

Beneficial effects of endophytic fungi from the *Anoectochilus* and *Ludisia* species on the growth and secondary metabolism of *Anoectochilus roxburghii*

Bingzhu Ye^{†#}, Yanbin Wu^{‡#}, Xin Zhai^{†#}, Ruoqing Zhang[§], Jinzhong Wu[‡], Chao Zhang[‡], Khalid Rahman[⊥], Luping Qin[†], Ting Han^{†*}, Chengjian Zheng^{†*}

[†]Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China

[‡]School of Pharmacy, Fujian University of Traditional Chinese Medicine, 1 Qiuyang Road, Fuzhou 350122, China

[§]Department of Pharmacy, Tianjin Rehabilitation and Recuperation Center, Tianjin 3000191, China

[⊥]Faculty of Science, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, England, U.K.

*Corresponding author:

E-mail addresses: zhengchengjian1984@126.com (C.-J. Zheng); Tel/fax: +86 21 81871308; *E-mail address:* than927@163.com (T. Han); Tel/fax: +86 21 81871306

These authors contributed equally to this work.

Tables and Figures Caption

Table S1. Information of collected samples of five *Anoectochilus* and one *Ludisia* species.

Table S2. Analysis of 227 isolates of endophytes from *Anoectochilus* and *Ludisia* species and their isolation information from different tissues

Table S3. Identification and GeneBank accessions of 53 strains of growth-promoting fungi

Table S4. Compositions of PCR reaction system (50 μ L)

Table S5. Parameters and process of PCR reaction

Table S6. Procedure of UPLC gradient program

Table S7. qRT-PCR primer sequences and GenBank accession numbers for tested genes in *A. roxburghii*

Figure S1. Plant structure of *Anoectochilus* and *Ludisia* species and representative isolated endophytic fungi

Figure S2. The effects of endophytic fungi on Arabidopsis roots (Ratio = treatment group/control; A, B. Root length; C, D. Lateral root number; E. The morphology on root hair formation in the root tip and root segment of Arabidopsis; Scale bars: 200 μ m.)

Table S1. Information of collected samples of five *Anoetochilus* and one *Ludisia* species.

Samples no.	Species	Code	Location of collection
1	<i>A. roxburghii</i>	AR-1	Guigang, Guangxi
2	<i>A. roxburghii</i>	AR-2	Ganzhou, Jiangxi
3	<i>A. roxburghii</i>	AR-3	Pingbian, Yunnan
4	<i>A. burmannicus</i>	AB-1	Dehong, Yunnan
5	<i>A. papillosus</i>	AP-1	Malipo, Yunnan
6	<i>Ludisia discolor</i>	LD-1	Nanning, Guangxi
7	<i>A. lylei</i>	AL-1	Laibin, Guangxi
8	<i>A. nandanensis</i>	AN-1	Nandan, Guangxi
9	<i>A. roxburghii</i>	AR-4	Nanjing, Fujian
10	<i>A. roxburghii</i>	AR-5	Nanping, Fujian

All samples are wild plants collected from difference provinces and cities in China (AR 1-5 Chinese name “Jinxianlian”).

Table S2. Analysis of 227 isolates of endophytes from *Anoectochilus* and *Ludisia* species and their isolation information from different tissues

Code	Tissues of <i>Anoectochilus</i> and <i>Ludisia</i>				No. of isolates
	Roots	Stems	Leaves	Flowers	
AR-1	8	6	7	0	21
AR-2	2	1	2	2	7
AR-3	1	1	3	0	5
AB-1	6	9	13	0	28
AP-1	11	5	11	0	27
LD-1	8	11	8	0	27
AL-1	17	1	27	0	45
AN-1	9	26	19	0	54
AR-4	7	27	14	0	48
AR-5	3	4	8	0	15
No. of total isolates	72	91	112	2	227

Table S3. Identification and GeneBank accessions of 53 strains of growth promoting
fungi

