

Niches of colonization of *Vitis vinifera* L. by an endophyte *Trichoderma* sp. T154 strain and its biocontrol activity against *Phaeoacremonium minimum*.

G. Carro-Huerga¹, S. Compant², M. Gorfer², R.E. Cardoza Silva^{1,3}, M. Schmoll², S. Gutiérrez^{1,3}, P.A. Casquero^{1*}

¹ Research Group of Engineering and Sustainable Agriculture, Natural Resources Institute, Universidad de León, 24071 Portugal Avenue 41, León, Spain.

² AIT Austrian Institute of Technology GmbH, Center for Health & Environment, Bioresources Unit, 3430 Tulln, Austria.

³ Area of Microbiology, University School of Agricultural Engineers, Universidad de León, Ponferrada Campus, 24401 Astorga Avenue s/n, Ponferrada, Spain.

* **Correspondence:** Pedro Antonio Casquero: pacasl@unileon.es

Data S2

This file contains:

Figures S3-S8

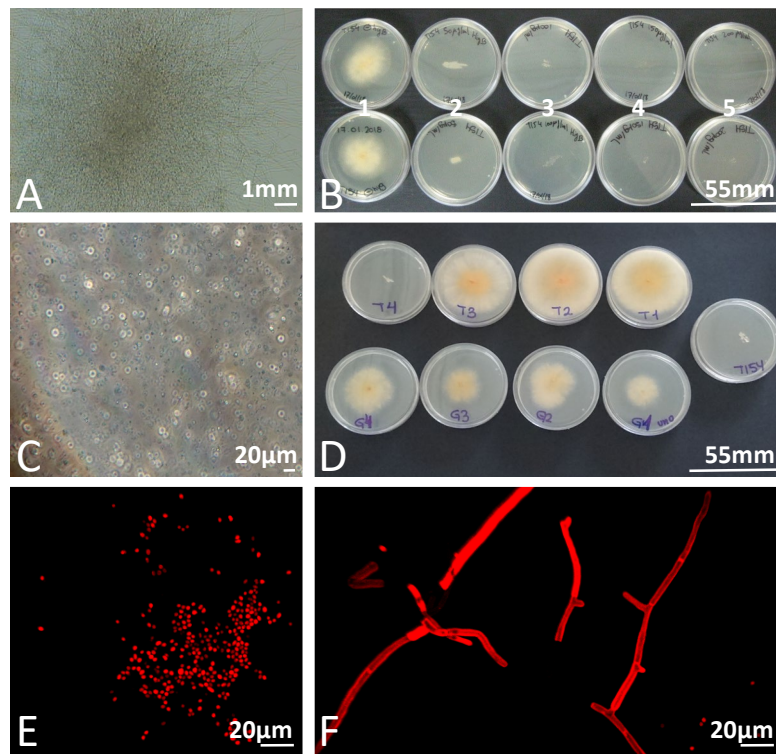


Figure S3 Fungal transformation: (A) Hyphae of *Trichoderma* sp. T154 strain after 24 hours (10x magnification). (B) Assay of hygromycin resistance by duplicate from (1) control to (2) 50 $\mu\text{g mL}^{-1}$, (3) 100 $\mu\text{g mL}^{-1}$, (4) 150 $\mu\text{g mL}^{-1}$ and (5) 200 $\mu\text{g mL}^{-1}$. (C) Protoplasts (20X magnification) of *Trichoderma* sp. T154. (D) Transformants after two selection rounds on selection medium. Transformed strains with *tdTom* gene T1, T2, T3, T4; strains G1, G2, G3, G4 and T154. (E) Spores of *Trichoderma* sp. T154::*tdTom3*. (F) Hyphae of *Trichoderma* sp. T154::*tdTom3*.

