Supplementary material

Friend or Foe? The Endophytic Fungus *Alternaria tenuissima* Might Be a Major Latent Pathogen Involved in Ginkgo Leaf Blight

Xiaojia Su, Ruirui Shi, Xiaobo Li, Zine Yu, Linfeng Hu, Haiyan Hu, Meng Zhang, Jingling Chang and **Chengwei Li**

Tables:

Table S1: Basic information of the samples used for ginkgo leaf blight pathogen isolation in this study.

Table S2: Endophytic fungi isolated from the ginkgo leaves in this study.

Table S3: Genbank accession numbers of the reference fungi used in phylogenetic analysis.

Table S4: The candidate fungi of ginkgo leaf blight pathogens and their pathogenicity analysis.

Figures:

Figure S1: Sampling information for ginkgo leaf blight pathogen isolation.

Figure S2: HPLC chromatograms showing flavonoid accumulation profiles in healthy leaves (H), healthy parts of sick leaves (H-S), and sick leaves (S).

Figure S3: Pathogenicity analysis of ginkgo leaf blight pathogens on tissue-cultured seedlings.

Figure S4: Agarose gel electrophoresis results after ITS amplification by PCR for the identification of ginkgo leaf endophytic fungi.

Figure S5: Phylogenetic analysis of the ITS sequences from ginkgo leaf endophytic fungi and the ginkgo leaf blight pathogen *Alternaria tenuissima*.

Figure S6: Flavonoid profile determination and analysis of the isolated ginkgo leaf endophytic fungi (GLEF).

Figure S7: Dual culture of ginkgo leaf endophytic fungi and ginkgo leaf blight pathogens in vitro.

Table S1 Basic information of the samples used for ginkgo leaf blight pathogen isolation in this study.

Number	Sampling leaf pairs (healthy/infected) / tree numbers	Leaf color	Crown shape
HNU-1	3/1	Dark green	Egg
HNU-2	3/1	Green	Egg
HNU-3	3/1	Yellow	Outspread
HNU-4	3/1	Green	Pyramidal
HNU-5	3/1	Edge yellow	Pyramidal
HNU-6	4/1	Green	Pyramidal
HNU-7	3/1	Yellow	Pyramidal
HNU-8	3/1	Yellow	Pyramidal
HNU-9	3/1	Yellow	Pyramidal
XMU-1	3/1	Yellow	Pyramidal
XMU-2	3/1	Edge yellow	Pyramidal
XMU-3	3/1	Yellow	Pyramidal
XMU-4	3/1	Edge yellow	Pyramidal
HIST-1	3/1	Yellow	Pyramidal
HIST-2	3/1	Edge yellow	Outspread
HIST-3	3/1	Yellow	Pyramidal
HIST-4	3/1	Dark green	Pyramidal

Table S2 Endophytic fungi isolated from the ginkgo leaves in this study.

