J Appl Genetics (2012) 53:343–354
DOI 10.1007/s13353-012-0093-1

MICROBIAL GENETICS · ORIGINAL PAPER

Trichoderma biodiversity in China

Rui-yan Sun · Zhi-cheng Liu · Kehe Fu · Lili Fan · Jie Chen

Received: 14 January 2012 / Revised: 20 February 2012 / Accepted: 14 March 2012 / Published online: 21 April 2012

Abstract In the present study, we made further investigation into the diversity of *Trichoderma* in China than previous ones utilizing comprehensive approaches of morphological microscopic observation and phylogenetic analysis by detecting molecular markers. One thousand nine hundred ten *Trichoderma* strains were isolated from soil or other materials in China: East (Anhui, Fujian, Jiangsu, Jiangxi, Shandong, Zhejiang province and Shanghai municipality), South-West (Guizhou, Qinghai, Shanxi, Sichuan and Yunnan province, Tibet Autonomous Region and Chongqing municipality), South-East (Guangdong, Guangxi, Hainan province), and Middle China (Henan, Hubei and Hunan province). Representative isolates were verified at the species level by morphological characters and the oligonucleotide barcode program TrichoOKey v.10 and the custom BLAST server TrichoBLAST, using sequence of the ITS 1 and 2 region of the rDNA cluster and partial sequences of translation elongation factor 1-alpha(*tef*1-α). A total of 23 *Trichoderma* species were identified : *T.asperellum*, *T.atrioviride*, *T.aureovriride*, *T. brevicompactum*, *T.citrioviride*, *T.erinaceum*, *T.gamsii*, *T. hamatum*, *T.harzianum* (*H.1ixii*), *T.intricatum*, *T.koningii* (*H. koningii*), *T.koningiopsis*, *T.longibranchiatum*, *T.pleuroticola*, *T.reeseii* (*H.jecorina*), *T.sinensis*, *T.spirale*, *T.stromaticum*, *T. tomentosum*, *T.velutinum*, *T.vermipilum*, *T.virens* (*H.virens*), *T.viride*. Among them, 3 species: *T.intricatum*, *T.stromaticum*, *T.vermipilum* were first reported in China; *T.harzianum* (*H,1ixii*) was the most widely distributed species in China. This study further shows that, the highest biodiversity of *Trichoderma* population appeared in South-West China.

Keywords Biogeography · Molecular identification · Phylogenetic analysis · *Trichoderma*

R.-y. Sun · Z.-c. Liu · K. Fu · L. Fan · J. Chen (✉)
Department of Resource and Environmental Science,
School of Agriculture and Biology, Shanghai Jiaotong University,
Shanghai 200240, China
e-mail: jiechen59@sjtu.edu.cn

R.-y. Sun · Z.-c. Liu · K. Fu · L. Fan · J. Chen
Key Laboratory of Urban Agriculture (South),
Ministry of Agriculture,
Shanghai, China

Introduction

As cosmopolitan soil-borne fungus genus, *Trichoderma* (*Ascomycetes*, *Hypocreales*) are remarkable for their rapid growth, capability of utilizing diverse substrates and resistance to noxious chemicals (Klein and Eveleigh 1998). In terms of the ecological importance of their production of enzymes and antibiotics (Kubicek and Penttilä 1998; Sivasithamparam and Ghisalberti 1998) and its application as a biocontrol agent against plant diseases (Hjeljord and Tronsmo 1998; Harman et al. 2004; Bailey et al. 2006), it is vital to identify accurately *Trichoderma* species and understand its biodiversity and biogeography.

Until recently, most of the known species have been isolated from China, over 40 *Trichoderma* species have been identified in China (Wen et al. 1992; Wen et al. 1993; Zhang et al. 1996; Luo and Wen 2003; Zhang and Xu 2004; Zhao et al. 2004; Zhang and Xu 2005; Sun et al. 2006a, b; Yu et al. 2007; He et al. 2008; Shao et al. 2008; Jia et al. 2009; Gao et al. 2007; Wu et al. 2008; Yuan et al. 2008; Xia and Chen 2009; He et al. 2010a, b; Li et al. 2010; Pan et al. 2010; Yu et al. 2010). Among them, 21 species of *Trichoderma* which are isolated from soil samples in different ecological environments (Wen et al. 1992; Zhang et al. 1996; Luo and Wen 2003; Zhao et al. 2004; Sun et al. 2006a, b; Jia et al. 2009; Pan et al. 2010; Yu et al. 2010). Furthermore, nine *Trichoderma* species are obtained from different spawn and fruiting bodies

of edible fungi based on morphological and colony characters (Gao et al. 2007; He et al. 2008; Shao et al. 2008; Wu et al. 2008). Besides, some species of *Trichoderma* are isolated from rhizosphere of plants (Ze et al. 2007; Yuan et al. 2008).

However previous studies in China lack systemic survey to *Trichoderma* population in East, South-East, South-West and Middle China, particularly in Sourth-East and South-West where the most regions are characterized with the most abundant biodiversity led by warm and moist climates, which thereby allow *Trichoderma* massive reproduction. The purpose of the present study was on a large scale to isolate *Trichoderma* from forest, garden and vegetable soil and other materials, and then to identify *Trichoderma* species and understand their geographic distribution state in China. The study would lead us to more reasonable exploration of *Trichoderma* sources, and also reveal partially the status of farming soil micro-ecological quality in China.

Materials and methods

Geography of sample sites

Trichoderma spp. were sampled in 20 regions of East, South-East, South-West and Middle China, which differ in their geographic location, altitude and climate: East (Anhui, Fujian, Jiangsu, Jiangxi, Shandong, Zhejiang province and Shanghai municipality), South-West (Guizhou, Qinghai, Shanxi, Sichuan and Yunnan province, Tibet Autonomous Region and Chongqing municipality), South-East (Guangdong, Guangxi, Hainan province), and Middle China (Henan, Hubei and Hunan province).

Substrates, storage and isolation of pure cultures

Samples of soil were kept in sterile polyethylene bags or kraft envelopes and stored at 4 °C until isolation. The soil dilution plating method was applied. PDAm (S Vargas Gil et al. 2009) was used as a selective medium for isolating *Trichoderma*. Putative *Trichoderma* colonies were purified by two rounds of subculturing on potato-dextrose agar (PDA) at 28 °C. Pure cultures were kept in 20 % (w/v) glycerol at -20 °C.

Morphological analysis

For morphological analysis, strains were grown on special nutrient agar (SNA), on 2 % (w/v) cornmeal dextrose agar (CMD), on 2 % (w/v) cornmeal sucrose agar (CMA), on 2 % (w/v) malt agar (MA), and on PDA at 25 °C under ambient daylight conditions for 7 days. *Trichoderma* species were identified according to Gams and Bissett (1998) and Samuels et al. (2002, 2009; http://nt.arsgrin.gov/taxadescriptions/keys/TrichodermaIndex.cfm).

