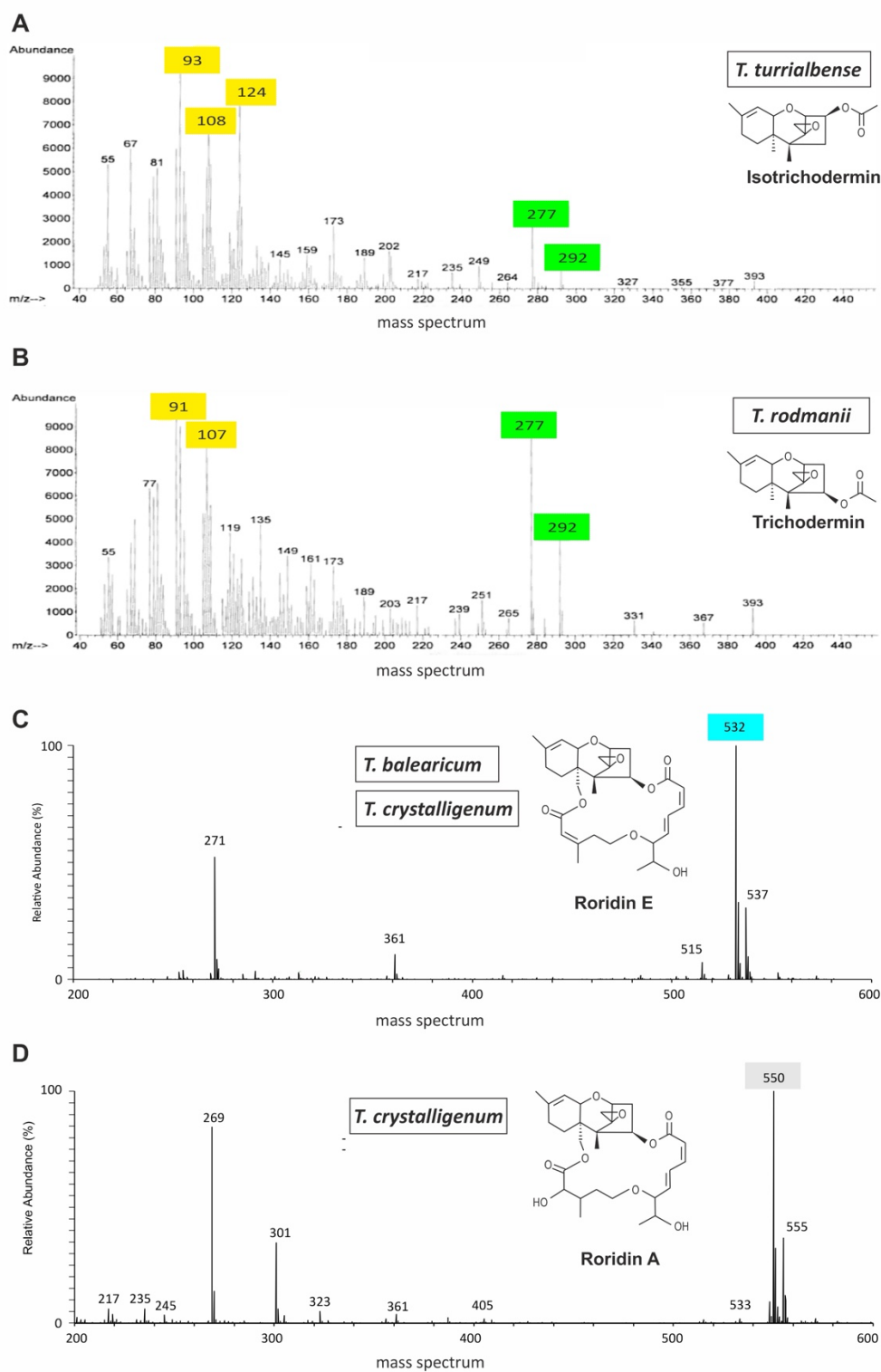
\*\*\*Values for Actin beta gamma 1 encoding gene were shaded in yellow, since they were used to normalize the gene expression data, but this gene is not located in the *tri5* genomic region.