Fungus name	Hits in the NCBI database	Similarity (%)	Accession number
GLEF01	*Unclutured Alternaria* sp.	99.63	OQ591934
GLEF02	*Phyllosticta capitalensis*	99.35	OQ591932
GLEF03	*Phyllosticta harai*	99.51	OQ591939
GLEF04	*Pleosporales* sp.	99.04	OQ591940
GLEF05	*Phyllosticta capitalensis*	99.50	OQ591941
GLEF06	*Alternaria alternata*	100	OQ591936
GLEF07	*Sarocladium* sp. *voucher*	99.82	OQ591933
GLEF08	*Phyllosticta capitalensis*	99.83	OQ591942
GLEF09	*Alternaria tenuissima*	99.81	OQ591937
GLEF10	*Phyllosticta capitalensis*	99.50	OQ591943
GLEF11	*Phyllosticta capitalensis*	99.83	OQ591944
GLEF12	*Dothideomycetes* sp.	99.23	OQ591945
GLEF13	*Dothideomycetes* sp.	99.43	OQ591935
GLEF14	*Peyronellaea* sp.	99.07	OQ591946
GLEF15	*Fusarium fujikuroi*	99.81	OQ591938
GLEF16	*Alternaria alternata*	99.81	OQ591947
GLEF17	*Uncultured soil fungus*	95.04	OQ591948
GLEF18	*Alternaria alternata*	99.81	OQ591949
GLEF19	*Colletotrichum gloeosporioides*	99.81	OQ591950
GLEF20	*Colletotrichum gloeosporioides*	99.81	OQ591951
GLEF21	*Alternaria alternata*	99.81	OQ591952
GLEF22	*Dothiorella* sp.	100	OQ591953
GLEF23	*Alternaria alternata*	100	OQ591954
GLEF24	*Alternaria tenuissima*	100	OQ591955
GLEF25	*Alternaria tenuissima*	100	OQ591956
GLEF26	*Alternaria alternata*	99.81	OQ591957
GLEF27	*Alternaria alternata*	99.45	OQ591958
GLEF28	*Alternaria tenuissima*	99.45	OQ591959
GLEF29	*Alternaria compacta*	99.63	OQ591960
GLEF30	*Alternaria alternata*	99.81	OQ591961
GLEF31	*Alternaria alternata*	100	OQ591962
GLEF32	*Alternaria* sp.	100	OQ591963
GLEF33	*Dothiorella gregaria isolate BJ17*	99.61	OQ591964
GLEF34	*Didymellaceae* sp.	99.80	OQ591965
GLEF35	*Phyllosticta* sp.	99.17	OQ591966

Fungus name	Hits in the NCBI database	Similarity (%)	Accession number
GLEF36	*Phyllosticta capitalensis*	99.02	OQ591967
GLEF37	*Alternaria* sp.	100	OQ591968
GLEF38	*Chaetomium globosum*	99.81	OQ591969
GLEF39	*Alternaria* sp.	99.63	OQ591970
GLEF40	*Phyllosticta capitalensis*	99.50	OQ591971
GLEF41	*Chaetomium globosum*	99.27	OQ591972
GLEF42	*Paraconiothyrium brasiliense*	99.82	OQ591973
GLEF43	*Phyllosticta capitalensis*	99.51	OQ591974
GLEF44	*Phyllosticta capitalensis*	99.83	OQ591975
GLEF45	*Nigrospora* sp.	99.42	OQ591976
GLEF46	*Chaetomium globosum*	99.27	OQ591977
GLEF47	*Alternaria tenuissima*	99.45	OQ591978
GLEF48	*Alternaria alternata*	99.63	OQ591979
GLEF49	*Alternaria* sp.	100	OQ591980
GLEF50	*Chaetomium globosum*	99.45	OQ591981
GLEF51	*Chaetomium globosum*	99.26	OQ591982
GLEF52	*Alternaria brassicae*	99.62	OQ591983
GLEF53	*Alternaria alternata*	99.45	OQ591984
GLEF54	*Aspergillus ruber*	99.43	OQ591985
GLEF55	*Alternaria tenuissima*	99.81	OQ591986
GLEF56	*Alternaria* sp.	99.81	OQ591987

Table S3 Genbank accession numbers of the reference fungi used in phylogenetic analysis.

