Table S1 Genomes of *Trichoderma* spp. used in the present *in silico* work

Strains[a]	**Genome ID**[b]	**References**
Trichoderma cf. *afroharzianum* T22	(1185335)	-
T. arundinaceum IBT 40837	GCA_003012105.1	(1)
T. asperellum B05	GCA_000733085.2	(2)
T. asperellum CBS 433.97	GCA_003025105.1	(3)
T. atrobrunneum ITEM 908	GCA_003439915.1	(2)
T. atroviride B10	(1185343)	-
T. atroviride IMI 206040	GCA_000171015.2	(4)
T. atroviride JCM 9410	GCA_001599035.1	(2)
T. cf. *atroviride* LY357	GCA_002916895.1	-
T. atroviride XS2015	GCA_000963795.1	(5)
T. brevicompactum IBT 40841	GCA_003012085.1	(1)
T. citrinoviride TUCIM 6016	GCA_003025115.1	(3)
T. gamsii A5MH	GCA_002894205.1	-
T. gamsii T6085	GCA_001481775.2	(6)
T. guizhouense NJAU 4742	GCA_002022785.1	(3)
T. hamatum GD12	GCA_000331835.2	(7)
T. harzianum B97	GCA_001990665.1	(8)
T. harzianum CBS 226.95	GCA_003025095.1	(3)
T. cf. *harzianum* M10	(1185333)	-
T. cf. *afroharzianum* T6776	GCA_000988865.1	(9)
T. cf. *pleuroti* Tr1	GCA_002894145.1	-
T. harzianum TR274	GCA_002838845.1	(10)
T. cf. *longibrachiatum* JCM 1883	GCA_001950475.1	(2)
T. koningiopsis POS7	GCA_002246955.1	(11)
T. longibrachiatum ATCC 18648	GCA_003025155.1	(3)
T. longibrachiatum MK1	(1185339)	-

T. longibrachiatum SMF2	GCA_000332775.1	(12)
T. parareesei CBS 125925	GCA_001050175.1	(13, 14)
T. pleuroti TPhu1	GCA_001721665.1	(2)
T. reesei CBS 999.97	GCA_001999515.1	(15)
T. reesei QM6a	GCA_000167675.2	(16)
T. reesei SS-II	GCA_004762065.1	(17)
T. cf. *harzianum* IMV 00454	GCA_001931985.1	(2)
T. virens FT-333	GCA_000800515.1	(2)
T. virens Gv29-8	GCA_000170995.2	(4, 14)
T. cf. *virens* IMI 304061	GCA_001835465.1	(18)
T. cf. *virens* Tv-1511	GCA_007896495.1	-

[a]The following strains including *T. harzianum* T22, *T. atroviride* LY357, *T. harzianum* T6776, *T. harzianum* M10, *T. harzianum* Tr1, *T. koningii* JCM 1883, *T.* sp. IMV 00454, *T. virens* IMI 304061 and *T. viride* Tv-1511 were manually updated for the putative right species identification based on the multilocus phylogram of *Trichoderma* spp. (Cai et al., unpublished data). [b]IDs of the genomes deposited in the Joint Genome Institute (JGI) database were bracketed, otherwise were given as in the National Centre for Biotechnology Information (NCBI) database.

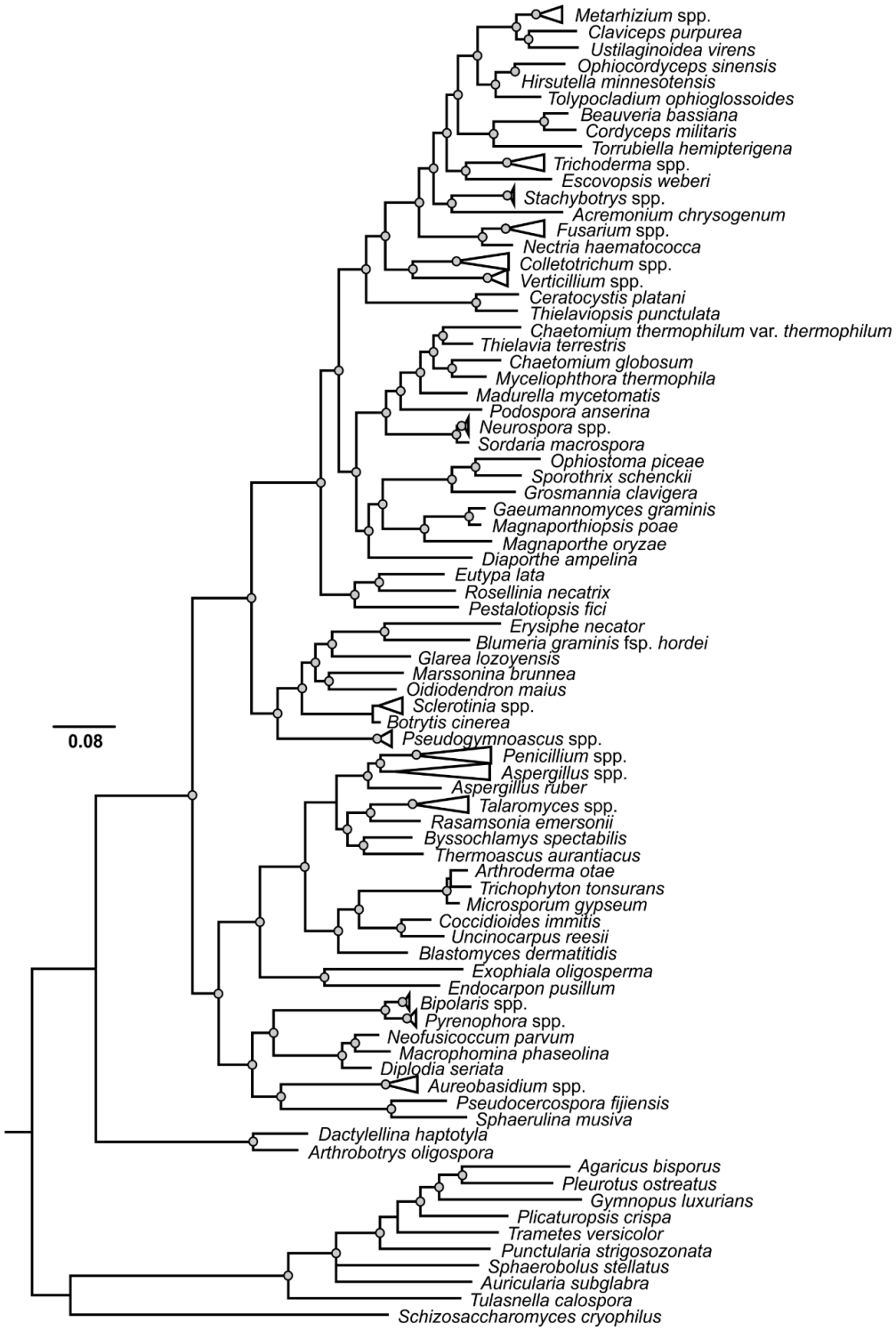
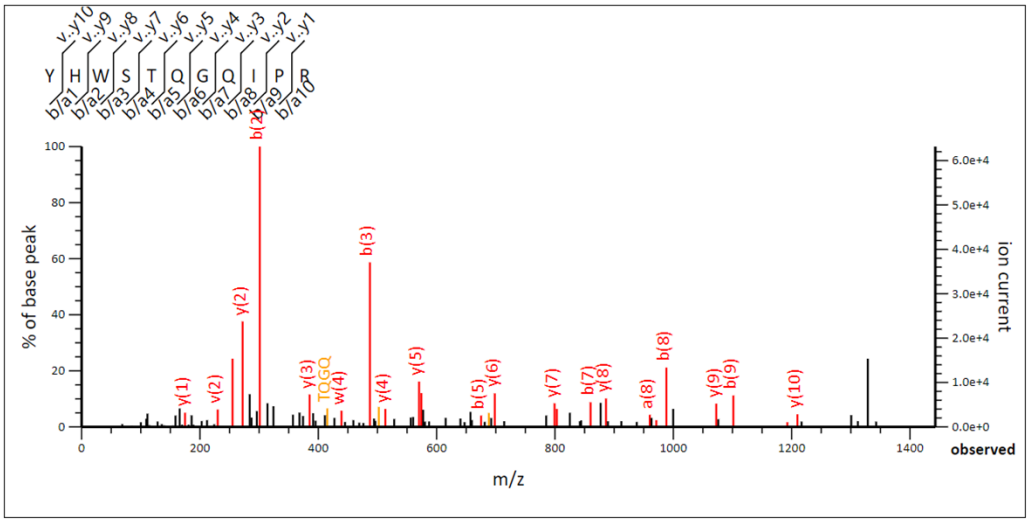