TPM= Transcripts per million of reads

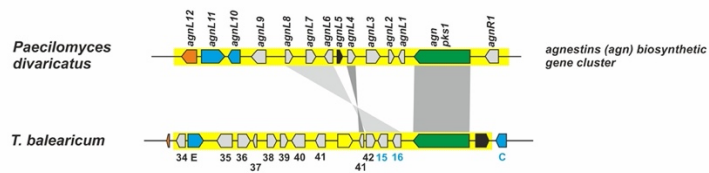
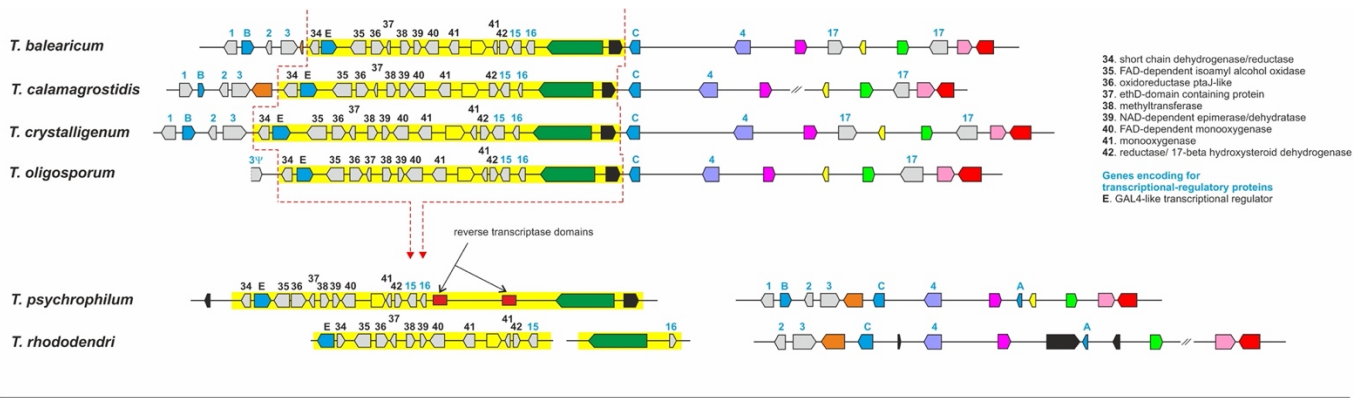
Lindo, L., McCormick, S.P., Cardoza, R.E., Brown, D.W., Kim, H.-S., Alexander, N.J., Proctor, R.H., Gutiérrez, S. (2018). Effect of deletion of a trichothecene toxin regulatory gene on the secondary metabolism transcriptome of the saprotrophic fungus *Trichoderma arundinaceum*. *Fungal Genet. Biol.* 119, 29-46. doi: 10-1016/j.fgb.2018.08.002



**Figure S1.** Plasmids used for *T. arundinaceum* *tri5* deletion (p $\Delta$ tri5) or for *tri5* gene overexpression (pTC\_T5#) studies. TARUN= *T. arundinaceum*, TBALE= *T. balearicum*, TDECI= *T. decipiens*, TFERT= *T. cf. fertile*, TGAMS= *T. gamsii* T065, TPOLY= *T. polysporum*, TSTRO= *T. stromaticum*.



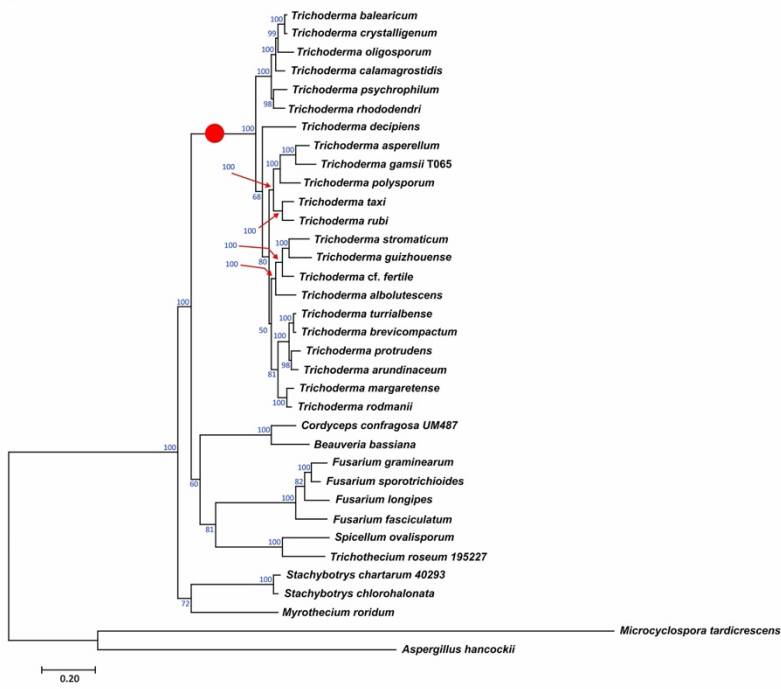
**Figure S2.** Total mass spectra of isotrichodermin (**A**), trichodermin (**B**); roridin E (**C**), and roridin A (**D**). Green rectangles in panels A and B correspond to common  $m/z$  peaks to isotrichodermin and trichodermin, while yellow rectangles indicated the differential  $m/z$  peaks between these two compounds. Blue and gray rectangles in panels C and D correspond to 532 and 550 ions for roridin E and roridin A, respectively, with an ammonium adduct.