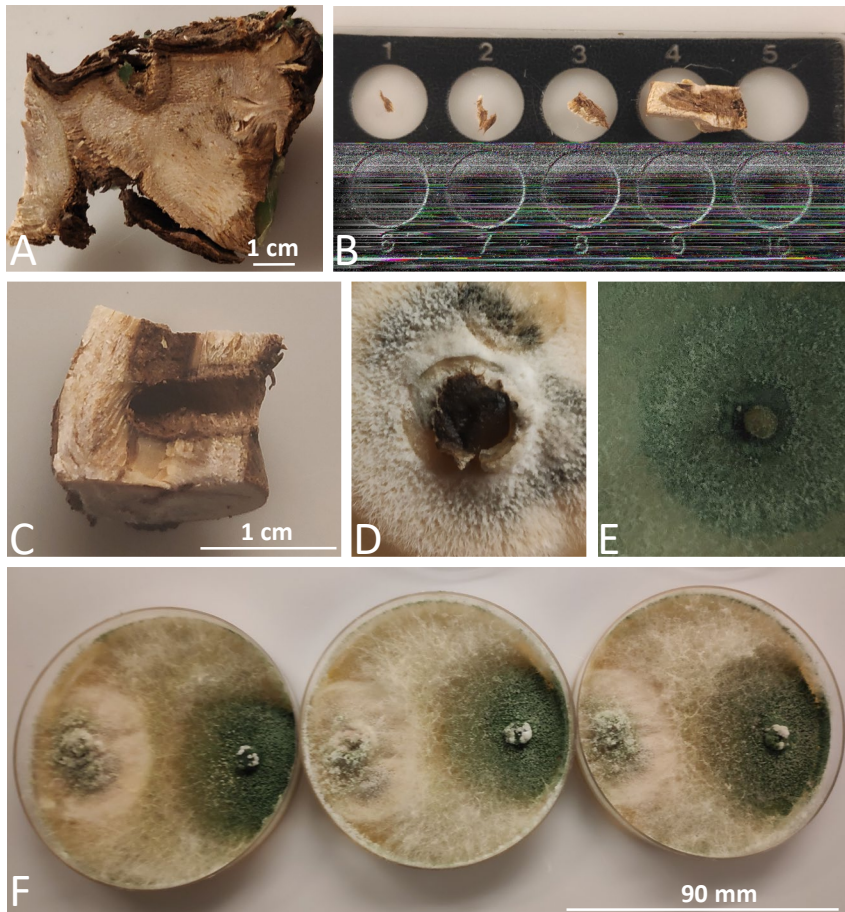


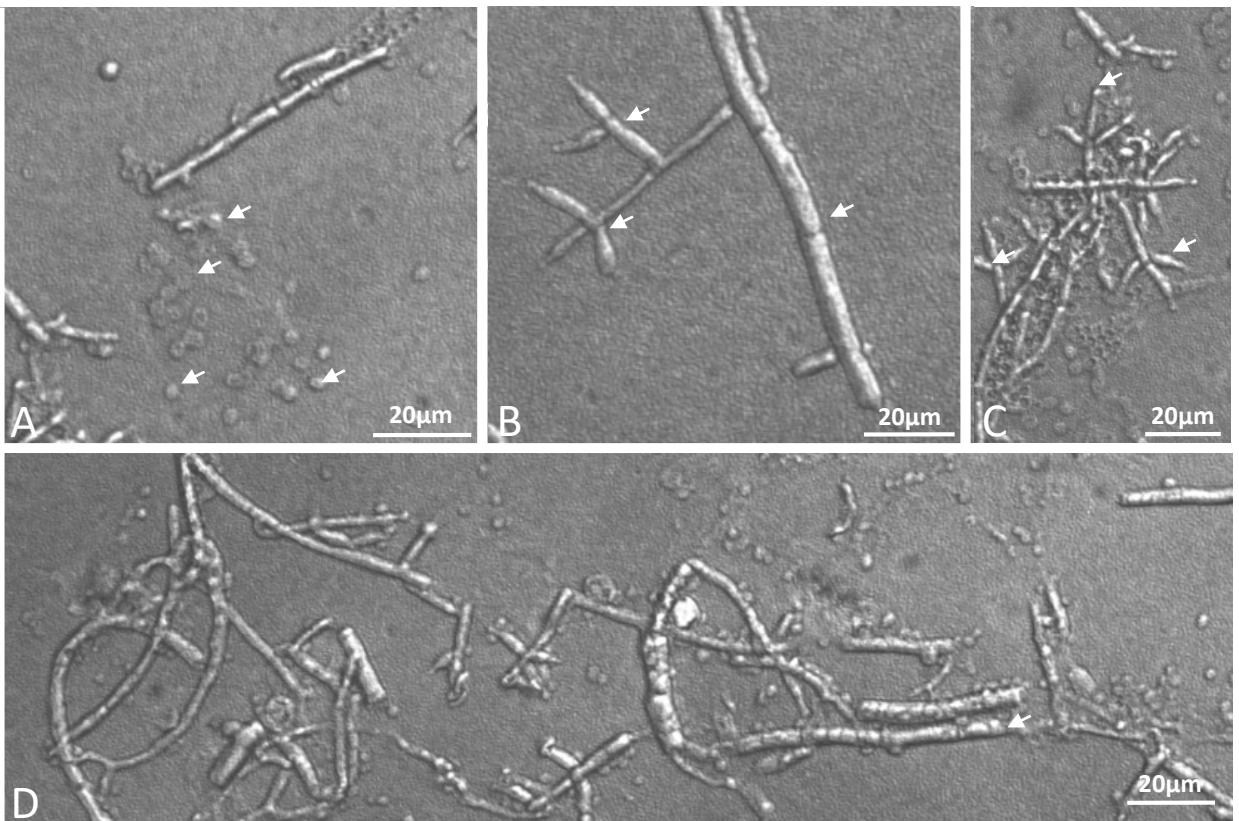
Figure S4 Sampling and evaluation of transformed fungi: (A) Overview, transversal section of vine plant. (B) Different repetitions of a sample for evaluation. (C) Transversal section for evaluation. (D) *Phaeoacremonium minimum::gfp7* after 14 days in pure culture. (E) *Trichoderma* sp. T154::tdTom3 after 7 days of growth. (F) Confrontation assays of *Trichoderma* sp. T154::tdTom3 (right) vs *P. minimum::gfp7* (left) after 10 days of growth.



Figure S5 Inoculation: (A) One year old grapevine grafted plants of Tempranillo/110 Richter were inoculated when at least six leaves were fully developed. (B) An internodal damage was performed with a driller over the woody scion Tempranillo cv. (C) A plug of fresh mycelium and spores of fungi was inoculated in the pruning injury. (D) Point of inoculum after putting plugs. (E) The inoculation point was covered with cellophane.

Pure culture after 7 days (CSLM)