PCR amplification and DNA sequencing

Mycelium for DNA extraction was obtained on PD through 3-day incubation at 28 °C on a rotary shaker (180 rpm). Mycelium was collected on filter paper in a Buchner funnel, washed with sterile water, frozen at -20 °C, and freeze-dried. Total DNA was extracted using the CTAB method (Doohan et al. 1998). Primary identification was based on the sequencing of internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of the rRNA gene cluster. In case of failure with ITS 1 and ITS 2 to take unambiguous species identification, we also sequenced a fragment of the translation-elongation factor 1-alpha (*tef*1-α) gene. The ITS region of the rDNA of 1910 isolates was amplified using primers ITS4, ITS5 (White et al. 1990). A fragment of *tef*1-α gene containing the 4th and 5th introns was amplified using the primers Ef728M (Carbone and Kohn. 1999) and tef1R (Kullnig-Gradinger et al. 2002). The PCR reaction was carried out in a 25-μl reaction mixture containing the following: 1 μl 50 ng/μl of DNA, 2.5 μl 10×PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.8, 0.1 % Triton X-100), 1.5 μl 10 mM dNTP Fermentas), 0.2 μl 100 mM of each primer, 19.35 μl MQ H_2O, 0.25 μl (2 U/μl) DyNAzymeTM II DNA Polymerase (Fermentas). Amplifications were performed in a Eppendorf Mastercycler pro Gradient thermal cycler (Shanghai Jiaotong University, China) under the following conditions: initial denaturation 5 min at 94 °C, 35 cycles of 60 s at 94 °C, 45 s at 50 °C (for the ITS region), or 55 °C (for the *tef*1-α fragment), 50 s at 72 °C, with the final extension of 7 min at 72 °C. Amplification products were separated on 1.2 % agarose gel (Invitrogen) in 1×TBE buffer (0.178 M Tris-borate, 0.178 M boric acid, 0.004 M EDTA) and stained with ethidium bromide. The 10-μl PCR products were combined with 2 μl of loading buffer (0.25 % bromophenol blue, 30 % glycerol). A 100-bp DNA Ladder Plus (Fermentas) was used as a size standard. PCR products were electrophoresed at 4 $V \cdot cm^{-1}$ for about 1.5 h, visualized under UV light, and photographed (Syngene UV visualizer). The 3-μl PCR products were purified with exonuclease I and shrimp alkaline phosphatase according to Chełkowski et al. (2003). The amplicons were sequenced with the aid of a MegaBACE 1000 DNA automatic sequencing system (Pharmacia), using cycle-sequencing with the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Pharmacia). Sequences were edited and assembled using Chromas v.1.43 (Applied Pharmacia). CLUSTALW (Thompson et al. 1994) and MUSCLE (Edgar 2004) were used to align the sequences; the resulting alignments were inspected and refined manually.

Phylogenetic analysis

For species identification, ITS1 and ITS2 sequences were submitted to the BLAST interface in NCBI (http://blast.ncbi.nlm.nih.gov/) and TrichOKEY (http://www.isth.info;

Druzhinina et al. 2005; Druzhinina and Kubicek 2005). In ambiguous cases, the result was rechecked using NCBI (http://blast.ncbi.nlm.nih.gov/) and the TrichoBLAST program based on *tef*1-α gene sequences (Druzhinina and Kopchinskiy 2004a, b). The NCBI GenBank accession numbers for some sequences obtained in this study are given in Table 1.

For phylogenetic analyses in MEGA4.0 (Tamura et al. 2004), 93 isolates included in 23 *Trichoderma* species, were selected randomly in different geographical locations. Both ITS1, ITS2 and *tef1*-α gene sequences were analyzed using the maximum parsimony (Eck and Dayhoff 1966) approach of close-neighbor-interchange algorithm with search level 3 (Nei and Kumar 2000), in which the initial trees were obtained with the random addition of sequences (1000 replicates). In total, there were 435 parsimony informative positions retained from an initial alignment of 996 for the ITS1, ITS2 sequences and 840 positions in the final dataset, of which 786 were parsimony informative for *tef1*-α gene sequences. In both cases, to infer the consensus, phylogenetic trees bootstrapping with 1000 data replicates was conducted (Felsenstein 1985).

A five-point sampling method was used. We collected soil core from each of the four corners and a center of the given field (10 m×10 m) by using a hand probe, then combined soil cores, these soil core samples were combined and mixed evenly, and took the central part of each combined sample into bags. The calculation formula of Margalef Species richness index: $d_{Ma}=(S-1)/\ln N$ (S: the total number of species in the community, i.e., species richness) (Hill et al. 2003).

Results

Species identification

A total of 1910 strains of *Trichoderma* were isolated from 491 soil samples collected from 18 provinces and two municipalities in China and then identified by using morphological and molecular methods. All *Trichoderma* strains were identified at species level by the analysis of ITS rDNA and translation-elongation factor 1-alpha (*tef1*-α) gene sequences. Identifications of species were completed by searching the BLAST interface in TrichOKEY, TrichoBLAST (http://www.isth.info) and NCBI. Those strains belonging to 23 taxa were eventually classified into species as follows: *Trichoderma asperellum* (430 isolates), *Trichoderma atrioviride* (25), *Trichoderma aureovriride* (14), *Trichoderma brevicompactum* (101), *Trichoderma citrioviride* (15), *Trichoderma erinaceum* (10), *Trichoderma gamsii* (1), *Trichoderma hamatum* (346), *Trichoderma harzianum* (*Hypocrea 1ixii*) (443), *Trichoderma intricatum* (1), *Trichoderma koningii* (*Hypocrea koningii*) (53), *Trichoderma koningiopsis* (55), *Trichoderma longibranchiatum* (59), *Trichoderma pleuroticola* (22), *Trichoderma reeseii* (*Hypocrea jecorina*) (1), *Trichoderma sinensis* (1), *Trichoderma spirale* (54), *Trichoderma stromaticum* (3), *Trichoderma tomentosum* (1), *Trichoderma velutinum* (4), *Trichoderma vermipilum* (1), *Trichoderma virens* (*Hypocrea virens*) (115), *Trichoderma viride* (155). The most abundant species (23.2 %) isolated from all regions was *T.harzianum* (*H.1ixii*). Besides, *T.intricatum*, *T. stromaticum*, *T.vermipilum* were first reported in China.

Phylogenetic analysis

The phylogenetic relation of 93 regional representative *Trichoderma* isolates was constructed based on the analysis of ITS1 and ITS2 sequences, *tef1*-α sequences (Figs. 1, 2). According to the ITS tree, the *Harzianum* clade, with *T. harzianum* (*H.1ixii*), *T.stromaticum*, *T.tomentosum*, *T.velutinum*; the *Longibrachiatum* Clade, with *T.citrinoviride*, *T. sinensis* and *T.longibrachiatum*, *T.reeseii* (*H.jecorina*); the *Viride* Clade, with *T.atrioviride*, *T.erinaceum*, *T.gamsii*, *T. koningii* (*H.koningii*), *T.koningiopsis*, *T.viride*; the *Pachybasium* A Clade, with *T.asperellum*, *T.hamatum*, *T.aureovriride*, *T.brevicompactum*, *T.intricatum*, *T.spirale*, *T.vermipilum* and *T.virens* (*H.virens*) were distinguished in a single moderately supported branch with bootstrap support of 80 %. 569 *Trichoderma* strains were identified as *T.harzianum* (*H.1ixii*), but this species is known to include several ITS alleles (Hermosa et al. 2004; Migheli et al. 2009) and is considered to be a species complex (Chaverri et al. 2003). In the present research, 12 haplotypes of *T.harzianum* (*H.1ixii*) were found (Fig. 1). With bootstrap support of only 53 %, 12 haplotypes of *T.harzianum* (*H.lixii*) formed a moderately well-supported (80 %) clade with *T.aureovriride*, *T.koningiopsis*, *T.pleuroticola*, *T.tomentosum* and an unresolved polytomy with *T. spirale*, *T.virens* (*H.virens*). Two groups were distinguished within the *Longibrachiatum* clade with moderate to good bootstrap support. One group, with a bootstrap value of 70 %, contains four strains of *T.longibrachiatum*. The second group, with a bootstrap value of 73 % included one strain of *T. longibrachiatum* and one strain of *T.reeseii* (*H.jecorina*). Sixteen strains of *Trichoderma* belonging to the *Viride* clade, formed a polytomy.