**Figure S3. Upper panel.** Analysis of genes found in the *tri5* genomic region in species of the *Psychrophila* lineage. Those genes shaded in yellow were only found in this clade among those *Trichoderma* clades included in the present study, among all the 35 species analyzed in the present work, and shown a significant degree of syntenicity with the *Paecilomyces divaricatus* agnestins biosynthetic cluster (**Lower panel**) (Szwalbe et al., 2018).

Szwalbe, A.J., Williams, K., Song, Z., de Mattos-Shiple, K., Vincent, J.L., Bailey, A.M., Willis, C.L., Cox, R.J., Simpson, T.J. (2018). Characterisation of the biosynthetic pathway to agnestins A and B reveals the reductive route to chrysophanol in fungi. *Chem. Sci.* 10, 233-238. doi: 10.1030/cBsc03778g

dpa1



fas1

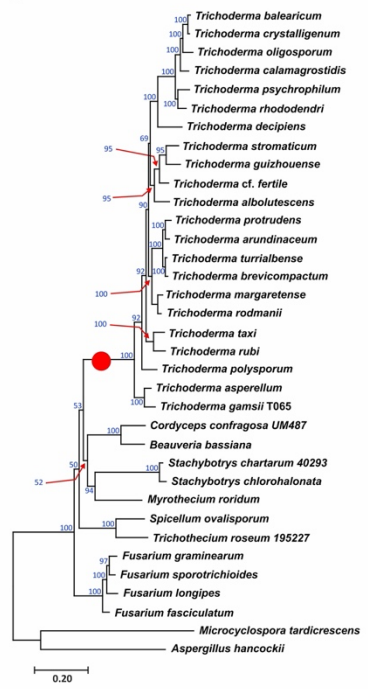
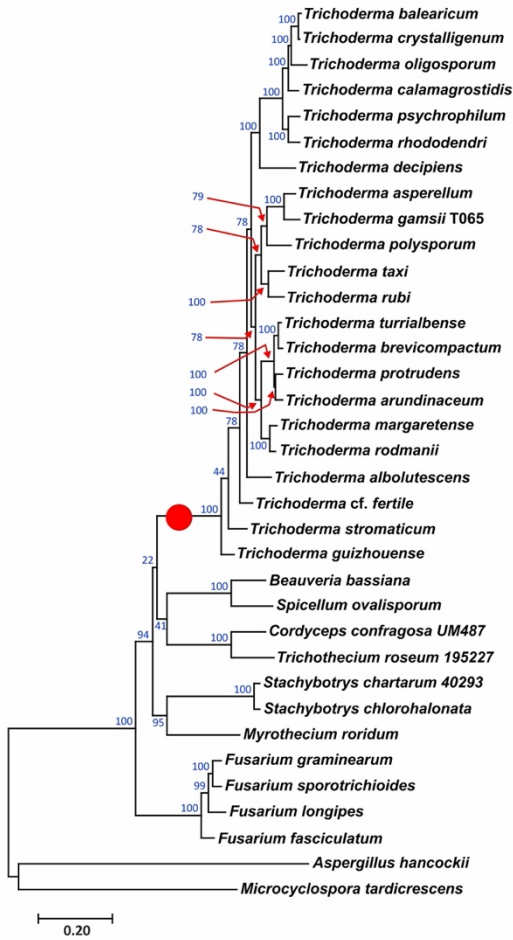


Figure S4. (Cont.)