Trichoderma sp. T154 strain



Trichoderma sp. T154::tdTom3

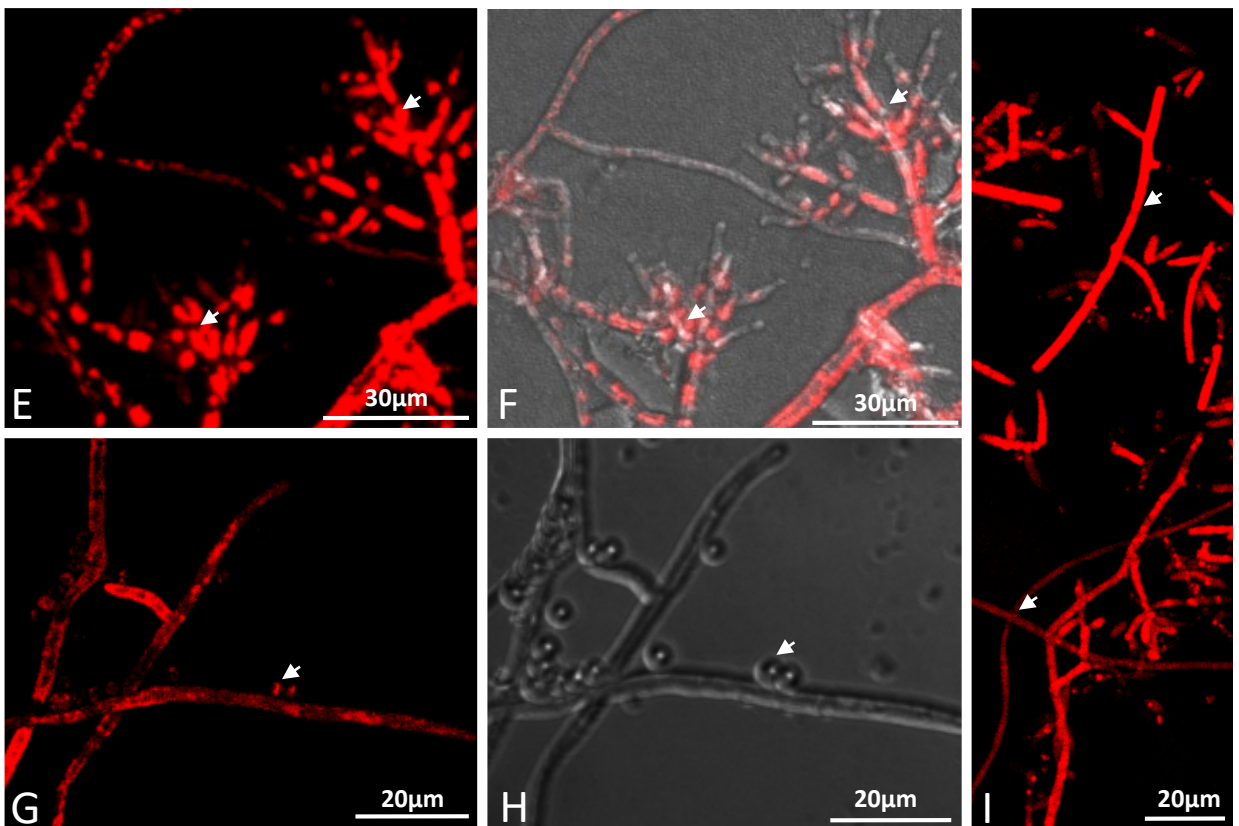


Figure S6 CSLM observation of *Trichoderma* sp. T154 and *Trichoderma* sp. T154::tdTom3 (arrowhead) from a pure culture: (A) Detail of spores of *Trichoderma* sp. T154. (B) Detail of *Trichoderma* sp. T154 strain T154's conidiophores. (C) General overview of characteristic *Trichoderma* structures in *Trichoderma* sp. T154. (D) General overview of *Trichoderma* sp. T154 strain with mycelium and spores. (E-F) Red fluorescent conidiophores and hyphae of *Trichoderma* sp. T154::tdTom3 and also showing conidiophores and hyphae under normal light. (G-H) Red fluorescent spores and hyphae of *Trichoderma* sp. T154::tdTom3 and also showing spores and hyphae of *Trichoderma* sp. T154::tdTom3 under normal light. (I) Red fluorescent hyphae and spores.

Pure culture after 14 days (CSLM)