Code of fungi	Taxa of endophytes	Host	Accession number in GeneBank
J2	<i>Bjerkandera adusta</i>	<i>Anectochilus roxburhii</i>	MH151056
J9	<i>Chaetomium globosum</i>	<i>Anectochilus roxburhii</i>	MH151040
J13	<i>Helminthosporium velutinum</i>	<i>Anectochilus roxburhii</i>	MH151012
J19	<i>Xylariaceae</i> sp.	<i>Anectochilus roxburhii</i>	MH151050
J22	<i>Gliomastix polychroma</i>	<i>Anectochilus roxburhii</i>	MH151033
J24	<i>Chaetomium murorum</i>	<i>Anectochilus roxburhii</i>	MH151020
J28	<i>Gliomastix polychroma</i>	<i>Anectochilus roxburhii</i>	MH151023
J29	<i>Fusarium solani</i>	<i>Anectochilus roxburhii</i>	MH151058
J30	<i>Gliomastix polychroma</i>	<i>Anectochilus roxburhii</i>	MH151044
J31	<i>Gliomastix polychroma</i>	<i>Anectochilus roxburhii</i>	MH151052
J32	<i>colletotrichum</i> sp.	<i>Anectochilus roxburhii</i>	MH151018
J33	<i>Chaetomium murorum</i>	<i>Anectochilus roxburhii</i>	MH151014
J35	<i>colletotrichum</i> sp.	<i>Anoectochilus burmannicus</i>	MH151013
J36	<i>Fusarium solani</i>	<i>Anoectochilus burmannicus</i>	MH151017
J41	<i>Gliomastix polychroma</i>	<i>Anoectochilus burmannicus</i>	MH151029
J46	<i>Gliomastix polychroma</i>	<i>Anoectochilus burmannicus</i>	MH151053
J69	<i>Gliomastix polychroma</i>	<i>Ludisia discolor</i>	MH151028
J72	<i>Glomerella cingulata</i>	<i>Ludisia discolor</i>	MH151037
J82	<i>Gliomastix polychroma</i>	<i>Ludisia discolor</i>	MH151016
J86	<i>Gliomastix polychroma</i>	<i>Ludisia discolor</i>	MH151024
J88	<i>Hypoxyton</i> sp.	<i>Ludisia discolor</i>	MH151011
J90	<i>Xylaria berteri</i>	<i>Anoectochilus papillosus</i>	MH151051
J91	<i>Xylariaceae</i> sp.	<i>Anoectochilus papillosus</i>	MH151010
J108	<i>Xylaria berteri</i>	<i>Anoectochilus papillosus</i>	MH151049
J112	<i>Xylaria berteri</i>	<i>Anoectochilus papillosus</i>	MH151022
J116	<i>Fusarium oxysporum</i>	<i>Anoectochchilus lylei</i>	MH151034
J118	<i>Gliomastix polychroma</i>	<i>Anoectochchilus lylei</i>	MH151045
J125	<i>colletotrichum</i> sp.	<i>Anoectochchilus lylei</i>	MH151047
J132	<i>Xylaria berteri</i>	<i>Anoectochchilus lylei</i>	MH151036
J134	<i>Gliomastix polychroma</i>	<i>Anoectochchilus lylei</i>	MH151026
J144	<i>Fusarium oxysporum</i>	<i>Anoectochchilus lylei</i>	MH151038
J148	<i>Fusarium</i> sp.	<i>Anoectochchilus lylei</i>	MH151032
J151	<i>colletotrichum</i> sp .	<i>Anoectochchilus lylei</i>	MH151009

J153	<i>colletotrichum</i> sp.	<i>Anoectochchilus lylei</i>	MH151031
J160	<i>Fusarium</i> sp.	<i>Anoectochchilus lylei</i>	MH151042
J162	<i>Chaetomium globosum</i>	<i>Anoectochilus nandanensis</i>	MH151027
J166	<i>Colletotrichum spaethianum</i>	<i>Anoectochilus nandanensis</i>	MH151057
J182	<i>Fusarium oxysporum</i>	<i>Anoectochilus nandanensis</i>	MH151025
J189	<i>Chaetomium murorum</i>	<i>Anoectochilus nandanensis</i>	MH151015
J193	<i>Phomopsis</i> sp.	<i>Anoectochilus nandanensis</i>	MH151048
J198	<i>Chaetomium murorum</i>	<i>Anoectochilus nandanensis</i>	MH151046
J209	<i>Auricularia polytricha</i>	<i>Anoectochilus nandanensis</i>	MH151043
J210	<i>Auricularia polytricha</i>	<i>Anoectochilus nandanensis</i>	MH151041
J211	<i>Colletotrichum gloeosporioides</i>	<i>Anoectochilus nandanensis</i>	MH151021
J212	<i>Auricularia polytricha</i>	<i>Anoectochilus nandanensis</i>	MH151030
J221	<i>Diaporthe eres</i>	<i>Anectochilus roxburhii</i>	MH151039
J223	<i>Colletotrichum</i> sp.	<i>Anectochilus roxburhii</i>	MH151054
J224	<i>Xylariaceae</i> sp.	<i>Anectochilus roxburhii</i>	MH151019
J248	<i>Xylariaceae</i> sp.	<i>Anectochilus roxburhii</i>	MH151035
J261	<i>Colletotrichum magnisporum</i>	<i>Anectochilus roxburhii</i>	MH151055
J94	<i>unknown</i>	<i>Anoectochilus papillosus</i>	
J105	<i>unknown</i>	<i>Anoectochilus papillosus</i>	
J218	<i>unknown</i>	<i>Anectochilus roxburhii</i>	