fas2



lcb2

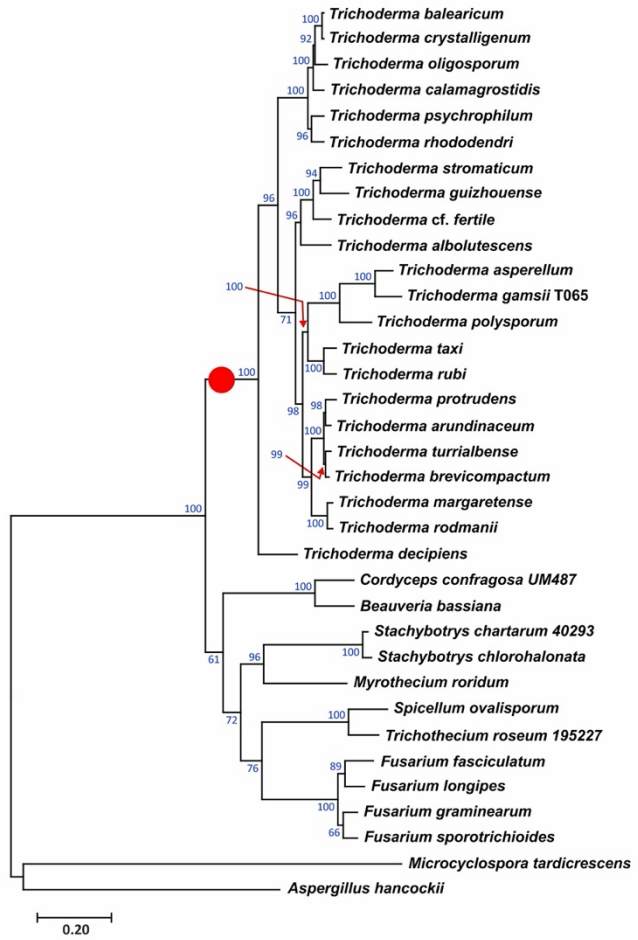
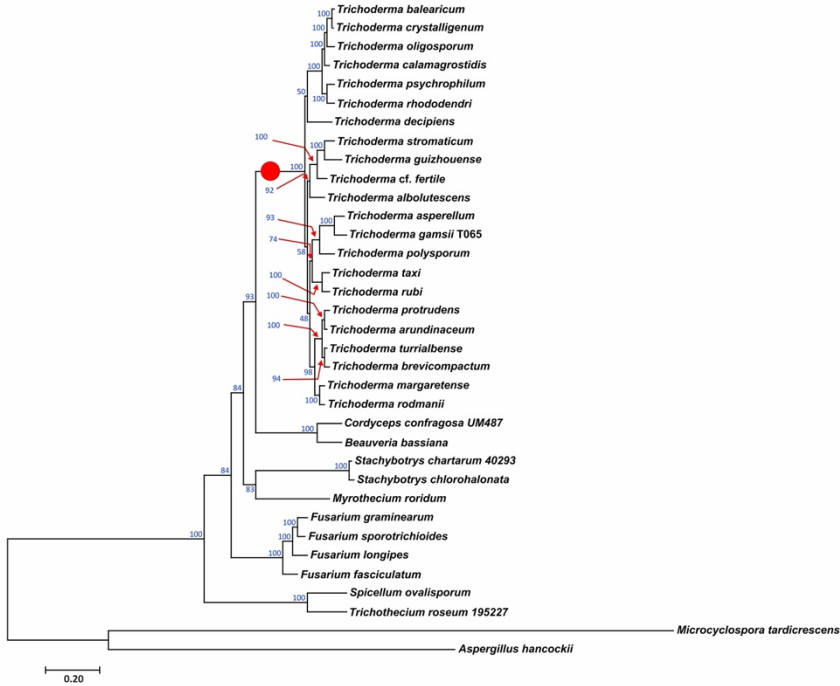
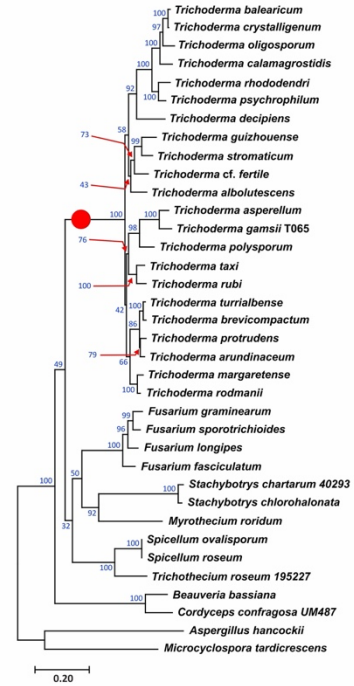


Figure S4. (Cont.)