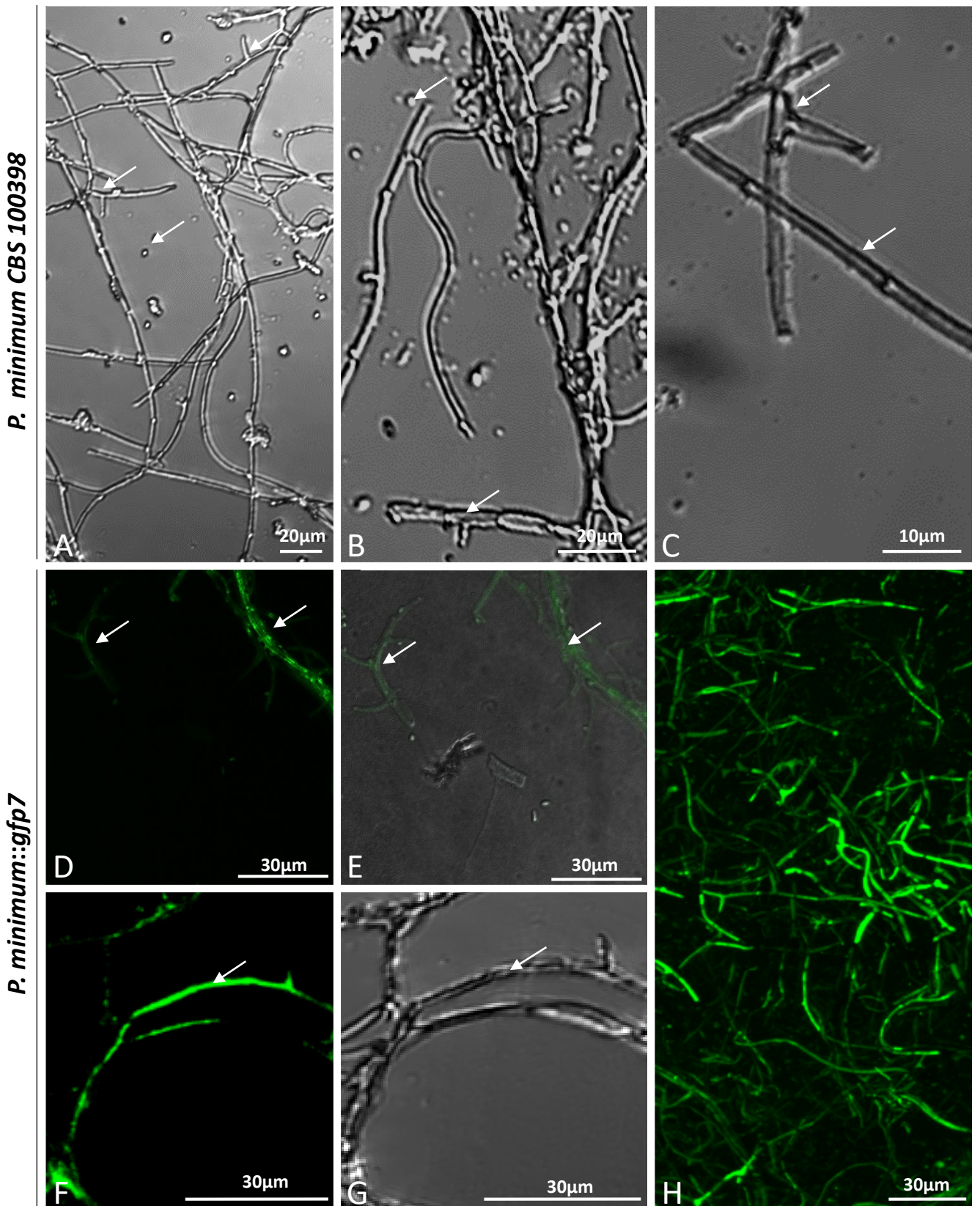


Figure S7 CSLM observation of *P. minimum* CBS 100398 and *P. minimum::gfp7* from (arrows) pure cultures: **(A)** General overview of mycelium and spores of *P. minimum* CBS 100398. **(B)** Magnification of hyphae and spores of *P. minimum* CBS 100398. **(C)** Detail of conidiophores of *P. minimum* CBS 100398. **(D-E)** Green fluorescent spores and hyphae of *P. minimum::gfp7* and also under normal light, evidencing some lost of signal with the green wavelength. **(F-G)** Green fluorescent Hyphae and conidiophore of *P. minimum::gfp7* showing some punctuated fluorescence and also under normal light. **(H)** Mycelium of *P. minimum::gfp7* showing a strong green fluorescence.

Pure culture (SEM)