Fig. S1 Multilocus maximum likelihood (ML) phylogram of Hypocreales and the representative Dikarya fungi. Genera containing multiple species were collapsed and represented by the genus name. The ML species tree was constructed by IQ-TREE 1.6.12 (N=1000) based on the concatenated alignment of four nuclear genes (histone acetyltransferase subunit of the RNA polymerase II holoenzyme, FG533; NAD-dependent glutamate dehydrogenase, FG570;

translation initiation factor eIF-5, FG832; and TSR1p, a protein required for processing of 20S pre-rRNA, MS277) described in Druzhinina et al. (3). Circles above nodes indicate the IQ-TREE ultrafast bootstrap values >95.

A



B

>OPB44018

MQLSSLFKLALFTAAVSADTVSYDTGYDDGSRSLNVVSCSDGPNGLETRYHWSTQGQIPRFPYIGGVQ
AVAGWNSASCGTCWKLSYSGHTIYVLAVDHAAAGFNIALDAMNALTGGQAVALGRVSATASQVAVK
NCGL

Fig. S2 The representative MS/MS fragmentation (**A**) of the most prominent tryptic peptide originated from the sample band (see Fig. 1) and its matched amino acid sequence (**B**) on EPL1 (GenBank: OPB44018) of *T. guizhouense* NJAU 4742 (3, 14). The matched peptide is shown in red. The signal peptide predicted by SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/) is underlined.