*rpb1*

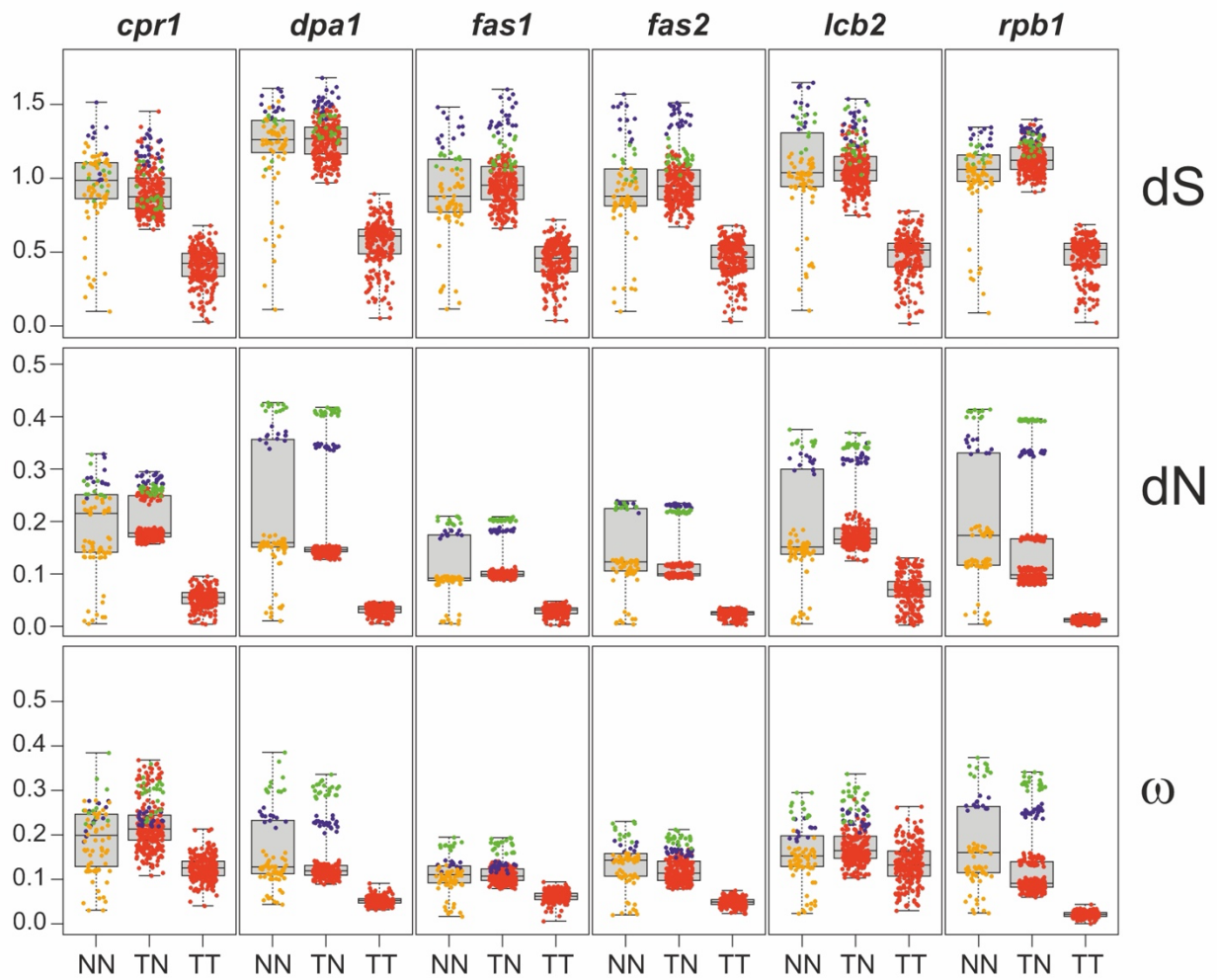


*cpr1*

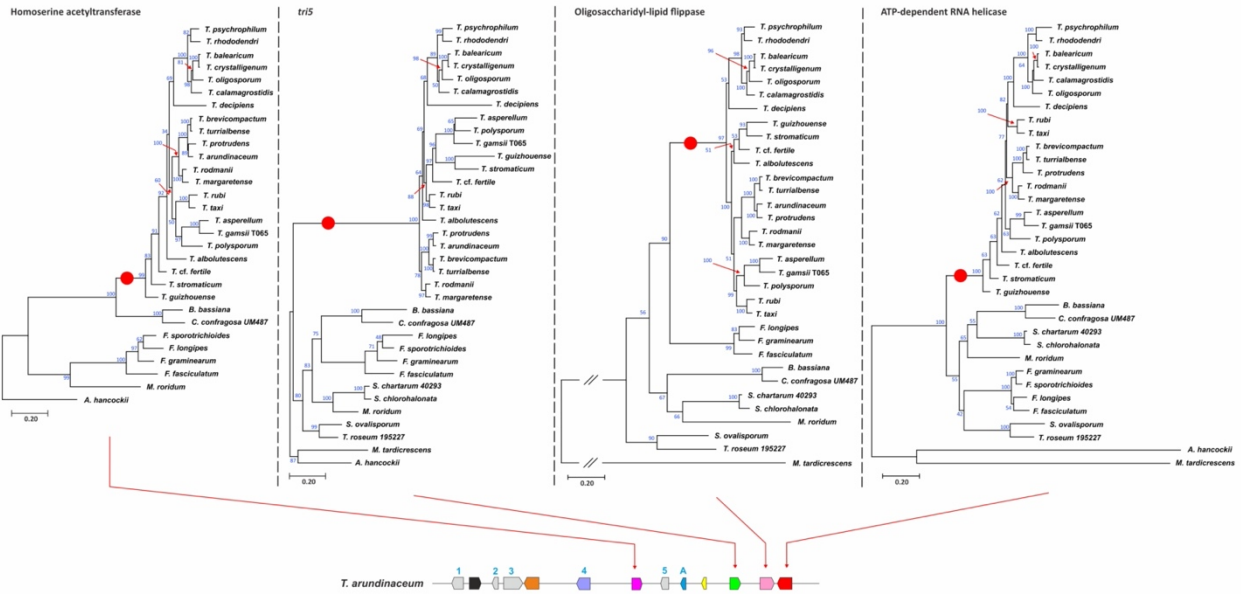


**Figure S4.** Individual ML Phylogenetic tree of the six genes (*cpr1*, *dpa1*, *fas1*, *fas2*, *lcb2*, and *rpb1*) used for *tri5* phylogenetic analysis, including all *Trichoderma* species studied in this work and other *tri5* gene-containing fungi. For details regarding the procedures used in the generation of these trees see legend to Fig. 4. The red circle denotes the connecting point between *Trichoderma* species and the other non-*Trichoderma* fungi for each of the genes analyzed.