A phylogenetic analysis based on *tef*1-α sequences indicated four groups were distinguished within the *Longibrachiatum* clade with moderate to good bootstrap support (Fig. 2). One group, with a bootstrap value of 68 % includes one strain of *T.longibrachiatum* and one strain of *T.reeseii* (*H.jecorina*). The second group, with a bootstrap value of 100 %, contains two strains of *T.citrinoviride.* The third group, with a bootstrap value of 61 %, contains two strains of *T.longibrachiatum*. The fourth group, with a bootstrap value of 100 %, contains two strains of *T.longibrachiatum*. *T.harzianum* (*H.1ixii*) include several *tef1*-α alleles, so itis considered to be a species complex, which is in accordance with Chaverri's view (Chaverri et al. 2003). In the present

Table 1 *Trichoderma* species identified in this study

Culture code	Species	Sources/localization	NCBI GenBank accession number	
			ITS1, ITS2	*tef1*-α
GDFS2012	*T.asperellum*	farmland soil, Guangdong	JQ040310	JQ040494
GXNN1001	*T.asperellum*	forest soil, Guangxi	JQ040311	JQ040493
HBJZ4001	*T.asperellum*	vegetable soil, Hubei	JQ040314	JQ040490
HNCS4014	*T.asperellum*	vegetable soil, Hunan	JQ040315	JQ040489
HNXY1	*T.asperellum*	garden soil, Henan	JQ040316	JQ040488
HNZZ4003	*T.asperellum*	pasture soil, Hainan	JQ040318	JQ040486
SCGA5008	*T.asperellum*	forest soil, Sichuan	JQ040319	JQ040485
SHBS2013	*T.asperellum*	farmland soil, Shanghai	JQ040320	JQ040484
XZNM4010	*T.asperellum*	farmland soil, Tibet	JQ040321	JQ040483
YNKM1067	*T.asperellum*	vegetable soil, Yunnan	JQ040322	JQ040482
ZJSX5001	*T.asperellum*	vegetable soil, Zhejiang	JQ040323	JQ040481
CQSQ5003	*T.atroviride*	vegetable soil, Chongqing	JQ040325	JQ040479
GXNN4017	*T.atroviride*	farmland soil, Guangxi	JQ040326	JQ040478
HNHK1004	*T.atroviride*	pasture soil, Hainan	JQ040327	JQ040477
SHFX3003	*T.atroviride*	forest soil, Shanghai	JQ040328	JQ040476
CQJB5002	*T.aureovriride*	farmland soil, Chongqing	JQ040329	JQ040405
XZNM5001	*T.aureovriride*	farmland soil, Tibet	JQ040330	JQ040404
GDZQ5008	*T.brevicompactum*	vegetable soil, Guangdong	JQ040331	JQ040474
GXNN2002	*T.brevicompactum*	vegetable soil, Guangxi	JQ040332	JQ040473
SHSJ2004	*T.brevicompactum*	forest soil, Shanghai	JQ040333	JQ040472
SXXL1001	*T.brevicompactum*	farmland soil, Shanxi	JQ040334	JQ040471
SHMH1002	*T.citrinoviride*	farmland soil, Shanghai	JQ040335	JQ040470
YNKM5010	*T.citrinoviride*	vegetable soil, Yunnan	JQ040336	JQ040469
GXNN4026	*T.erinaceum*	forest soil, Guangxi	JQ040337	JQ040468
HBWH1	*T.erinaceum*	farmland soil, Hubei	JQ040338	JQ040467
HNHK4002	*T.erinaceum*	pasture soil, Hainan	JQ040339	JQ040475
HNZZ2002	*T.erinaceum*	garden soil, Hainan	JQ040340	JQ040466
SHBS2002	*T.erinaceum*	farmland soil, Shanghai	JQ040341	JQ040465
CQBN3005	*T.gamsii*	garden soil, Chongqing	JQ040342	JQ040464
CDCH1003	*T.hamatum*	forest soil, Sichuan	JQ040343	JQ040463
CQJB2001	*T.hamatum*	garden soil, Chongqing	JQ040344	JQ040462
GDHN1008	*T.hamatum*	garden soil, Guangdong	JQ040345	JQ040461
GXNN1002	*T.hamatum*	farmland soil, Guangxi	JQ040346	JQ040460
HBJZ1001	*T.hamatum*	forest soil, Hubei	JQ040347	JQ040459
HNHK3007	*T.hamatum*	garden soil, Hainan	JQ040348	JQ040458
XZNM4002	*T.hamatum*	pasture soil, Tibet	JQ040349	JQ040457
ASHX4004	*T.harzianum*	farmland soil, Guizhou	JQ040350	JQ040456
CDJJ2006	*T.harzianum*	forest soil, Shanghai	JQ040351	JQ040455
CQSQ3002	*T.harzianum*	vegetable soil, Fujian	JQ040352	JQ040454
FZ1302	*T.harzianum*	vegetable soil, Fujian	HQ259308	HQ222307
GDHN3002	*T.harzianum*	forest soil, Jiangxi	JQ040353	JQ040453
GYHB1005	*T.harzianum*	forest soil, Guiyang	JQ040354	JQ040412
HBXF4018	*T.harzianum*	vegetable soil, Hubei	JQ040355	JQ040452
HNHK5001	*T.harzianum*	vegetable soil, Hainan	JQ040356	JQ040451
NC3206	*T.harzianum*	vegetable soil, Jiangxi	HQ259313	HQ222311
NMMX3008	*T.harzianum*	farmland soil, Neimeng	JQ040357	JQ040450
SH3204	*T.harzianum*	vegetable soil, Shanghai	HQ259316	HQ222315
SHMH1012	*T.harzianum*	vegetable soil, Shanghai	JQ040358	JQ040449

Table 1 (continued)