Fungus	Accession number
Alternaria burnsii isolate BJ7	OP897264.1
Phyllosticta capitalensis strain KACC48659	MK396601.1
Phyllosticta horai (nom. inval.)	KU363980.1
Pleosporales sp. isolate 201805255-2	MH395164.1
Alternaria alternata isolate NSH-1	MN173832.1
Sarocladium sp. voucher GJB	MH890606.1
Phyllosticta capitalensis strain JL6	KM513574.1
Alternaria tenuissima isolate dzdh4	FJ949080.1
Fungal sp. RTL3.1	KF688123.1
Nothophoma spiraeae strain CFCC 53929	MN737834.1
Fusarium fujikuroi isolate LrBF9	MG543727.1
uncultured fungus	OU939442.1
Colletotrichum gloeosporioides	MN298754.1
Dothiorella sp. isolate	OM237283.1
Dothiorella gregaria isolate BJ17	MN685280.1
Coniothyrium pyrinum strain 19LGLF012	MW132013.1
Fungal endophyte isolate 9178	EF419948.1
Chaetomium globosum strain 812-1	MZ648237.1
Nigrospora oryzae isolate NAU-3	OP735562.1
Aspergillus ruber strain OUCMBI110098	KP269010.1

Table S4 The candidate fungi of ginkgo leaf blight pathogens and their pathogenicity analysis.

Number	Pathogenicphenotype	Candidate fungus	Identity (%)	Reisolated	Identity (%)	ITS sequences Accession number
1	None	*Botryosphaeria dothidea* SL	99%-99.28%	/	/	KX024705.1
2	**Dark black patch**	***Alternaria tenuissima***	**98% -90.07%**	***Alternaria tenuissima***	**100%**	**OQ591986**
3	Very weak yellow patch	*Nothophoma quercina*	98%-99.60%	/	/	MN049540.1
4	None	*Alternaria tamaricis* 16P4	98%-99.63%	/	/	KY400101.1
5	**Blackish yellow patch**	**Uncultured *Dothiorella***	**61%-98.11%**	***Dothiorella gregaria***	**99.22%**	**OQ591964**
6	None	*Alternaria alternata*	99%-99.62%	/	/	KU728353.1
7	None but vigorous hypha	*Cladosporium chasmanthicola*	74%-99.74%	/	/	OQ625740
8	**Yellow patch**	***Botryosphaeria dothidea* YCB17**	**99%-99.64%**	***Botryosphaeria dothidea* YCB17**	**99%-99.64%**	**MG462852.1**