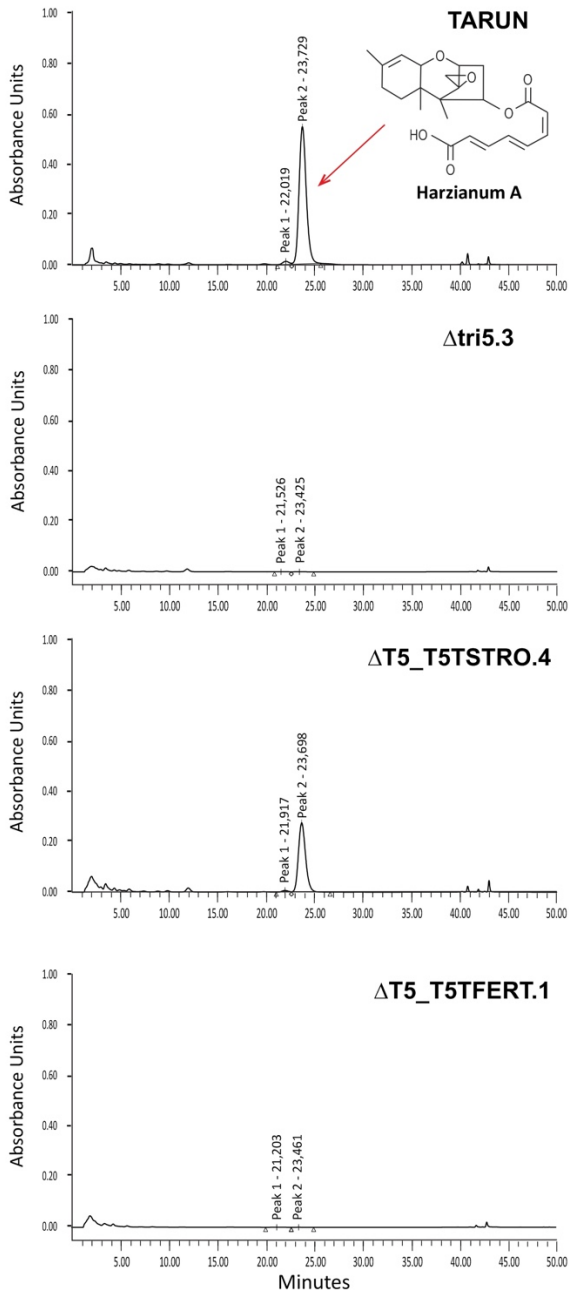




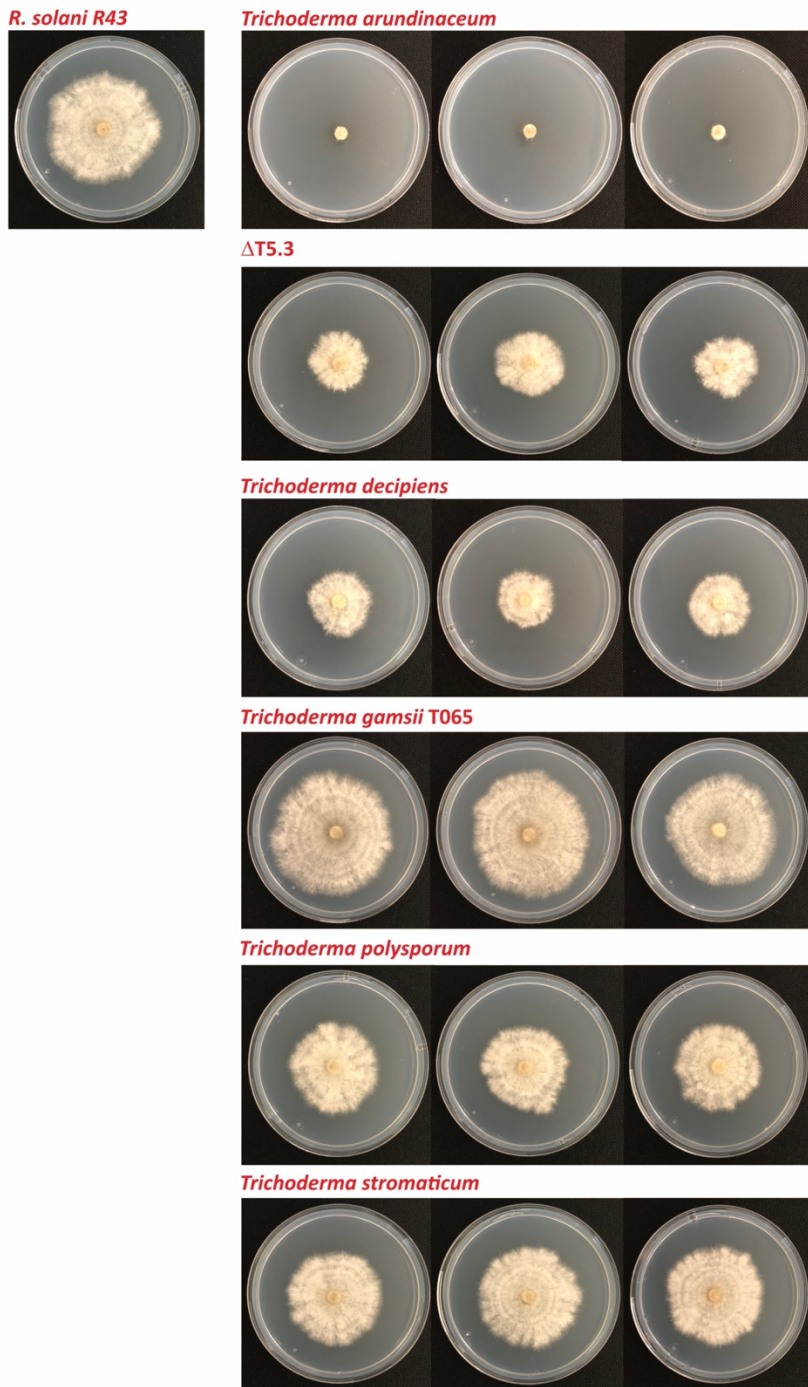
**Figure S5.** Boxplots and scatterplots of the distances between sequence pairs. The pairs analyzed were between non-*Trichoderma* (NN), *Trichoderma* and non-*Trichoderma* (TN), and *Trichoderma* (TT) genes. The distances of synonymous changes per synonymous site (**dS**); non-synonymous changes per non-synonymous site (**dN**), and their rates ( $\omega$ ) are included. Six housekeeping (HK) genes (*cpr1*, *dpa1*, *fas1*, *fas2*, *lcb2*, and *rpb1*) (Table S2) were considered in this analysis. Green and blue points in the scatterplots represent those that involve *Aspergillus hancockii* or *Microcyclospora tardicrescens* sequences, respectively, while red points correspond to comparisons between all the other sequences used in this study. As it can be observed, the dN, dS, and  $\omega$  pattern is similar in all HK genes for all comparisons.