Culture code	Species	Sources/localization	NCBI GenBank accession number	
			ITS1, ITS2	*tef1*-α
SZ1201	*T.harzianum*	vegetable soil, Shanghaii	HQ259314	HQ222317
XZNM2001	*T.harzianum*	garden soil, Tibet	JQ040360	JQ040447
YNDL1001	*T.harzianum*	vegetable soil, Yunnan	JQ040361	JQ040446
YZ2203	*T.harzianum*	vegetable soil, Jiangsu	HQ259312	HQ222318
ZQ2302	*T.harzianum*	farmland soil, Shandong	HQ259307	HQ222320
CM01	*T.intricatum*	farmland soil, Shanghai	JQ040362	JQ040445
CQSQ5001	*T.koningii*	pasture soil, Chongqing	JQ040363	JQ040444
GDGZ2010	*T.koningii*	pasture soil, Guangdong	JQ040364	JQ040443
CQSQ4004	*T.koningiopsis*	forest soil, Chongqing	JQ040366	JQ040441
GDZQ2006	*T.koningiopsis*	vegetable soil, Guangdong	JQ040367	JQ040440
GXSS1002	*T.koningiopsis*	forest soil, Guangxi	JQ040368	JQ040439
SHSJ8001	*T.koningiopsis*	forest soil, Shanghai	JQ040369	JQ040438
ZJSX4010	*T.koningiopsis*	vegetable soil, Zhejiang	JQ040370	JQ040437
GXBS7002	*T.longibranchiatum*	pasture soil, Guangxi	JQ040371	JQ040436
GYHB2036	*T.longibranchiatum*	pasture soil, Guidong	JQ040372	JQ040435
NJJL501	*T.longibranchiatum*	vegetable soil, Jiangsu	JQ040373	JQ040434
SHQP3006	*T.longibranchiatum*	vegetable soil, Shanghai	JQ040374	JQ040433
SZHQ5	*T.longibranchiatum*	vegetable soil, Jiangsu	JQ040375	JQ040432
XZNM4003	*T.longibranchiatum*	garden soil, Tibet	JQ040376	JQ040431
CQJB5015	*T.pleuroticola*	vegetable soil, Chongqing	JQ040377	JQ040430
FJFZ1004	*T.pleuroticola*	garden soil, Fujian	JQ040378	JQ040429
GXNN4056	*T.reeseii*	forest soil, Guangxi	JQ040380	JQ040427
SH4206	*T.sinensis*	vegetable soil, Jiangxi	JQ040381	JQ040426
ASYJ1006	*T.spirale*	forest soil, Guizhou	JQ040382	JQ040425
GDHN4007	*T.spirale*	forest soil, Guangdong	JQ040383	JQ040424
HNZZ1007	*T.spirale*	forest soil, Hainan	JQ040384	JQ040423
CQSQ1032	*T.stromaticum*	vegetable soil, Chongqing	JQ040385	JQ040422
GXNN7006	*T.stromaticum*	pasture soil, Guangxi	JQ040386	JQ040421
SCGA5003	*T.stromaticum*	forest soil, Sichuan	JQ040387	JQ040407
CQSQ5023	*T.tomentosum*	garden soil, Chongqing	JQ040388	JQ040403
GDZQ5003	*T.velutinum*	vegetable soil, Guangdong	JQ040389	JQ040410
SCGA5001	*T.velutinum*	pasture soil, Sichuan	JQ040390	JQ040409
ZQ3206	*T.velutinum*	vegetable soil, Shandong	JQ040391	JQ040408
GYHB2006	*T.vermipilum*	forest soil, Guangxi	JQ040402	JQ040495
CQJB2002	*T.virens*	forest soil, Chongqing	JQ040392	JQ040411
GDZQ5014	*T.virens*	vegetable soil, Guangdong	JQ040393	JQ040406
GXBS3003	*T.virens*	garden soil, Guangxi	JQ040394	JQ040420
GZGH4004	*T.virens*	forest soil, Guangdong	JQ040395	JQ040419
HNCD2002	*T.virens*	vegetable soil, Hunan	JQ040396	JQ040418
HNSY3018	*T.virens*	garden soil, Hainan	JQ040397	JQ040417
SCGA4002	*T.virens*	vegetable soil, Sichuang	JQ040398	JQ040416
SHSJ1006	*T.virens*	vegetable soil, Shanghai	JQ040399	JQ040415
XZNM2007	*T.virens*	garden soil, Tibet	JQ040400	JQ040414
GDZQ5005	*T.viride*	vegetable soil, Guangdong	JQ040401	JQ040413

research, 17 haplotypes of *T.harzianum* (*H.1ixii*) were found (according to Fig. 2). With bootstrap support of only 53 %, these haplotypes of *T.harzianum* (*H.1ixii*) formed a moderately well-supported clade with *T.citrinoviride*, *T.erinaceum*,

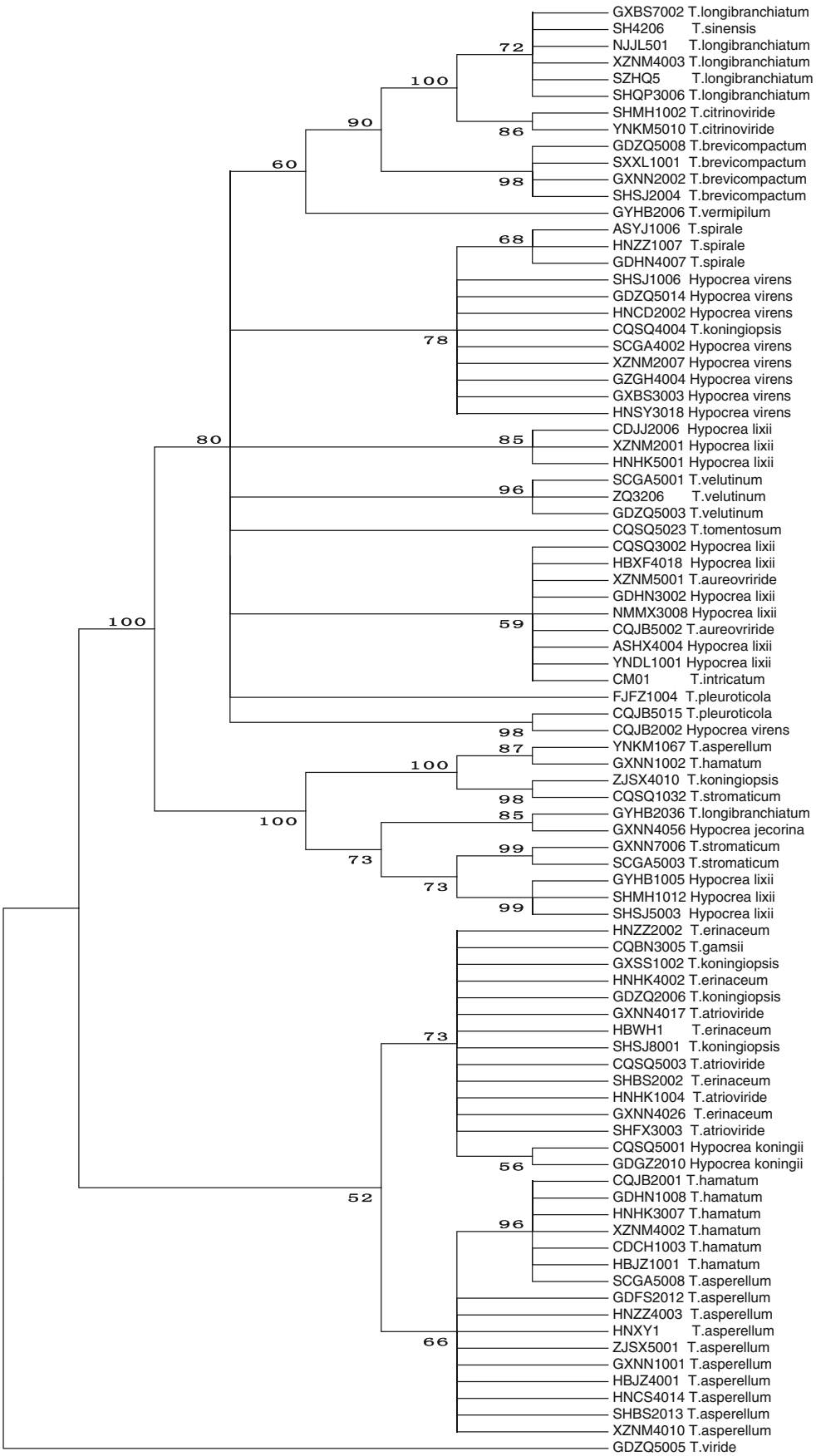

Fig. 1 Phylogenetic tree of the 93 regional representative *Trichoderma* isolates inferred by parsimony analysis of ITS1, ITS2 sequences. Sequences obtained during this study are listed by their GenBank numbers in Table 1. The numbers given over branches indicate bootstrap coefficient >50 %