Table S4. Compositions of PCR reaction system (50.0 μL)

Composition	Volume
DNA template	2.0 μL
Primer ITS4	1.0 μL
Primer ITS5	1.0 μL
2 \times TransTap PCR SuperMix	25.0 μL
ddH ₂ O	21.0 μL

Table S5. Parameters and process of PCR reaction

Procedure	Temperature(°C)	time
1.Initial denaturation	94	4 min
2. Degeneration	94	50s
3. Annealing	54	50s
4. Elongation	72	90 s
5. Degeneration-Annealing-Elongation, 30 cycles		
6. Elongation	72	7 min
7. Finish	4	∞

Table S6. Procedure of UPLC gradient program

Time(min)	Acetonitrile(%)	0.1% formic acid in water(%)
0	10	90
1	11	89
6	20	80
9	35	65
13	33	67
15	37	63
18	65	35
19	10	90

Table S7. qRT-PCR primer sequences and GenBank accession numbers for tested

genes in *A. roxburghii*

Gene name	Primer sequence (5'-3')	Accession
Uracil phosphoribosyl transferase	F: AGAGTGCAATGGCAGAGCTT R: AATCGTCACAGGCTCCCTTG	GR410424
Amino acid transmembrane transporter	F: GGTCGATCGCCGGAGTTATT R: AGCACGATAGAGCGCTACAC	GR410425
Maturase K	F: TGCCGGAATTCTGGTCATCC R: AGGATCCGCTGTGATAACGAC	GR410426

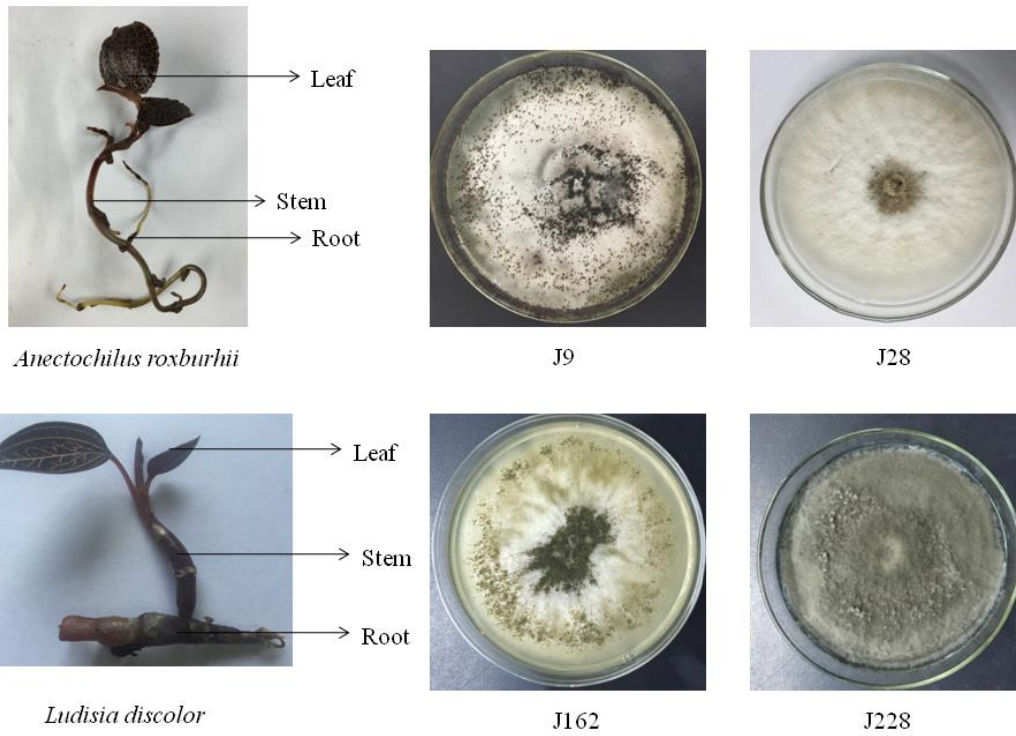


Figure S1. Plant structure of *Anectochilus* and *Ludisia* species and representative isolated endophytic fungi