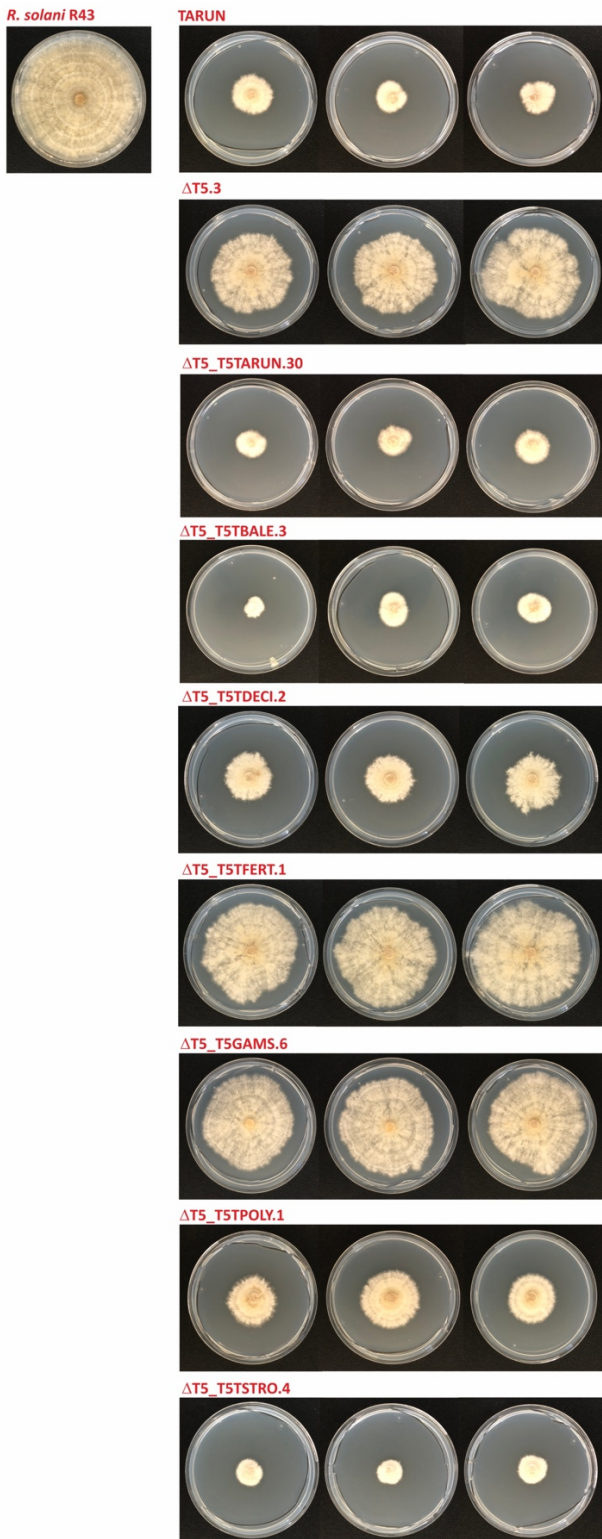
**Figure S6.** ML Phylogenetic trees of (left to right) the homoserine acetyltransferase, *TRI5*, oligosaccharidyl-lipid flippase and the ATP-dependent RNA helicase encoding genes located in the *tri5* gene region (see lower panel), including all *Trichoderma* species studied in this work and other *tri5* gene-containing fungi. For details regarding the procedures used in the generation of these trees see legend to Fig. 4. *tri5* tree has been included in the present Figure only for comparative purposes. Note the long *Trichoderma* branch in the *tri5* tree compared to the other trees.



**Figure S7.** HPLC chromatograms showing production of harzianum A (HA) by *T. arundinaceum* (TARUN) and  $\Delta T5\_T5TSTRO.4$ , a  $\Delta tri5.3$  complemented transformant with the plasmid overexpressing *T. stromaticum tri5* gene, and the lack of HA production in  $\Delta tri5.3$  mutant, and in  $\Delta T5\_T5TFERT.1$ , a transformant of  $\Delta tri5.3$  with plasmid overexpressing *T. cf. fertile tri5*.



**Figure S8.** Cellophane membrane assays to analyze the antifungal activity against the fungal phytopathogen *Rhizoctonia solani* R43 of *T. arundinaceum* wild type (TARUN), mutant  $\Delta tri5.3$  ( $\Delta T5.3$ ), *T. decipiens*, *T. gamsii* T065, *T. polysporum* and *T. stromaticum*. Note: In this experiment plates were incubated for 5 days after the placement of the pathogen plug.



**Figure S9.** Cellophane membrane assays to analyze the antifungal activity against the fungal phytopathogen *Rhizoctonia solani* R43 of *T. arundinaceum* wild-type (TARUN), mutant  $\Delta tri5.3$  ( $\Delta T5.3$ ) and transformants of the latter with plasmids overexpressing *tri5* gene from *T. arundinaceum* ( $\Delta T5\_T5STARUN\#$ ), *T. balearicum* ( $\Delta T5\_T5TBAL.\#$ ), *T. decipiens* ( $\Delta T5\_T5TDECI.\#$ ), *T. cf. fertile* ( $\Delta T5\_T5TFERT.\#$ ), *T. gamsii* T065 ( $\Delta T5\_T5TGAMS.\#$ ), *T. polysporum* ( $\Delta T5\_T5TPOLY.\#$ ), and *T. stromaticum* ( $\Delta T5\_T5TSTRO.\#$ ). Note: In this experiment plates were incubated for 8 days after the placement of the pathogen plug.