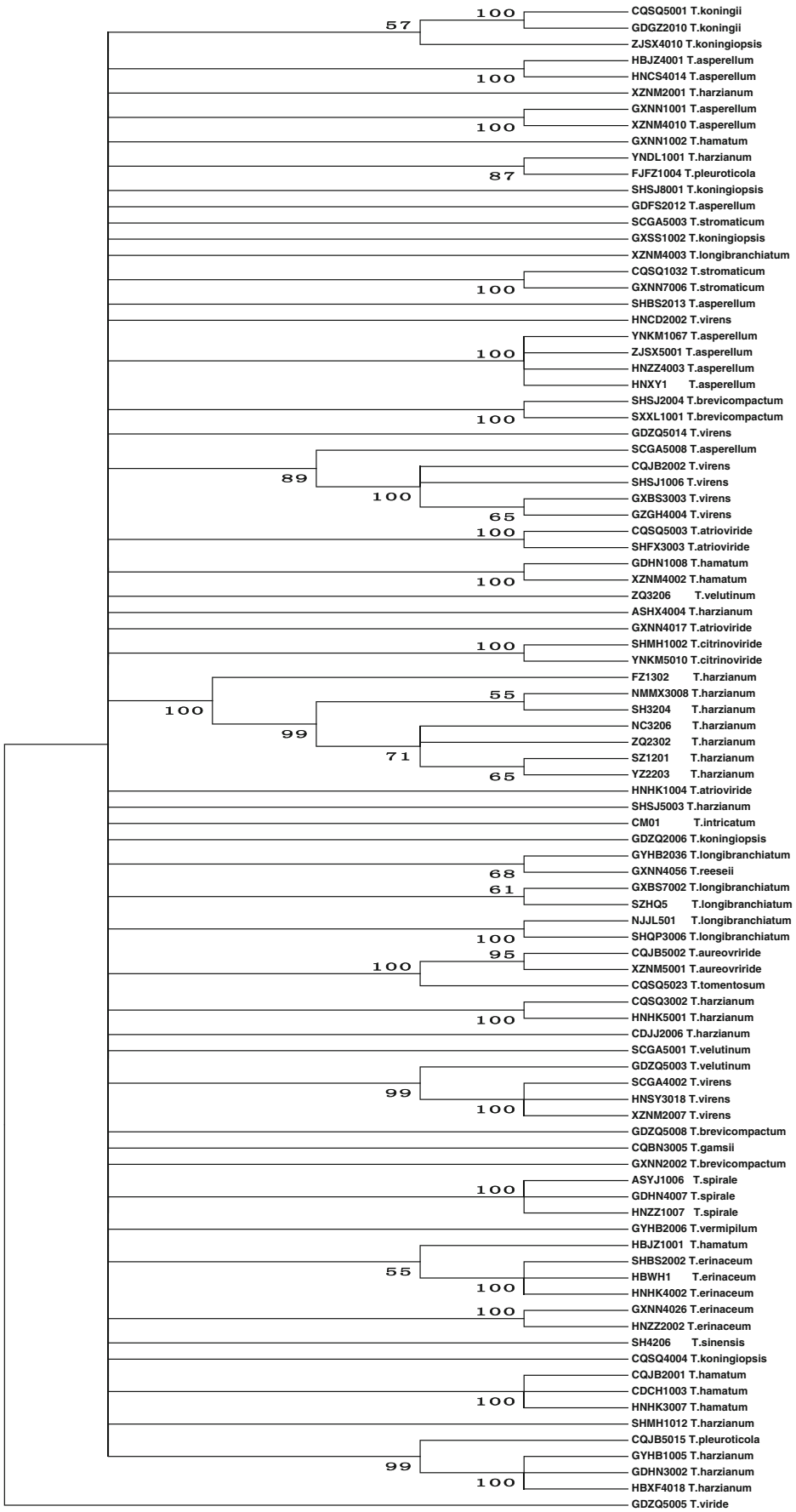

Fig. 2 Phylogenetic tree of the 93 regional representative *Trichoderma* isolates inferred by parsimony analysis of *tef1*-α sequences. Sequences obtained during this study are listed by their GenBank numbers in Table 1. The numbers given over branches indicate bootstrap coefficient >50 %

T.hamatum, *T.koningii* (*H.koningii*), *T.longibrachiatum*, *T. pleuroticola*, *T.reeseii* (*H.jecorina*), *T.tomentosum*, and an unresolved polytomy with *T.aureovriride*, *T.spirale*, *T.virens* (*H.virens*). As a result, the eight species including *T.aureovriride*, *T.citrinoviride*, *T.erinaceum*, *T.koningii*, *T.pleuroticola*, *T.reeseii* (*H.jecorina*), *T.spirale*, *T.tomentosum* were resolved with high bootstrap support.

Species geographic distribution

Twenty three species of *Trichoderma* were identified among 1910 isolates collected from varied soil of 20 different regions in China by using both morphological and molecular analysis, those species were *T.asperellum*, *T.atrioviride*, *T.aureovriride*, *T.brevicompactum*, *T.citrioviride*, *T.erinaceum*, *T.gamsii*, *T. hamatum*, *T.harzianum* (*H.1ixii*), *T.intricatum*, *T.koningii* (*H. koningii*), *T.koningiopsis*, *T.longibranchiatum*, *T.pleuroticola*, *T.reeseii* (*H.jecorina*), *T.sinensis*, *T.spirale*, *T.stromaticum*, *T. tomentosum*, *T.velutinum*, *T.vermipilum*, *T.virens* (*H.virens*) and *T.viride*. Diversity distribution of the species according to their geographic origin revealed significant differences (Table 2, Table 3, Table 4 and Table 5). The species richness of *Trichoderma* according to their geographic origin, see Table 6 for details.

The 94 strains from Middle China (Henan, Hubei and Hunan province) comprised 11 species: *T.asperellum*, *T.citrinoviride*, *T.erinaceum, T.hamatum*, *T.harzianum* (*H.1ixii*), *T. koningii* (*H.koningii*), *T.koningiopsis*, *T.longibranchiatum*, *T. spirale*, *T.virens* (*H.virens*), *T.viride*, in which *T.asperellum* was a dominant species in this region. From East China 1281 strains (Anhui, Fujian, Jiangsu, Jiangxi, Shandong, Zhejiang province and Shanghai municipality) comprised 16 species: *T. asperellum*, *T.atrioviride*, *T.aureovriride*, *T.brevicompactum*, *T.citrinoviride*, *T.erinaceum*, *T.hamatum*, *T.harzianum* (*H.1ixii*), *T.intricatum*, *T.koningii* (*H.koningii*), *T.koningiopsis*, *T.longibranchiatum*, *T.pleuroticola*, *T.sinensis*, *T.virens* (*H.virens*), *T.viride*, in which *T.asperellum* was dominant species in this region. In South-East China (Guangdong, Guangxi, Hainan province), 218 strains comprised 15 species of *Trichoderma*: *T.asperellum*, *T.atrioviride*, *T.aureovriride*, *T.brevicompactum*, *T.citrinoviride*, *T.hamatum*, *T.harzianum* (*H.1ixii*), *T.koningii* (*H.koningii*), *T.koningiopsis*, *T.longibranchiatum*, *T.reeseii* (*H.jecorina*), *T.spirale*, *T.velutinum*, *T. virens* (*H.virens*), *T.viride*, in which *T.harzianum* (*H.1ixii*) was a dominant species. From South-West China 317 isolated (Guizhou, Qinghai, Shanxi, Sichuan and Yunnan province, Tibet Autonomous Region and Chongqing municipality) comprised 20 species: *T.asperellum*, *T.atrioviride*, *T.aureovriride*, *T.brevicompactum*, *T.citrinoviride*, *T.erinaceum*, *T. gamsii*, *T.hamatum*, *T.harzianum* (*H.1ixii*), *T.koningii* (*H. koningii*), *T.koningiopsis*, *T.longibranchiatum*, *T.pleuroticola*, *T.spirale*, *T.stromaticum*, *T.tomentosum*, *T.velutinum*, *T.vermipilum*, *T.virens* (*H.virens*) and *T.viride*, *in which T.harzianum* (*H.1ixii*) was a dominant species.