File S1 Data regarding CPs of *T. harzianum* generated in this study

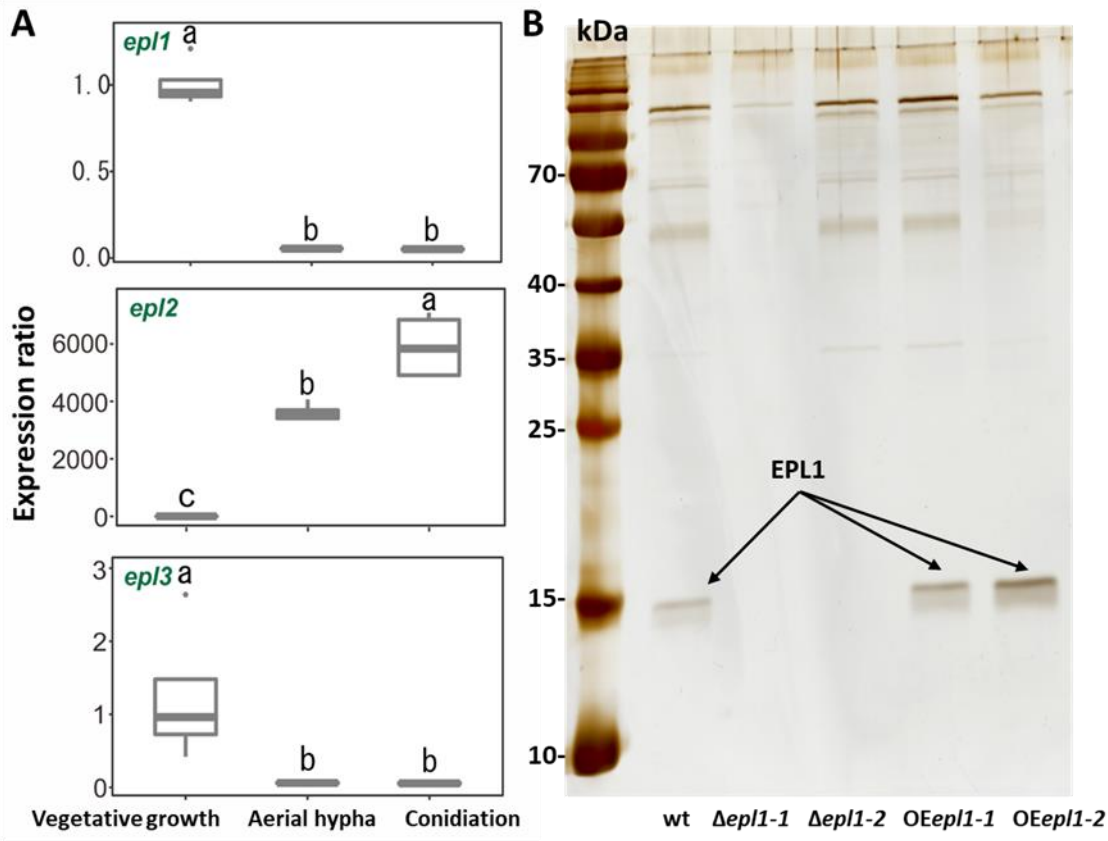


Figure S1.1 Transcriptional determination of CPs in *T. harzianum* CBS 226.95 and proteomic assay for its *epl1* mutants. **A**, expression dynamics of *epl*s at the three developmental stages of the fungus: submerged vegetative growth, aerial hypha formation, and conidiation; **B**, silver stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the culture filtrates of *T. harzianum* strains cultivated in 30% MS medium (including 1% glucose, MSG) at 25 ℃ (170 rpm) for 72 h. wt, represents the wild type strain of *T. harzianum* CBS 226.95; Δ*epl1*, represents *epl1*-deleted mutants; OE*epl1*, represents mutants overexpressing *epl1*. PageRuler™ Prestained Protein Ladder (Fermentas, USA) was used in the gel electrophoresis.

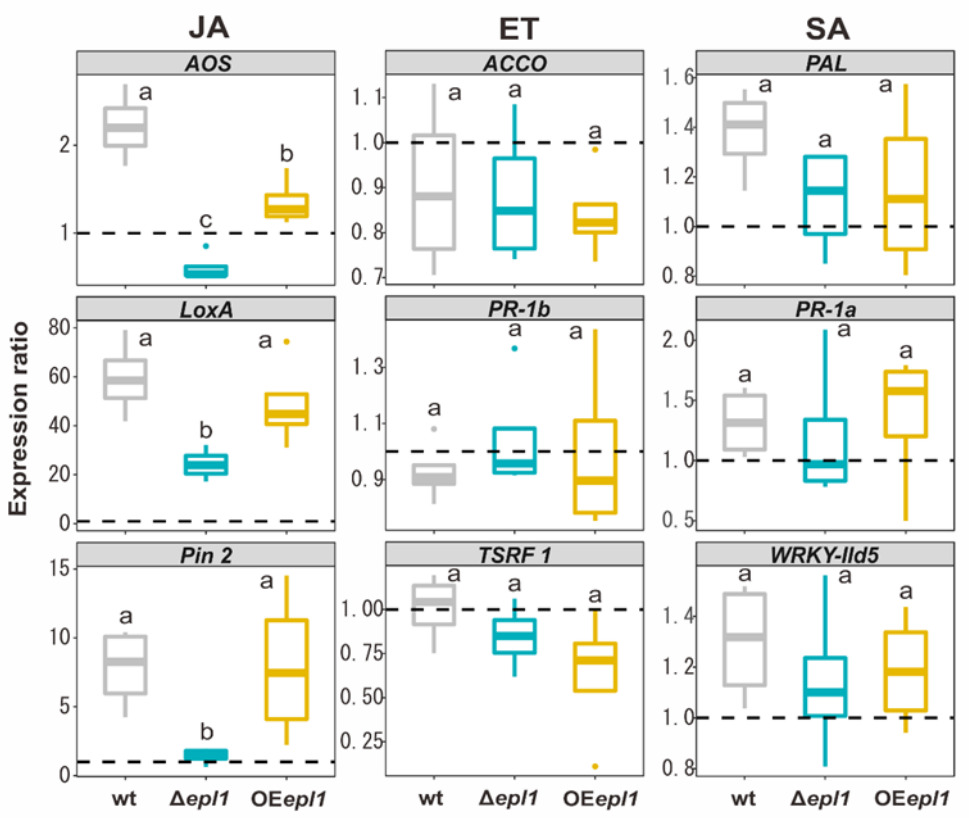


Figure S1.2 Immune response of tomato seedlings to *T. harzianum* colonization. JA, jasmonic acid-mediated signaling pathway; ET, ethylene-mediated signaling pathway; and SA, salicylic acid-mediated signaling pathway. Expression ratio of the immune defense genes is the fold change calculated using the $2^{-\Delta\Delta Ct}$ method (n= 12) relative to the control sample that was not colonized by *T. harzianum* CBS 226.95. PGK gene was used as the internal housekeeping gene. The dashed line represents the expression rate of the corresponding gene in the control samples grown *T. harzianum*. Boxes without a same letter indicate a significant difference at p <0.05 (Tukey multiple comparison test).