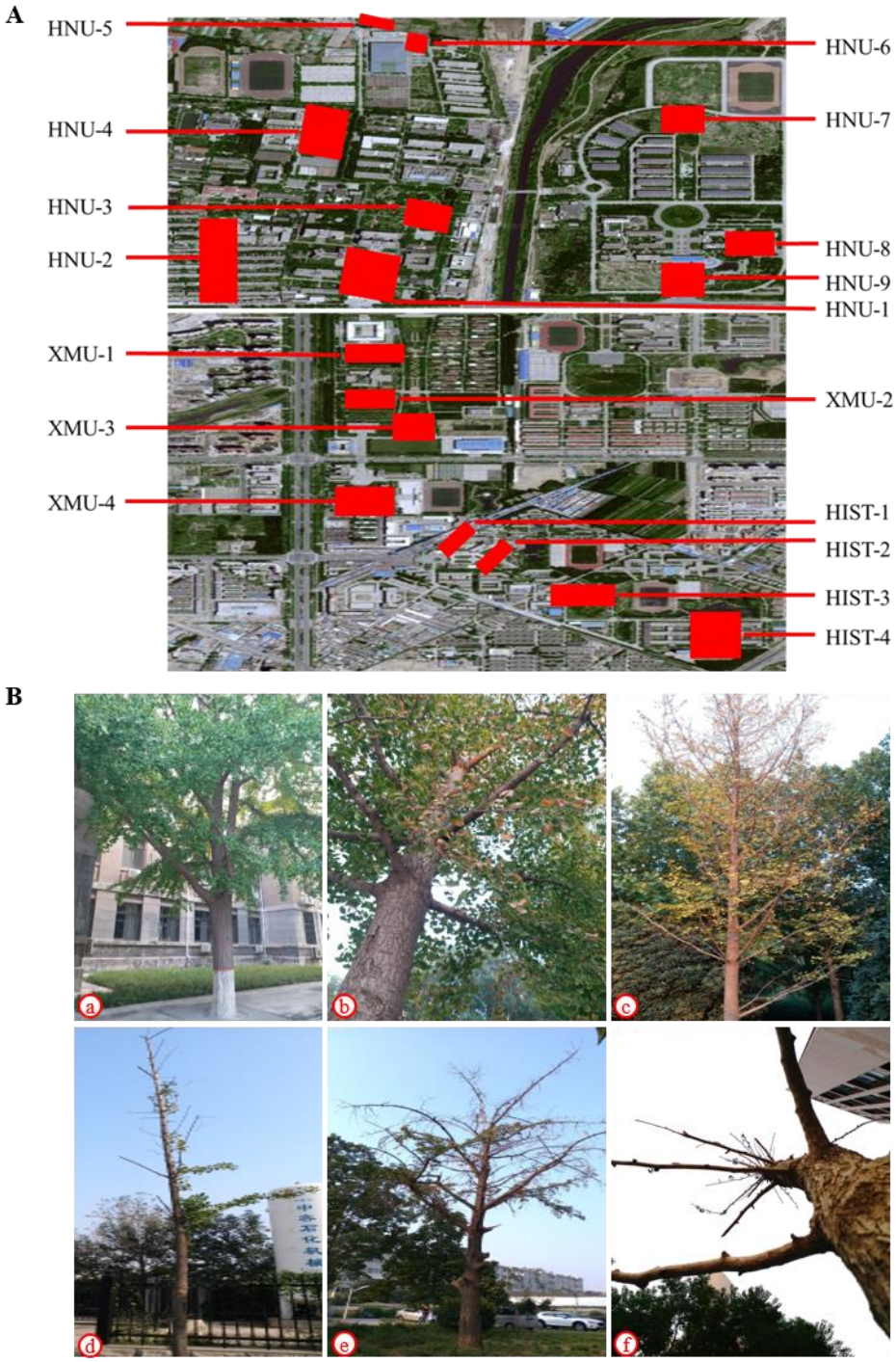

Figure S1. **Sampling information for ginkgo leaf blight pathogen isolation.**

A, Sampling sites distribution of ginkgo leaves used for ginkgo leaf blight pathogen isolations in Xinxiang (～35° 18’ 13.71” N 113° 55’ 15.05” E) (Henan province, China). HNU, Henan Normal University; XMU, Xinxiang Medical University; HIST, Henan Institute of Science and Technology.

B, Examples of ginkgo leaf blight disease development at different infection periods. **a**, the healthy stage; **b**, the early stage; **c**, the middle stage; **d**-**f**, the serious stage.