In terms of geographic distribution level, *T.harzianum* was the most abundant species (23.2 %) distributed widely in China, and followed successively by *T.asperellum* (22.5 %), *T.hamatum* (18.1 %) and *T.viride* (8.1 %). *T.aureovriride*, *T. citrinoviride*, *T.erinaceum*, *T.gamsii*, *T.intricatum*, *T.reeseii* (*H.jecorina*), *T.sinensis*, *T.stromaticum*, *T.tomentosum*, *T.velutinum* and *T.vermipilum* were barely isolated among identified species (≤1 %) of all isolates from the collection.

Table 2 Diversity distribution of *Trichoderma* species in East China

Species	Number of strains isolated in East China						
	Anhui	Fujian	Jiangsu	Jiangxi	Shandong	Shanghai	Zhejiang
T.asperellum	54	61	56	12	39	21	73
T.atrioviride	0	2	4	1	4	0	1
T.aureovriride	0	0	0	1	1	0	0
T.brevicompactum	29	3	13	3	18	3	20
T.citrioviride	0	0	1	0	0	2	0
T.erinaceum	0	0	1	0	0	2	0
T.hamatum	37	61	46	25	58	39	14
T.harzianum	35	59	29	17	38	36	32
T.intricatum	0	0	0	0	0	1	0
T.koningii	6	11	6	7	8	0	0
T.koningiopsis	1	0	0	0	0	3	0
T.longibranchiatum	0	0	14	0	0	29	1
T.pleuroticola	0	16	0	0	0	1	0
T.sinensis	0	0	0	0	0	1	0
T.virens	11	32	8	4	13	4	8
T.viride	13	0	25	48	56	2	1

Table 3 Diversity distribution of *Trichoderma* species in South-West China

Species	Number of strains isolated in South-West China						
	Chongqing	Guizhou	Qinghai	Shanxi	Sichuan	Tibet	Yunnan
T.asperellum	7	6	1	2	8	3	9
T.atrioviride	4	0	0	2	1	0	1
T.aureovriride	2	1	0	1	2	2	1
T.brevicompactum	3	2	0	2	0	0	0
T.citrioviride	0	0	0	0	0	0	1
T.erinaceum	2	2	0	0	1	0	1
T.gamsii	1	0	0	0	0	0	0
T.hamatum	12	5	0	0	8	4	3
T.harzianum	25	8	1	2	53	7	8
T.koningii	2	4	0	0	1	0	2
T.koningiopsis	7	11	0	0	8	3	6
T.longibranchiatum	2	3	0	0	1	3	0
T.pleuroticola	5	0	0	0	0	0	0
T.spirale	3	9	0	0	4	0	4
T.stromaticum	0	2	0	0	1	0	0
T.tomentosum	1	0	0	0	0	0	0
T.velutinum	0	0	0	0	2	0	0
T.vermipilum	0	1	0	0	0	0	0
T.virens	7	4	0	0	6	3	3
T.viride	2	2	0	0	0	0	1

Discussion

The present study was a preliminary domestic assessment of the occurrence of *Trichoderma* diversity in China. Sampling regions were mainly distributed in East, South-East, South-West and middle China; Northern China was not included since a similar study has been completed by other authors in those areas. Our results showed that *Trichoderma* distribution were remarkably diversified, the dominant species and proportion of each species were significantly different in a given area, so much varied distribution might be associated with obviously different ecological environments of vast land in China. On the other hand, the variable character of *Trichoderma* made itself more adaptive to diversified

Table 4 Diversity distribution of *Trichoderma* species in South-East China

Species	Numbers of strains isolated in South-East China		
	Guangdong	Guangxi	Hainan
T.asperellum	22	19	1
T.atrioviride	0	0	5
T.aureovriride	1	2	0
T.brevicompactum	1	3	1
T.citrioviride	2	2	5
T.hamatum	10	5	2
T.harzianum	26	28	10
T.koningii	3	1	0
T.koningiopsis	7	6	2
T.longibranchiatum	3	1	1
T.reeseii	0	0	1
T.spirale	6	4	23
T.velutinum	1	0	0
T.virens	6	2	2
T.viride	2	1	1

Table 5 Diversity distribution of *Trichoderma* species in Middle China

Species	Numbers of strains isolated in Middle China		
	Henan	Hubei	Hunan
T.asperellum	11	16	9
T.citrioviride	0	0	2
T.erinaceum	0	1	0
T.hamatum	2	4	11
T.harzianum	7	17	6
T.koningii	0	0	2
T.koningiopsis	0	1	0
T.longibranchiatum	0	0	1
T.spirale	0	1	0
T.virens	0	0	2
T.viride	0	1	0

Table 6 Species richness of *Trichoderma* according to geographic origin

Geographic origin	Species richness (d_{Ma})	Geographic origin	Species richness(d_{Ma})
East China: Anhui	2.23	South-West China: Chongqing	4.78
East China: Fujian	2.23	South-West China: Guizhou	4.15
East China: Jiangsu	2.87	South-West China: Qinghai	0.32
East China: Jiangxi	2.55	South-West China: Shanxi	1.28
East China: Shandong	2.23	South-West China: Sichuan	3.83
East China: Shanghai	2.55	South-West China: Tibet	1.91
East China: Zhejiang	2.23	South-West China: Yunnan	3.51
Middle China: Henan	0.64	South-East China: Guangdong	3.83
Middle China: Hubei	1.91	South-East China: Guangxi	3.51
Middle China: Hunan	1.91	South-East China: Hainan	3.51

Note: $d_{Ma}=(S-1)/\ln N$ (S: the total number of species in the community, i.e., species richness)

environment. The similar results about *Trichoderma* biodiversity have been found in South-East Asia (Kubicek et al. 2003), Austria (Wuczkowski et al. 2003), South America (Druzhinina et al. 2005), on Sardinia in Italy (Migheli et al. 2009), and in Poland (Błaszczyk et al. 2011). The *Trichoderma* biodiversity extent depended upon the difference level among geographic areas (Hoyos-Carvajal et al. 2009).

Geographical distribution of *T.harzianum* (*H.1ixii*) in China

Based on previous study, *T.harzianum* (*H.1ixii*) has been commonly considered as predominant taxa (Kubicek et al. 2003; Wuczkowski et al. 2003; Druzhinina et al. 2005, 2010; Zhang et al. 2005; Migheli et al. 2009). Using gene sequence analysis, we identified 88 strains as *T.harzianum* (*H.1ixii*) from a total of 382 strains. *T.harzianum* (*H.1ixii*) was the most commonly reported species in the genus, occurring in diverse ecosystems and ecological niches. Since *T.harzianum* (*H.1ixii*) historically originated from China, thus it would be rational that the species distributes most widely in China. Besides, *T.asperellum* and *T.harzianum* (*H.1ixii*), the global distribution species (Danielson and Davey 1973; Chaverri and Samuels 2003), were also identified in our work.