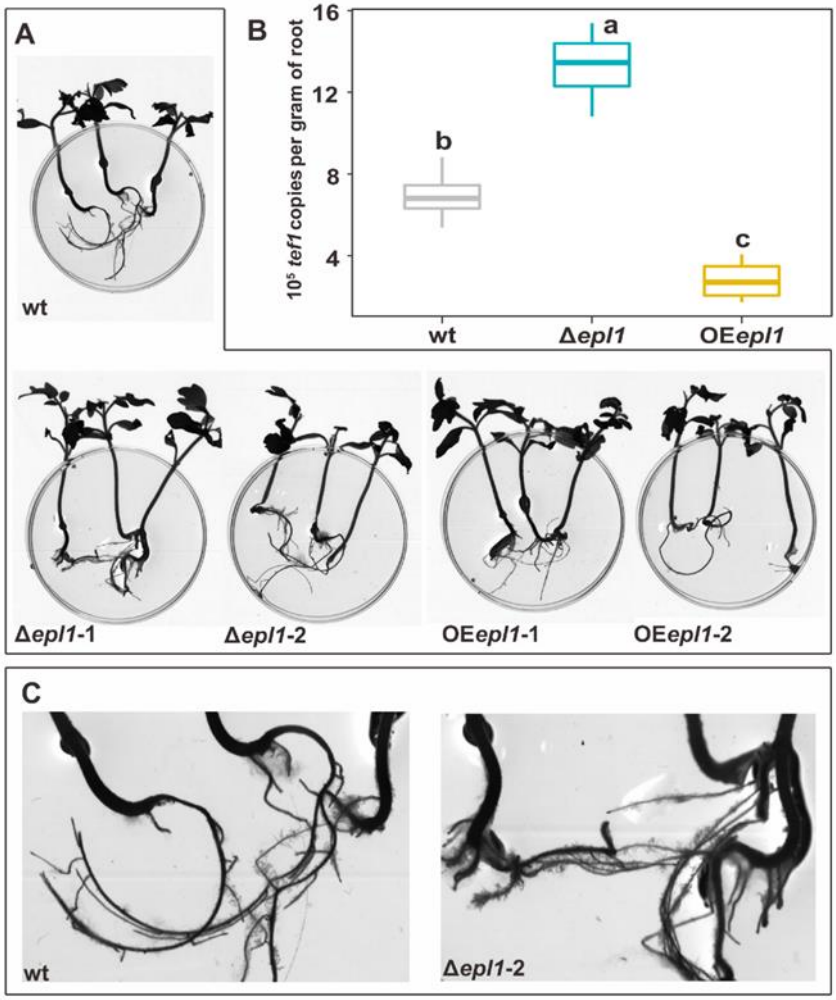


Figure S1.3 Quantitative determination of *T. harzianum* colonization on tomato roots. **A**, scanned images of tomato seedlings colonized by *T. harzianum*. Diameter of Petri plate is 6 cm. **B**, a box plot showing the quantification of *T. harzianum tef1* gene copies per gram of root. The values were obtained by RT-qPCR with isolated RNA from the roots (n≥ 4). Boxes without a same letter indicate a significant difference at p <0.05 (Tukey multiple comparison test). **C**, a magnified field of the roots colonized by the wild type strain of *T. harzianum* CBS 226.95 (left) and its *epl1*-deleted mutant (right).

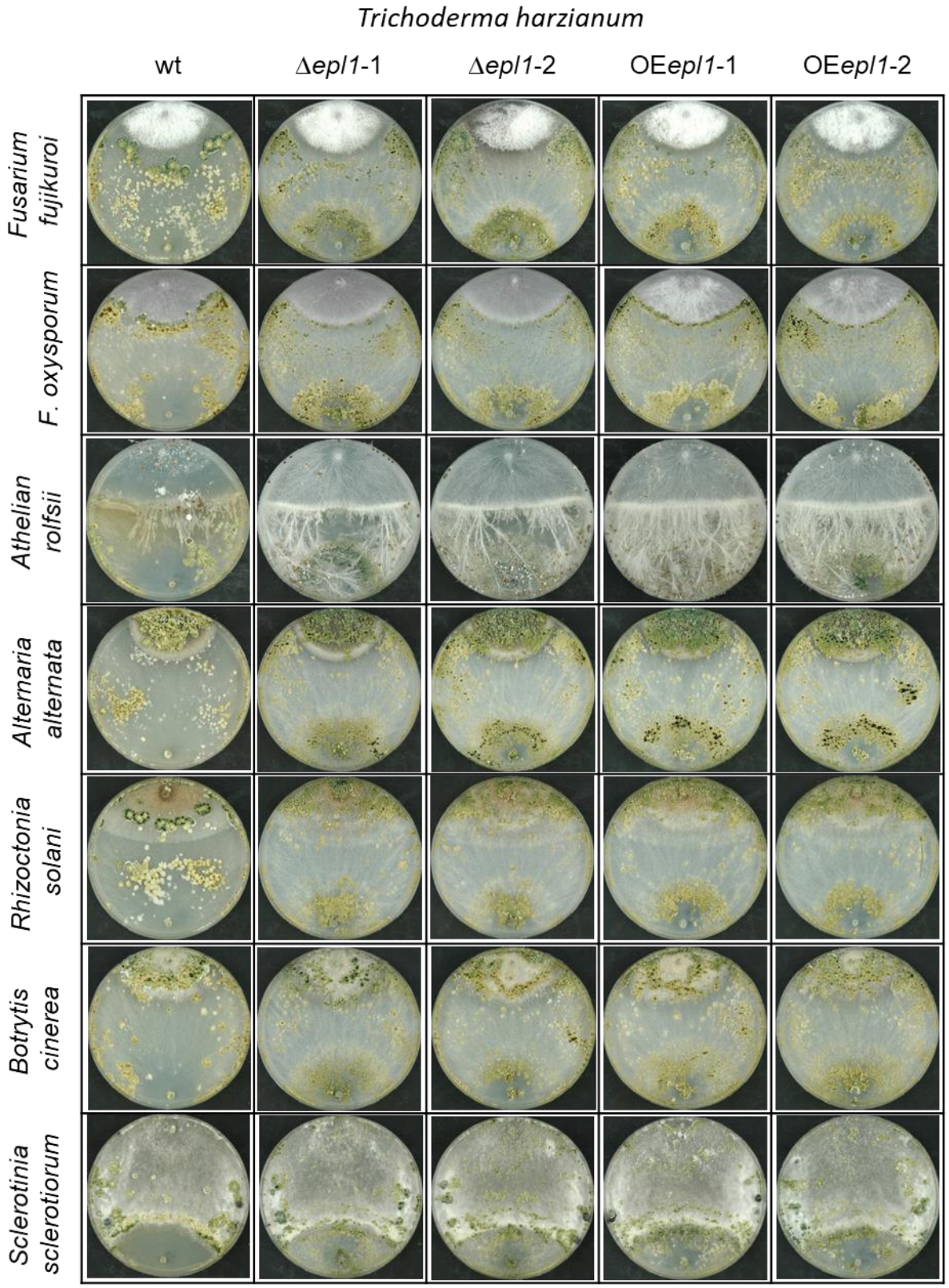


Figure S1.4 Dual confrontation assay between *T. harzianum* and other fungi.