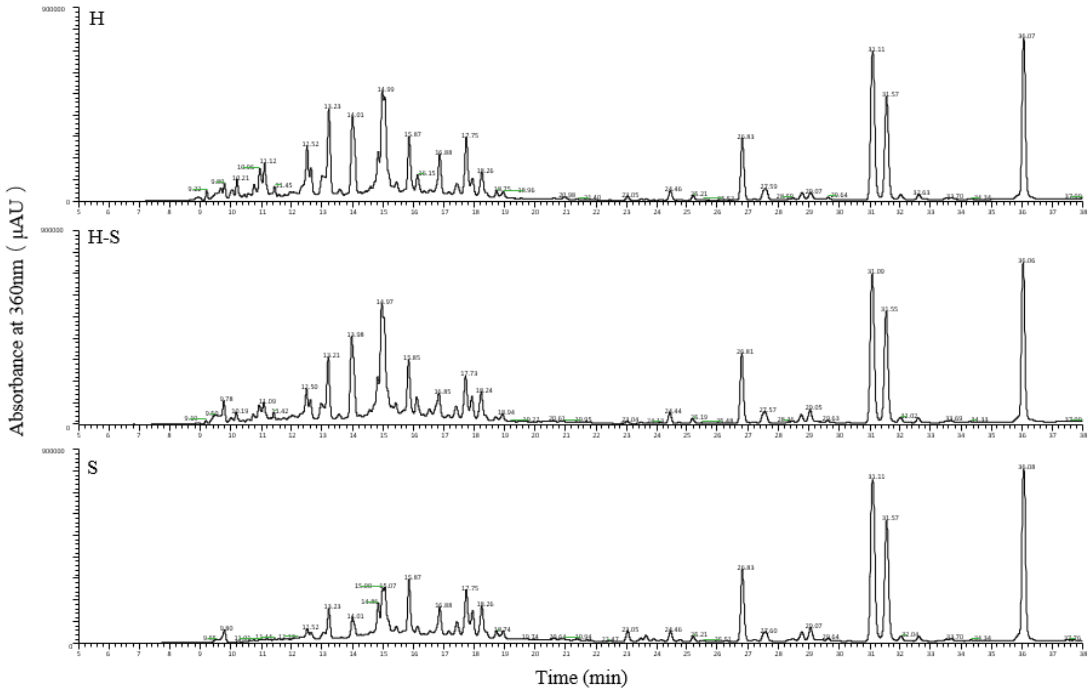

Figure S2. HPLC chromatograms showing flavonoid accumulation profiles in healthy leaves (H), healthy part of the sick leaves (H-S), and sick leaves (S).

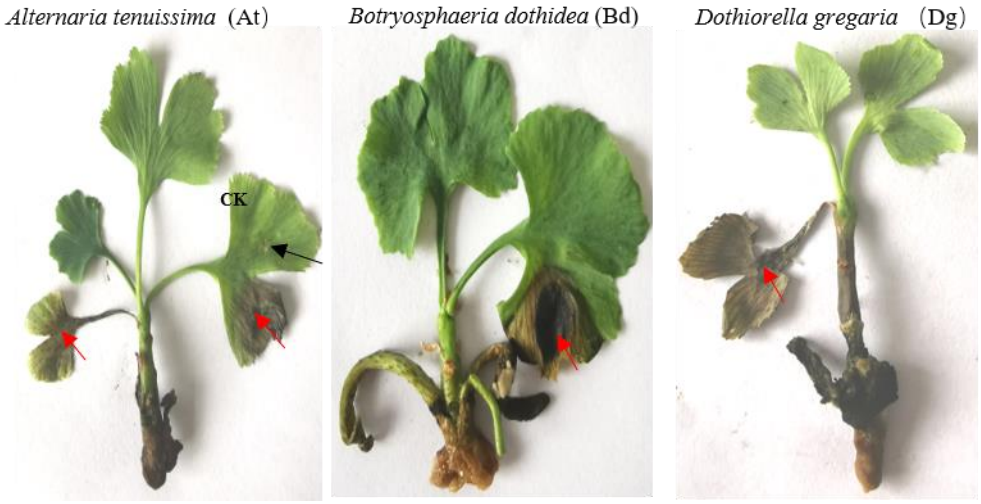

Figure S3. **Pathogenicity analysis of ginkgo leaf blight pathogens on tissue cultured seedlings.** Morphologies of sterile ginkgo seedlings infected by candidate pathogens for 14 days. The black represents the points of infected sites that were marked by different color arrows (black, CK; red, pathogens-infected).