Species difference between East and West China

Different *Trichoderma* species usually require different living environments, and different geographic regions and climate types determined features of *Trichoderma* population and its distribution. Apart from *T.harzianum* (*H.1ixii*), there was much significant difference in *Trichoderma* species distribution between East and West in China. For instance, *T.gamsii*, *T.stromaticum*, *T.tomentosum*, *T.vermipilum* and the majority of strains of *T.aureoviride*, *T.erinaceum*, *T.koningiopsis* were mainly distributed in South-West China. Moreover, *T.reeseii* (*H.jecorina*) and the majority of strains of *T.citrinoviride*, *T. spirale* were found in South-East China. In addtion, *T.intricatum*, *T.sinensis* and the majority of strains of *T.asperellum*, *T. brevicompactum*, *T.hamatum*, *T.koningii* (*H.koningii*), *T.longibranchiatum*, *T.pleuroticola*, *T.virens* (*H.virens*), *T.viride* were found in East China.

This was probably due to climatic preferences of these species. These species are representative of a temperate *Trichoderma* biota (Kubicek et al. 2008). For example, *T. viride* survived better at lower temperatures, however, *T. koningii* and *T.hamatum* seem to exhibit a somewhat higher stress-tolerance than the other species, and tend to be more adaptive to colder soils, winter conditions and acid soils (Widden and Scattolin 1988). In contrast, *T.sinensis*, a species ever isolated from tree bark in Taiwan, China (Bissett et al. 2003), was also found in samples collected from East China, which was demonstrated to be commonly distributed species in South-East Asia (Zhang et al. 2005).

High abundance of *Trichoderma* present in South-West China

We found that the abundance of *Trichoderma* was largest in South-West China among all investigated regions, the phenomenon is understandable because South-West China is a place where varied geographic environments offered plenty of ecological habitats to foster the abundant species resources (http://www.biodiversityhotspots.org/xp/Hotspots/China). Tai and Wen's earlier studies (Tai 1979; Wen et al. 1993) also stressed the abundant diversity of *Trichoderma* spp. in South-West China.

More *Trichoderma* species to be explored in China

Until now, 44 species of *Trichoderma* have been isolated from diversified habitats in China, however, most of the 23 species we identified in this study were mainly included in those known species. In our study, *Trichoderma* strains were mainly sourced from forest soil (22 species/698 isolates), garden soil (20 species/496 isolates) and vegetable soil (13 species/415 isolates), in which 20 species of *Trichoderma* again were isolated from 491 soil samples collected from 18

provinces and two municipalities in China. Furthermore, three species, *T.intricatum*, *T.stromaticum*, *T.vermipilum* were first reported in China.

Some habitats might nurture a very abundant *Trichoderma* population such as crop and forest roots, mushroom, grain crop soil, etc. On the other hand, *Trichoderma* population might dynamically change or vary with seasons, therefore for the next step we will collect samples in different seasons, which will allow us to find species of *Trichoderma*, and a global picture of *Trichoderma* population in China will be generated.

Acknowledgements This work was supported by key grant from Shanghai Committee of Science and Technology (09391910900), 948 project of Ministry of Agriculture (2011 - G4), the National Department Public Benefit Research Foundation of China (200903052), and China Agriculture Research System (CARS - 2).

References

Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Crozier J, Samuels GJ, Choi I-Y, Holmes KA (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta 224:1449–1464

Bissett J, Szakacs G, Nolan CA, Druzhinina I, Kullnig-Gradinger C, Kubicek CP (2003) New species of *Trichoderma* from Asia. Can J Bot 81:570–586

Błaszczyk L, Popiel D, Chełkowski J, Koczyk G, Samuels GJ, Sobieralski K, Siwulski M (2011) Species diversity of *Trichoderma* in Poland. J Appl Genet 52:233–243

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Chaverri P, Samuels G (2003) *Hypocrea*/*Trichoderma* (*ascomycota*, *hypocreales*, *hypocreaceae*): species with green ascospores: Centraalbureau voor Schimmelcultures, pp 1–36

Chaverri P, Castlebury LA, Samuels GJ, Geiser DM (2003) Multilocus phylogenetic structure within the *Trichoderma harzianum*/*Hypocrea lixii* complex. Mol Phylogenet Evol 27:302–313

Chełkowski J, Golka L, Stępień Ł (2003) Application of STS markers for leaf rust resistance genes in near-isogenic lines of spring wheat cv. Thatcher. J App Genet 44:323–338

Danielson R, Davey C (1973) The abundance of *Trichoderma* propagules and the distribution of species in forest soils. Soil Biol Biochem 5:485–494

Doohan FM, Parry DW, Jenkinson P, Nicholson P (1998) The use of species-specific PCR-based assays to analyse Fusarium ear blight of wheat. Plant Pathol 47:197–205

Druzhinina I, Kopchinskiy A (2004a) TrichoBLAST version 1.0, Multiloci database of phylogenetic markers and similarity search. Published online by the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (ISTH). Home page at: http://www.isth.info/tools/blast/index.php

Druzhinina IS, Kopchinskiy AG (2004b) TrichoBLAST version 1.0, *Trichoderma* oligonucleotide key. Published online by the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (ISTH). Home page at: http://www.isth.info/tools/molkey/index.php

Druzhinina IS, Kubicek CP (2005) Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters. J Zhejiang Univ Sci B 6:100–112

Druzhinina IS, Kopchinskiy AG, Komoń M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. Fungal Genet Biol 42:813–828

Druzhinina IS, Kubicek CP, Komoń-Zelazowska M, Mulaw TB, Bissett J (2010) The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. BMC Evol Biol 10:94

Eck RV, Dayhoff MO (1966) Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Springs, MD

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32(5):1792–1797

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Gams W, Bissett J (1998) Morphology and identification of *Trichoderma*. In: Kubicek CP, Hannan GE (eds) *Trichoderma* and *Gliocladium*, vol 1: basic biology, taxonomy and genetics. Taylor and Francis, London, pp 3–34

Gao W, Baoju L, Sun J, Yanxia S (2007) Inderntificaotion of *Trichoderma* Speices from Contaminnated Substrate During Cultivation of *Pleurotus ostreatus*. Acta Edulis Fungi 14(3):81–85

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56

He Z, Sun H, Gao Y (2008) Identification of *Trichoderma* Species on Mushrooms. J Hebei Normal Univ Sci Technol 22(4):41–45

He Z, Gao Z, Gao Y, Zhao B, Zhang X (2010a) ITS sequence of *Trichoderma* species in soil planted vegetables in the greenhouse and UP-PCR analysis on genetic diversity. Acta Phytophylacica Sinica 37(5):459–465

He Z, Gao Z, Hou D (2010b) Study on species of *Trichoderma* on edible fungi and conidiospore production on the edible fungi residue with cotton seed shell as fermentation medium. Jiangsu Agric Sci 3:440–442

Hermosa MR, Keck E, Chamorro I, Rubio B, Sanz L, Vizcaíno JA, Grondona I, Monte E (2004) Genetic diversity shown in *Trichoderma* biocontrol isolates. Mycol Res 108:897–906

Hill TCJ, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. FEMS Microbiol Ecol 43:1–11