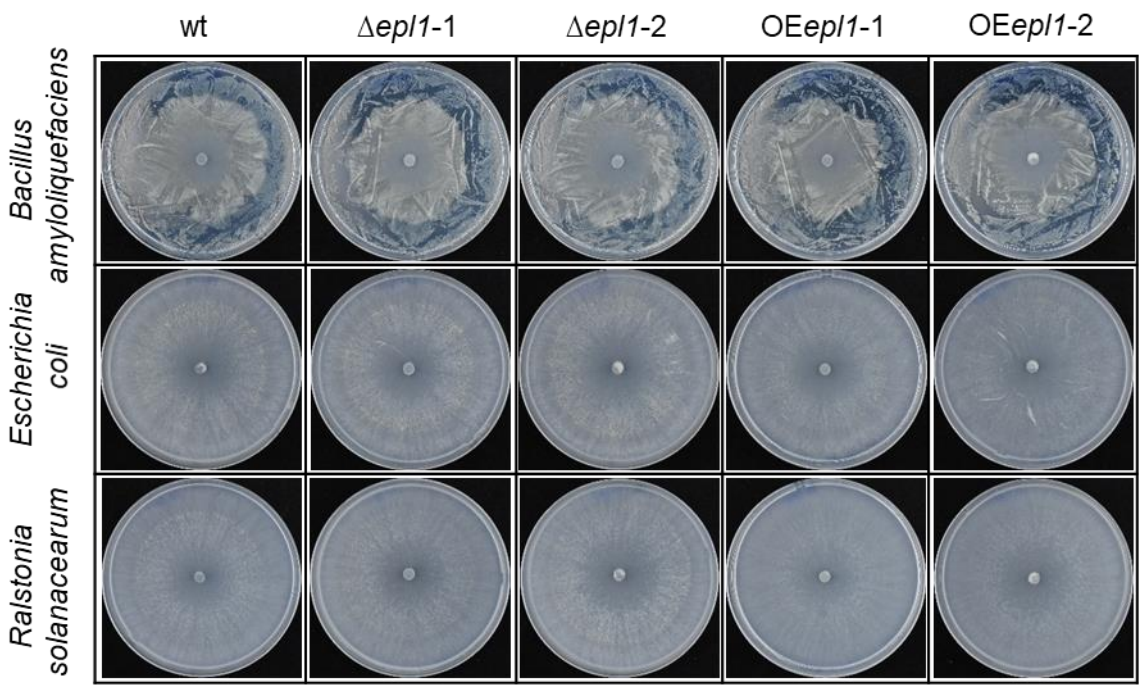


Figure S1.5 Fungal-bacterial interaction between *T. harzianum* and three bacteria from three genera.

File S2 Detailed procedures of mutant construction

Text S2.1 Construction of *epl1*-deletion mutants of *Trichoderma* spp.

The vector for *epl1* gene deletion was constructed as shown in **Figure S2.1** by overlapping PCR (see primers in **Table 4**). The *epl1* gene was designed to be replaced by the *hph* cassette. Hygromycin B-resistant transformants were first screened for the presence of the marker (*hph*) right at the downstream of the 5' flanking arm by using the primer pair F2 and R2. The positive ones were then screened for the occurrence of homologous recombination by using the primer pair F4 and R4. Positive mutants were purified by the method of single spore isolation and confirmed for the absence of the target gene by using the primer pair F3 and R3. The PCR results of two randomly selected *epl1* deletion (Δ*epl1*) mutants are shown in **Figure S2.2**. All vectors and PCR products were confirmed by SANGER sequencing.

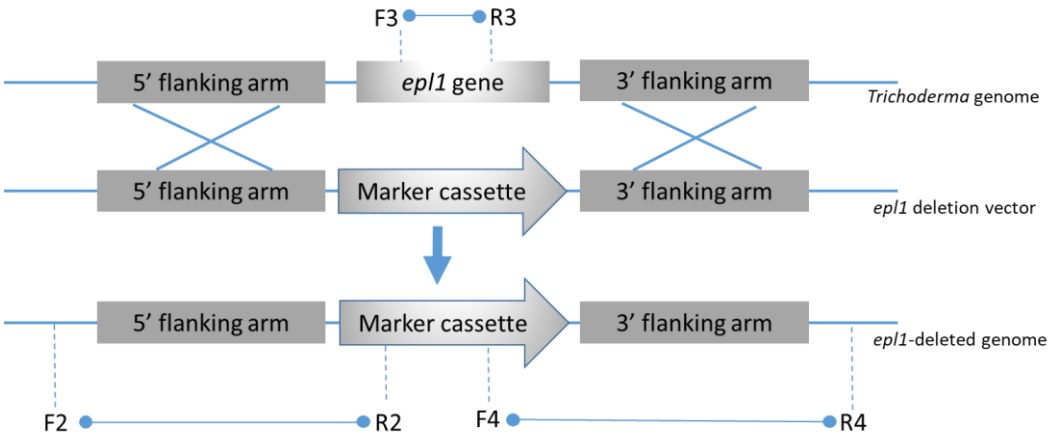


Figure S2.1 Schematic diagram of gene deletion via homologous recombination and mutant screening.