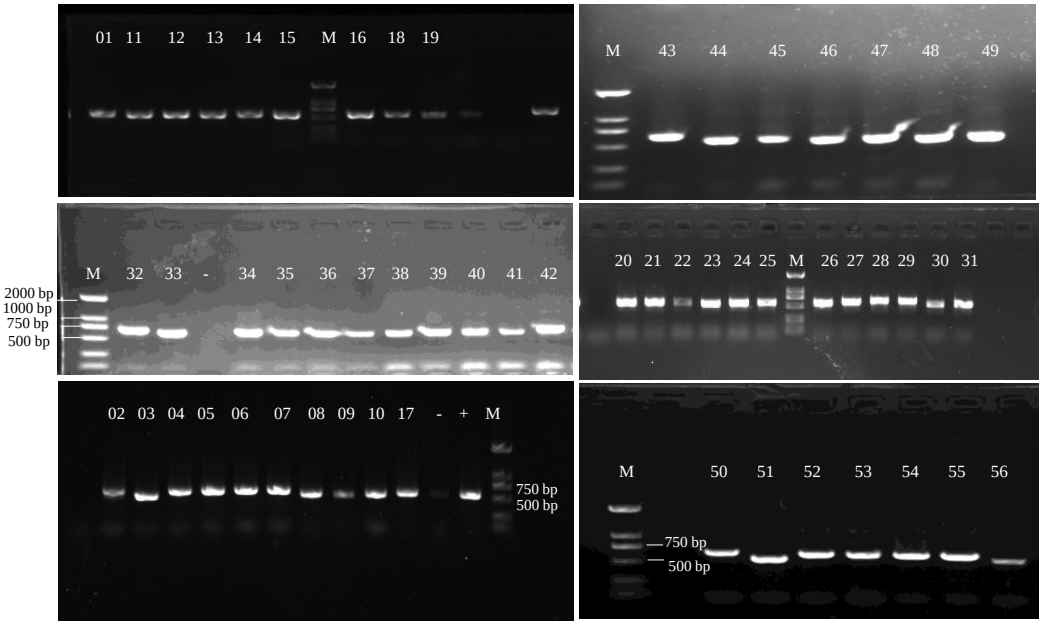

Figure S4 Agarose gel electrophoresis results after ITS amplification by PCR for the identification of ginkgo leaf endophytic fungi. "-" represents negative control; "+" represents positive control with the genomic DNA of *Fusarium graminearum*.

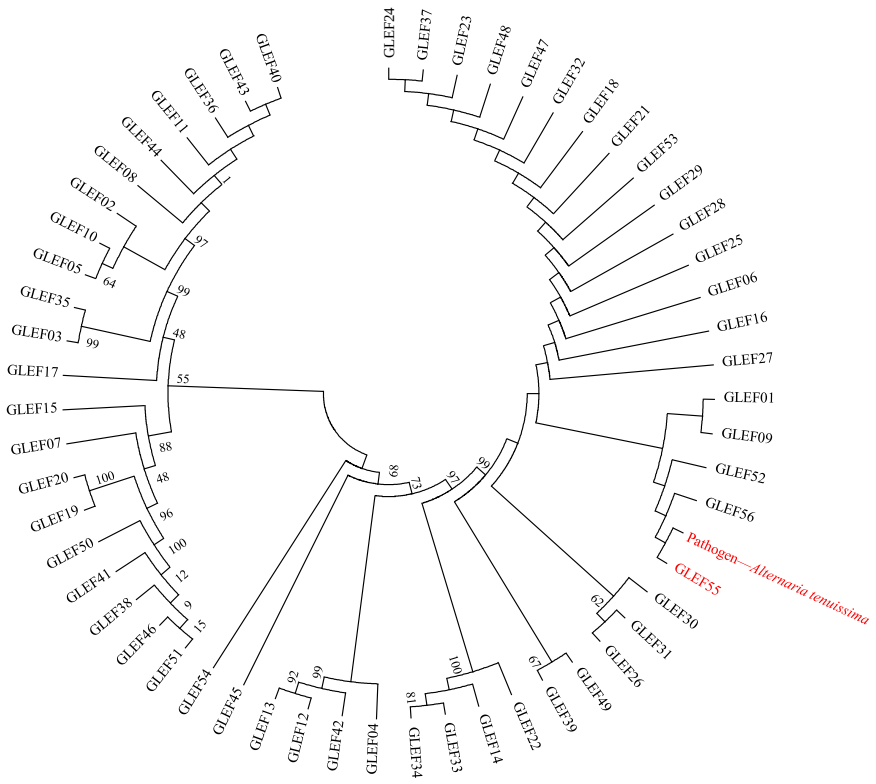

Figure S5: Phylogenetic analysis of the ITS sequences from ginkgo leaf endophytic fungi and the ginkgo leaf blight pathogen *Alternaria tenuissima*.