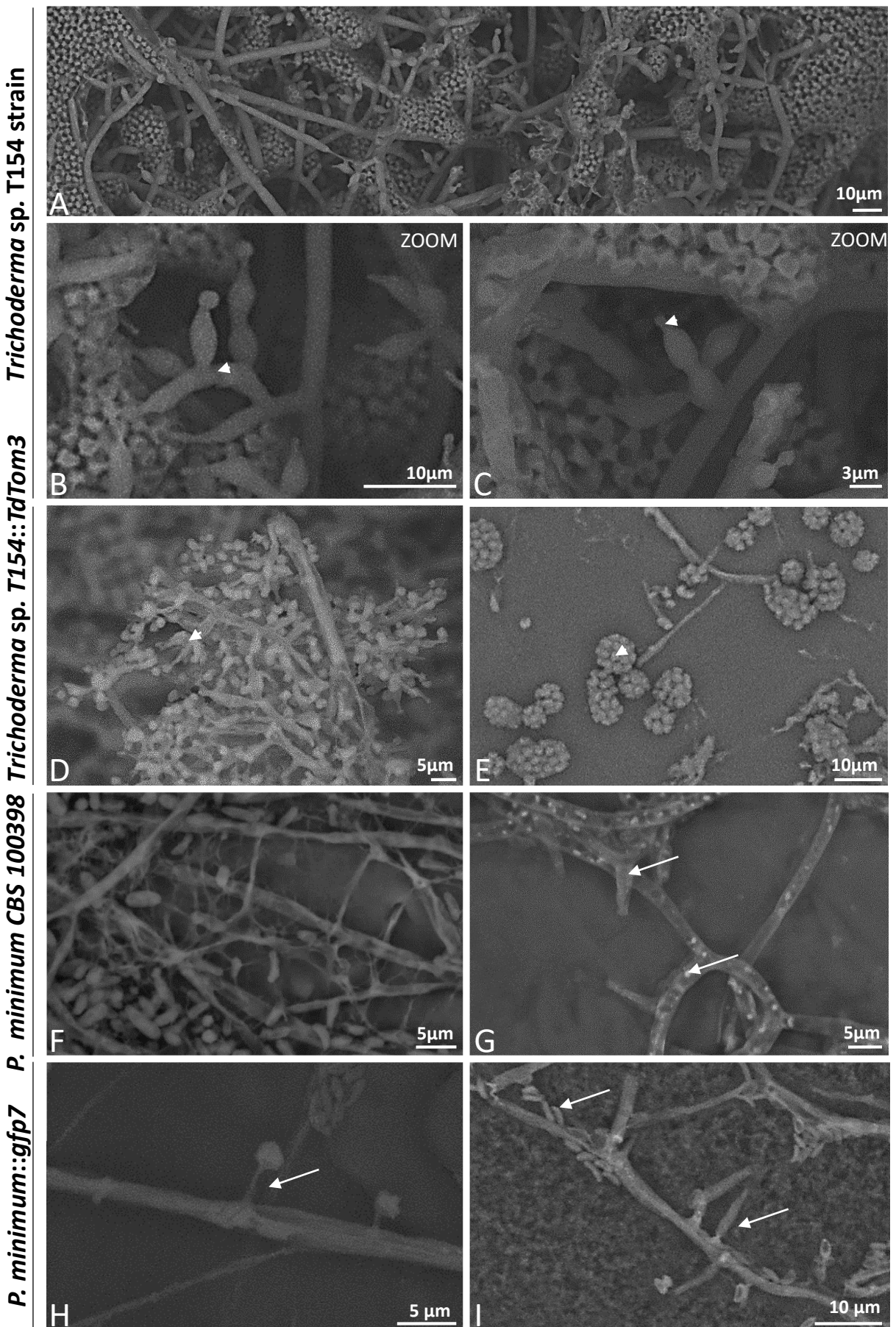


Figure S8 SEM observation of *Trichoderma* sp. T154 (arrowhead), *Trichoderma* sp. T154::tdTom3 (arrowhead), *P. minimum* CBS 100398 (arrows) and *P. minimum*::gfp7 (arrows), from pure culture: **(A)** General overview of spores and mycelium of *Trichoderma* sp. T154 after 7 days of growth in pure culture. **(B-C)** Details of conidiophores and phialides of *Trichoderma* sp. T154. **(D)** View of conidiophores and typical structures of *Trichoderma* with spores of *Trichoderma* sp. T154::tdTom3. **(E)** Phialides full of spores of *Trichoderma* sp. T154::tdTom3. **(F)** Mycelium and spores of *P. minimum* CBS 100398. **(G)** Phialides of *P. minimum* CBS 100398. **(H)** Hyphae with a phialide full of spores of *P. minimum*::gfp7. **(I)** Spores and mycelium of *P. minimum*::gfp7.