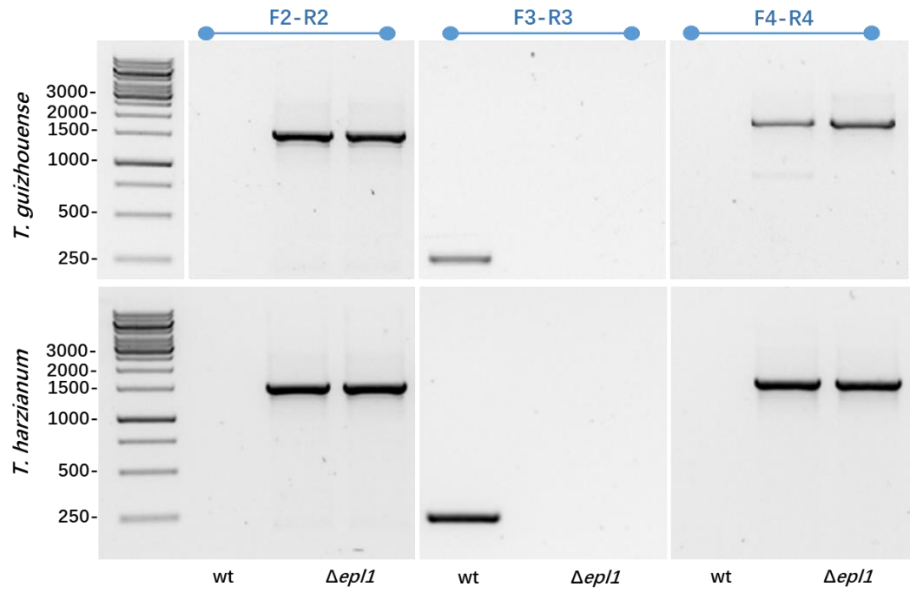


Figure S2.2 PCR verification of the *epl1*-deletion mutants. wt represents the wild type strain of *T. guizhouense* NJAU 4742 or *T. harzianum* CBS 226.95; Δ*epl1* represents the *epl1*-deleted mutants. GeneRuler 1 kb DNA Ladder (Thermo Fischer Scientific, USA) was used in the gel electrophoresis.

Text S2.2 Construction of *epl1*-overexpression mutants of *Trichoderma* spp.

The *epl1*-overexpressing vector was constructed by cloning the open reading frame of *epl1* gene and its native terminator region (1.2 kb) to the downstream of a constitutive promoter P_{cdna1} from *T. reesei* QM6a (19).as shown in **Figure S2.3**. PCR results of two randomly selected *epl1* overexpression (OE*epl1*) mutants are shown in **Figure S2.4**.

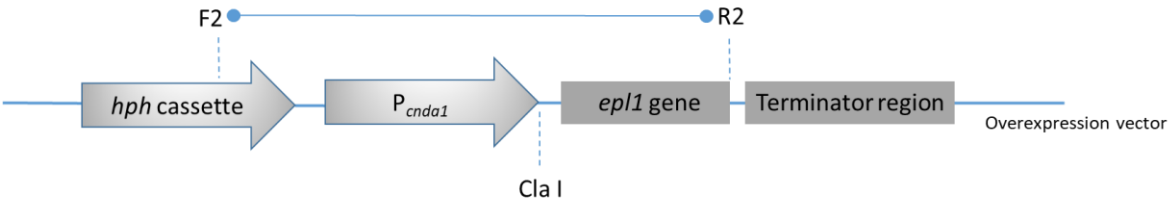


Figure S2.3 Schematic diagram of overexpressing *epl1* under a constitutive promoter P_{cnda1} and mutant screening.

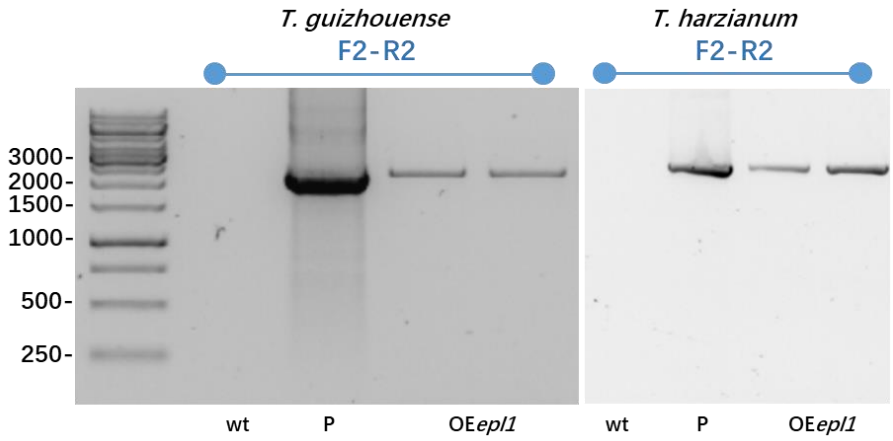


Figure S2.4 PCR verification of *epl1* overexpressing mutants. wt, represents the wild type strain of *T. guizhouense* NJAU 4742 or *T. harzianum* CBS 226.95; OE*epl1*, represents *epl1*-overexpression mutants; P, positive control cloning from the constructed plasmid. GeneRuler 1 kb DNA Ladder (Thermo Fischer Scientific, USA) was used in the gel electrophoresis.

Text S2.3 Construction of *epl1*-expression mutants of *Pichia pastories*

The EasySelect™ *Pichia* Expression Kit (Invitrogen, Thermo Fisher Scientific, USA) was used to express *epl1* from *T. guizhouense* NJAU 4742 in *P. pastoris* KM71H strain, according to the manufacturer's instructions. To express *epl1* with its native N-terminus, we cloned the gene flush (without the signal peptide or the intron sequences) with the Kex2 cleavage site and inserted it into position between the restriction site of Xho I and Xba I of the plasmid pPICZαA (**Figure S2.5**). PCR results of two randomly selected *epl1* expression ($_{Pp}$*epl1*) mutants of *P. pastories* are shown in **Figure S2.6**.