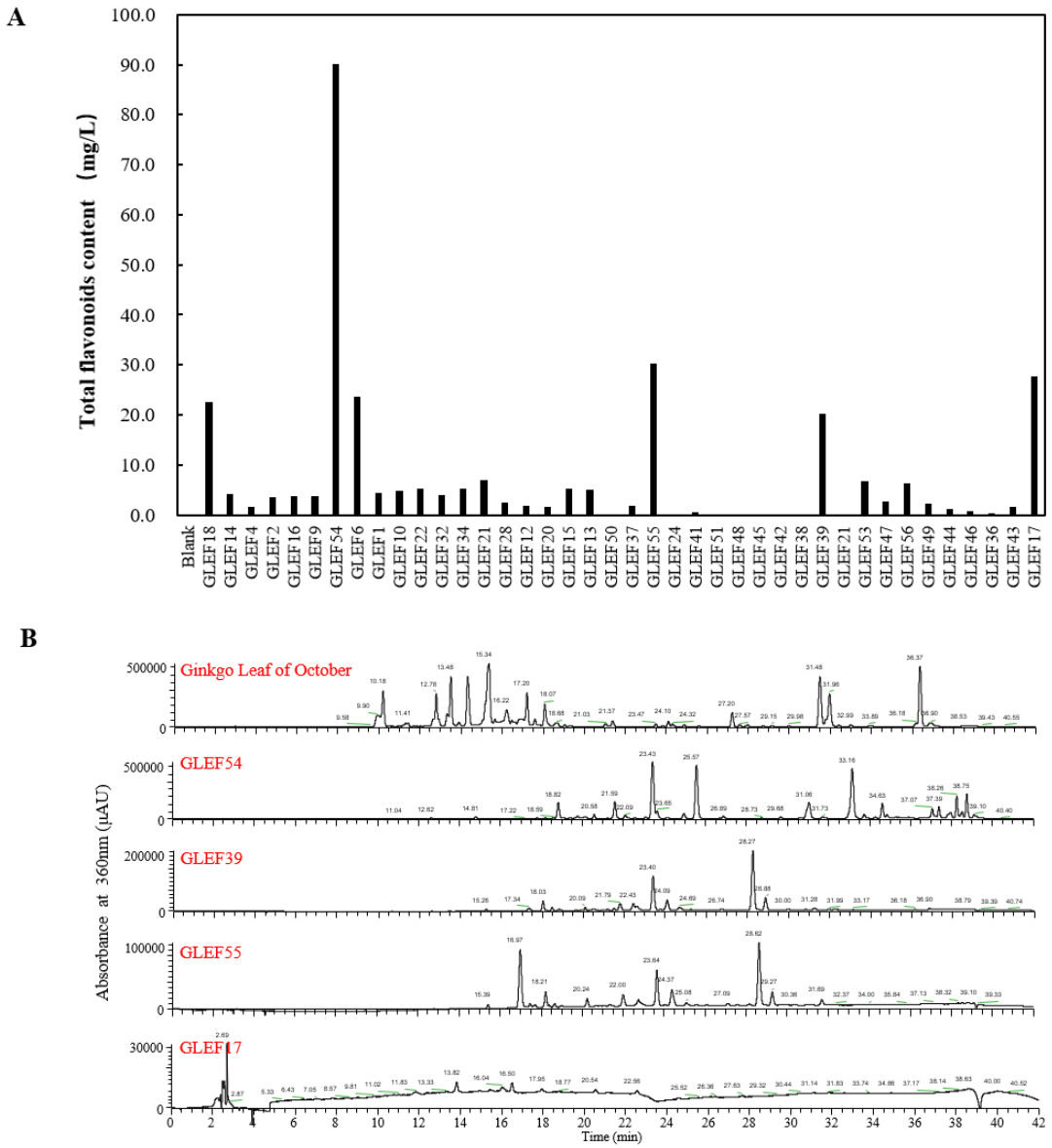

Figure S6. Flavonoid profile determination and analysis of the isolated ginkgo leaf endophytic fungi (GLEF).