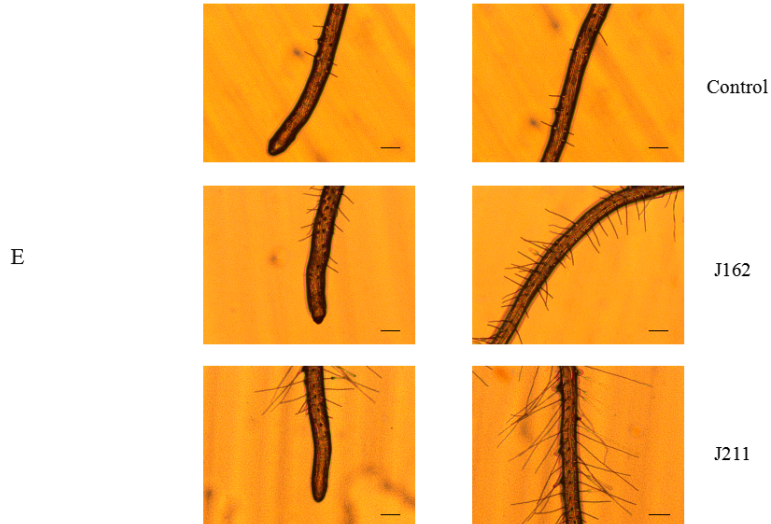
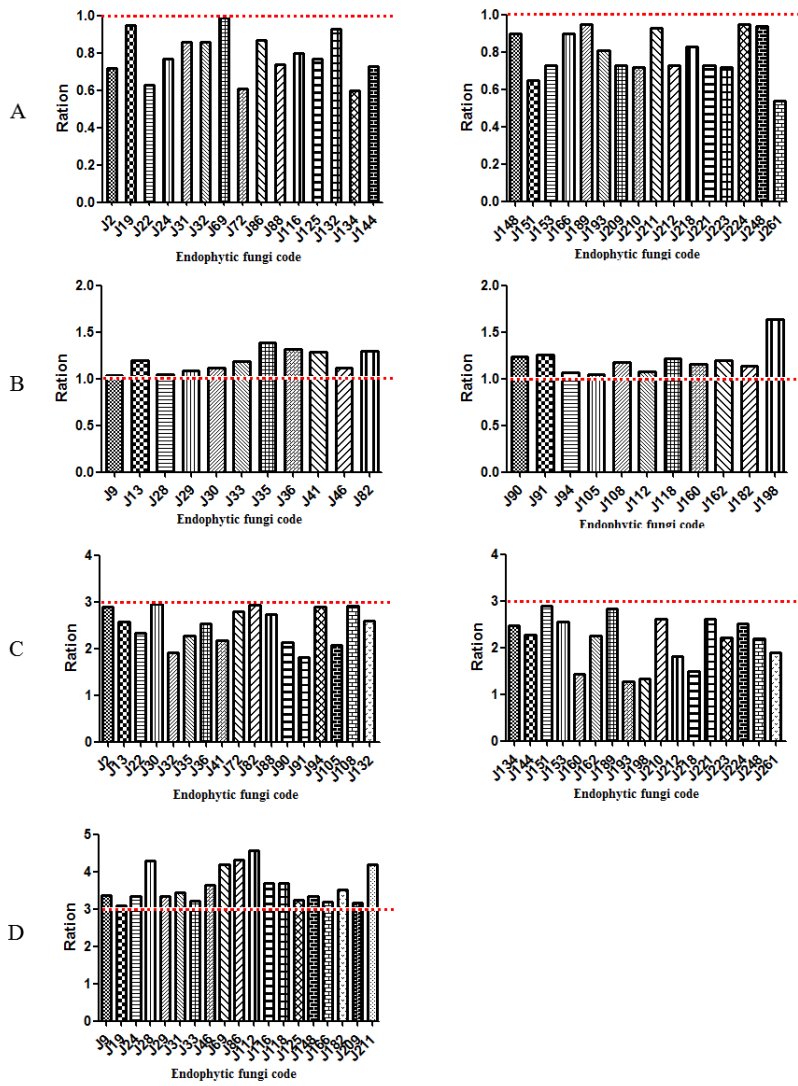


Figure S2. Effects of endophytic fungi on *Arabidopsis* roots (Ratio = treatment group/control; A, B. Root length; C, D. Lateral root number; E. The morphology on root hair formation in the root tip and root segment of *Arabidopsis*; Scale bars: 200 μm .)