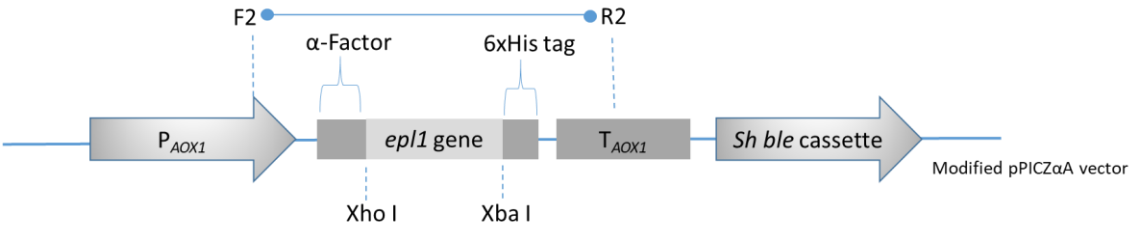


Figure S2.5 Schematic diagram of expressing *epl1* under a methanol-inducible promoter P_{AOX1} in *P. pastories* and mutant screening. α-Factor, native *S. cerevisiae* α-factor secretion signal; T_{AOX1}, native transcription termination from *AOX1* gene of *P. pastoris*; *Sh ble* cassette, *Streptoalloteichus hindustanus ble* gene driving resistance to Zeocin.

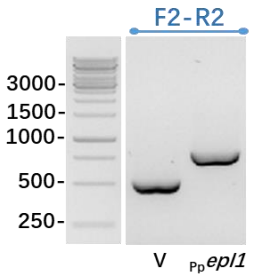


Figure S2.6 PCR verification of mutant expressing *epl1* (from *T. guizhouense* NJAU 4742) in *P. pastoris*. V, represents mutants transformed with the original vector pPICZαA without *epl1*. $_{Pp}$*epl1*, represents Pichia mutant transformed with the vector pPICZαA::*epl1*; GeneRuler 1 kb DNA Ladder (Thermo Fischer Scientific, USA) was used in the gel electrophoresis.

Text S2.4 Proteomic and immune blotting verification of mutants

The proteomic verification of the *epl1*-deletion and overexpression mutants were performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (**Figure S2.7**). Protein samples were collected from the *Trichoderma* culture broth by cultivating the mutants in 30% MS medium (including 1% glucose, MSG) at 25 °C (170 rpm) for 72 h. The *Pichia*-produced EPL1 ($_{Pp}$EPL1) was also confirmed by SDS-PAGE and further investigated by immune blotting as described by our previous research (20). The results shown in **Figure S2.8** gave the corrected band (ca. 40 KDa) of the recombinant $_{Pp}$EPL1 produced by *P. pastories*.

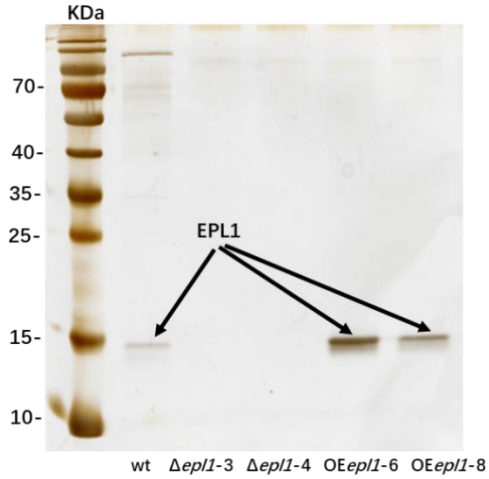


Figure S2.7 Silver stained SDS-PAGE of *epl1* mutants of *T. guizhouese*. wt, represents the wild type strain of *T. guizhouense* NJAU 4742; Δ*epl1*, represents *epl1*-deleted mutants; OE*epl1*, represents mutants overexpressing *epl1*. PageRuler™ Prestained Protein Ladder (Fermentas, USA) was used in the gel electrophoresis.

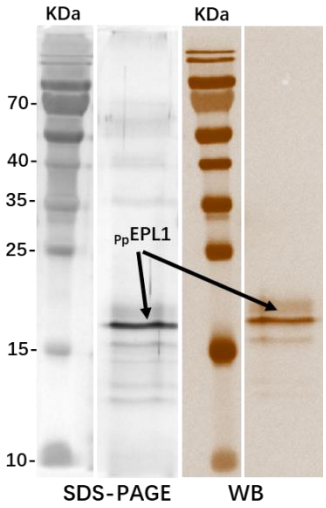


Figure S2.8 Silver stained SDS-PAGE and Western Blot (WB) confirmation of *P. pastories* expressing EPL1 under the induction by 0.5 % methanol. $_{Pp}$EPL1, represents *P. pastories* mutants producing recombinant EPL1. PageRuler™ Prestained Protein Ladder (Fermentas, USA) was used in the gel electrophoresis.