A, Total flavonoid content determined by chemical colorimetry methods. Rutin was used as the standard, and all the absorption values were obtained at 510 nm.

B, HPLC chromatograms of flavonoid accumulation profiles in natural ginkgo leaf of October and the extracts of candidate GLEF.

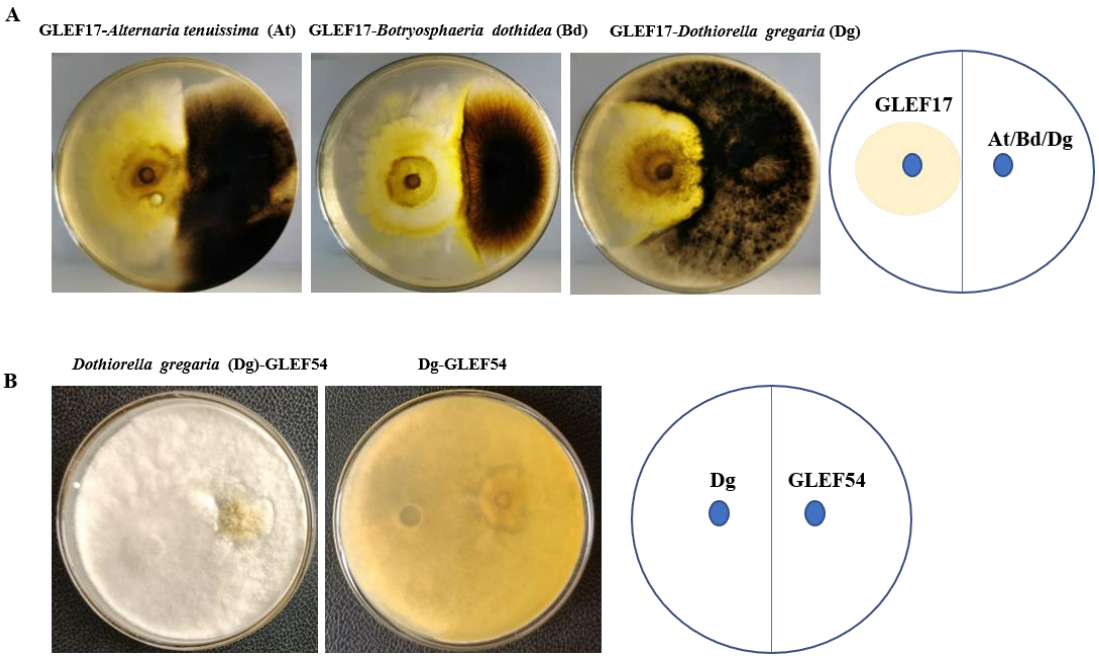

Figure S7. Confrontation culture of ginkgo leaf endophytic fungi and ginkgo leaf blight pathogens in vitro.

A, Confrontation culture of GLEF17 at mycelia proliferation phase and ginkgo leaf blight pathogens**,** *Alternaria tenuissima* (left), *Botryosphaeria dothidea* (middle), *Dothiorella gregaria* (right). Ginkgo leaf blight pathogens were co-cultured for 7 days with GLEF17 under vigorous growth stage on PDA medium.

B, Dual culture of GLEF54 and *D. gregaria* (Dg). The front (left) and back (right) of the plate. The far right was simulation diagrams of inoculation.