Hjeljord L, Tronsmo A (1998) *Trichoderma* and *Gliocladium* in biological control: an overview. In: Harman GE, Kubicek CP (eds) *Trichoderma* and *Gliocladium*, vol 2: enzymes, biological control and commercial application. Taylor and Francis, London, pp 131–151

Hoyos-Carvajal L, Orduz S, Bissett J (2009) Genetic and metabolic biodiversity of *Trichoderma* from Colombia and adjacent neotropic regions. Fungal Genet Biol 46:615–631

Jia DC, Yu ZF, Qiao M, Zhang KQ (2009) A new Chinese record of the genus *Trichoderma*. Mycosystema 28(6):860–862

Klein D, Eveleigh DE (1998) Ecology of *Trichoderma*. In: Harman GE, Kubicek CP (eds) *Trichoderma* and *Gliocladium*, vol 1: basic biology, taxonomy and genetics. Taylor and Francis, London, pp 57–74

Kubicek CP, Penttilä ME (1998) Regulation of production of plant polysaccharide degrading enzymes by *Trichoderma*. In: Harman GE, Kubicek CP (eds) *Trichoderma* and *Gliocladium*, vol 1: basic biology, taxonomy and genetics. Taylor and Francis, London, pp 49–71

Kubicek CP, Bissett J, Druzhinina I, Kullnig-Gradinger C, Szakacs G (2003) Genetic and metabolic diversity of *Trichoderma*: a case study on South East Asian isolates. Fungal Genetics Biol 38:310–319

Kubicek CP, Komoń-Zelazowska M, Druzhinina IS (2008) Fungal genus *Hypocrea*/*Trichoderma*: from barcodes to biodiversity. J Zhejiang Univ Sci B 9(10):753–763

Kullnig-Gradinger CM, Szakacs G, Kubicek CP (2002) Phylogeny and evolution of the genus *Trichoderma*: a multigene approach. Mycol Res 106:757–767

Li G, Chen J, Liu T, Liu L (2010) *Trichoderma koningiopsis*: A New Chinese Record of the Genus *Trichoderma*. Microbiol China 37 (11):1663–1665

Luo X, Wen C (2003) Studies on Eeology and Biological Control of Antagonisti *Trichoderma* spp. Against *Phytophthora capsici* in ya'an. Sichuan Agricultural University, ya'an Sichuan, China

Migheli Q, Balmas V, Komoñ-Zelazowska M, Scherm B, Fiori S, Kopchinskiy AG, Kubicek CP, Druzhinina IS (2009) Soils of a Mediterranean hot spot of biodiversity and endemism (Sardinia, Tyrrhenian Islands) are inhabited by pan-European, invasive species of *Hypocrea*/*Trichoderma*. Environ Microbiol 11(1):35–46

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, New York

Pan Z, Fu J, Ma L, Wang H (2010) Diversity of *Trichoderma* spp. isolated from medicinal plant rhizosphere soils in Liaoning Province. J Zhejiang Univ Agric and Life Sci 36(4):405–410

Samuels GJ, Chaverri P, Farr DF, McCray EB (2009) *Trichoderma* online systematic mycology and microbiology laboratory home page at: http://ntars-gringov/taxadescriptions/keys/TrichodermaIndexcfm

Samuels GJ, Dodd SL, Gams W, Castlebury LA (2002) *Trichoderma* species associated with the green mold epidemic of commercially grown Agaricus bisporus. Mycologia 94:146–170

Shao L, Shi Y, Guo L (2008) *Trichoderma* Species Isolated from Contaminated Mushrooms and Cultivation Substrste in the Beijing Region. Acta Edulis Fungi 15(1):86–90

Sivasithamparam K, Ghisalberti EL (1998) Secondary metabolism in *Trichoderma* and *Gliocladium*. In: Harman GE, Kubicek CP (eds) *Trichoderma* and *Glicoladium*, vol 1: basic biology, taxonomy and genetics. Taylor and Francis, London, pp 139–191

Sun J, Duan Y-X, Lǔ G-Z (2006a) Morphological Identification of *Trichoderma* Species in Liaoning Province. J Fungal Res 4(2):38–44

Sun J, Duan X-X, Lǔ G-Z (2006b) Primary Research On the Diversity Resource of *Trichoderma* in Liaoning Province. J Anhui Agri Sci 34(17):4193–4195, 4197

Tai FL (1979) Sylloge fungorum sinicorum. Science Press, Academia Sinica, Peking, China

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA 101:11030–11035

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Vargas Gil S, Pastor S, March GJ (2009) Quantitative isolation of biocontrol agents *Trichoderma* spp., *Gliocladium* spp. and Actinomycetes from soil with culture media. Microbiol Res 164 (2):196–205

Wen C, Chen W, Tao J (1992) Distribution of *Trichoderma* spp. in the Cotton Soil and Their Biological Activities. Acta Phytopathologica Sinica 4:306

Wen C, Chen W, Tao J (1993) Studies on The Taxonomy of The Genus *Trichoderma* in Southwestern China. Acta Myeologlca Sinlea 12 (2):118–130

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Shinsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322

Widden P, Scattolin V (1988) Competitive interactions and ecological strategies of *Trichoderma* species colonizing spruce litter. Mycologia 80:795–803

Wu X, Wu X, Hu F, He H, Xie B (2008) Identification of *Trichoderma* Species Associated with Cultivated Edible Fungi. J Agric Biotochnol 16(6):1048–1055

Wuczkowski M, Druzhinina I, Gherbawy Y, Klug B, Prillinger HJ, Kubicek CP (2003) Species pattern and genetic diversity of *Trichoderma* in a mid-European, primeval floodplain-Forest. Microbiol Res 158:125–133

Xia C, Chen Y (2009) Biodiversity Research of the Genus *Trichoderma* from Yunnan Province: Data from Morphology and ITS Sequence. Southwest Forestry University, China

Yu H-l, Wei J-g, Su X-l (2010) Investigation on *Trichoderma* resources in forest soil of Northern Guangxi. Guangxi Agric Sci 41(7):703–706

Yu Z-F, Qiao M, Zhang Y, Zhang K-Q (2007) Two new species of *Trichoderma* from Yunnan, China. Antonie van Leeuwenhoek 92:101–108

Yuan Z-L, Chen Y-C, Zhang C-L, Lin F-C, Chen L-Q (2008) *Trichoderma chlorosporum*, a new record of endophytic fungl from Dendrobium in China. Mycosystema 27(4):608–610

Ze F, Qiao Y-M, Zhang Y, Zhang K-Q (2007) Two new species of *Trichoderma* from Yunnan, China. Antonie van Leeuwenhoek 92:101–108

Zhang C-L, Xu T (2004) *Trichoderma* species from China. J Zhejiang Univ Agric and Life Sci 30(4):464

Zhang C-L, Xu T (2005) Records of *Trichoderma* species from Hebei, Zhejiang, Yunnan and Tibet of China. Mycosystema 24(2):184–192

Zhang E, Zhou B, Yang L (1996) Ecological distribution of *Trichoderma* propagules in main vegetation soils of Yunnan. Chin J Appl Ecol 7:78–82

Zhang C-L, Druzhinina IS, Kubicek CP, Xu T (2005) *Trichoderma* biodiversity in China: Evidence for a North to South distribution of species in East Asia. FEMS Microbiol Lett 251:251–257

Zhao Z, Sun X, Yang R, Yang H, Lǔ G (2004) Diversity of *Trichoderma* in greenhouse soil. J Zhejiang Univ Agric and Life Sci 30 (4):467