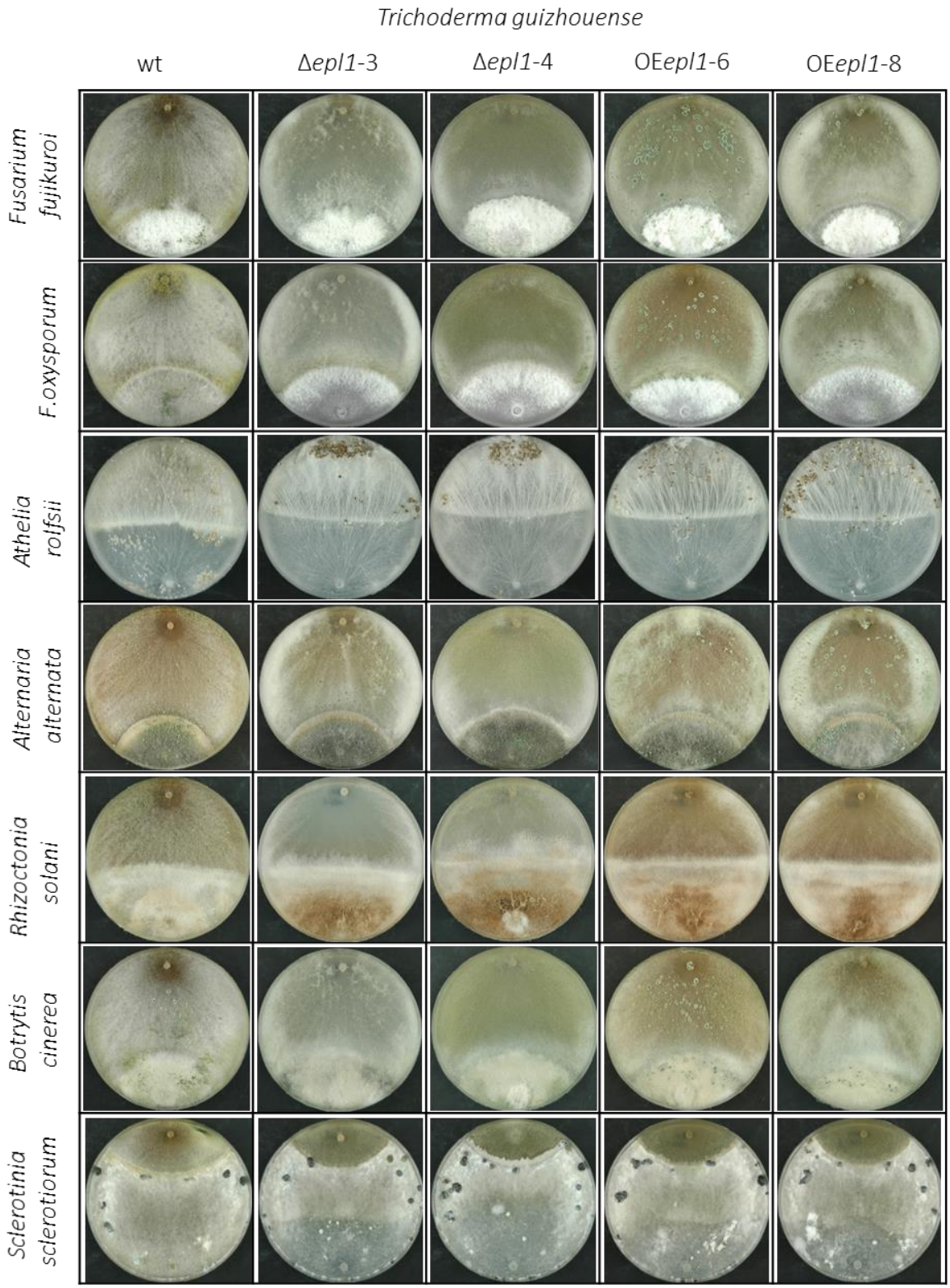


Fig. S3 Dual confrontation assay between *T. guizhouense* and other fungi. Images were recorded after inoculation on PDA (25 °C in darkness) for 14 days. wt, represents the wild type strain of *T. guzihouense* NJAU 4742; Δe*pl1*, represents the *epl1*-deleted mutants; OE*epl1*, represents *epl1*-overexpression mutants.

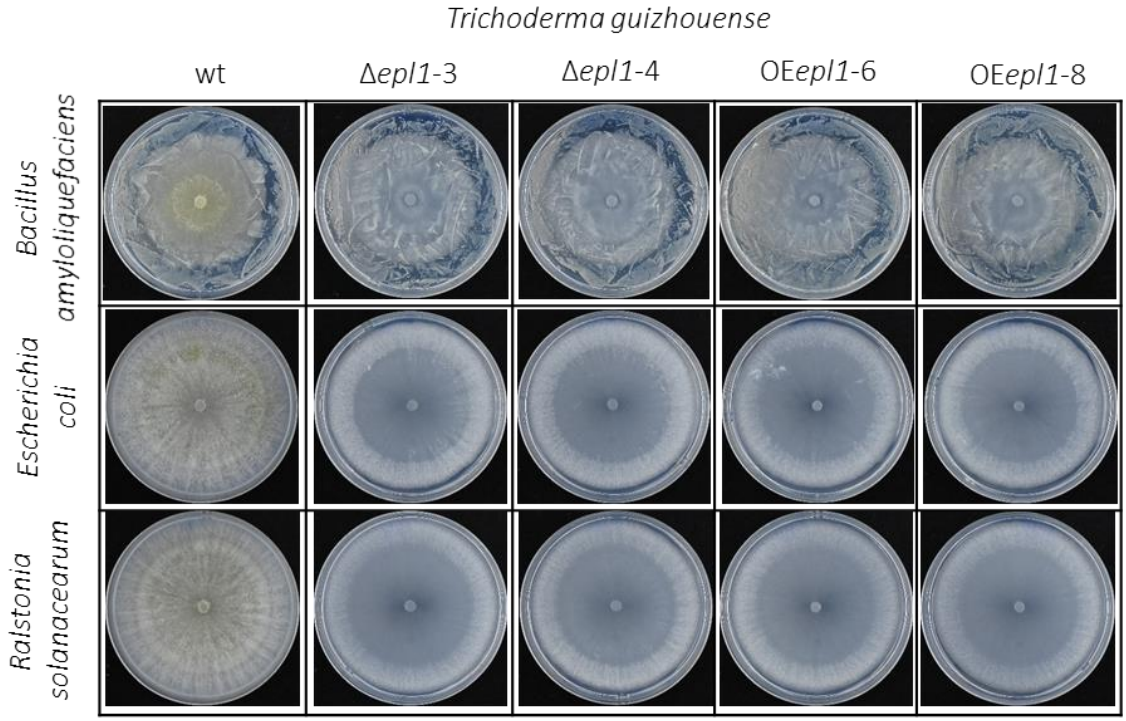


Fig. S4 Fungal-bacterial interaction between *T. guizhouense* and three bacteria from three genera. Images were recorded after inoculation on PDA (25 °C in darkness) for 3 days. wt, represents the wild type strain of *T. guzihouense* NJAU 4742; Δe*pl1*, represents the *epl1*-deleted mutants; OE*epl1*, represents *epl1*-overexpression mutants.

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