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The Diversity, Growth Promoting Abilities and Anti-microbial Activities of Bacteria Isolated from the Fruiting Body of *Agaricus bisporus*

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Abstract

Agaricus bisporus plays an important role in ecological processes and is one of the most widely cultivated mushrooms worldwide. Mushroom growth-promoting bacteria have been isolated from casing soil and compost, but microorganisms in the fruiting body have received only a little attention. To get an overview of phylogenetic diversity of microorganisms in the fruiting body of *A. bisporus*, as well as to screen antimicrobial and mushroom growth-promoting strains, and eventually intensify mushroom production, we isolated and characterized microorganisms from the fruiting body of *A. bisporus*. In total, 55 bacterial strains were isolated, among which nine isolates represented Actinomycetes. All the isolates were analyzed by 16S rRNA gene RFLP and sixteen representative strains by 16S rRNA gene sequencing. According to the phylogenetic analysis, eleven isolates represented the Gram-positive *Bacillus*, *Lysinibacillus*, *Paenibacillus*, *Pandorea* and *Streptomyces* genera, and five isolates belonged to the Gram-negative *Alcaligenes* and *Pseudomonas* genera. The bacteria isolated from the fruiting body of *A. bisporus* had broad-spectrum antimicrobial activities and potential mushroom growth-promoting abilities.

Key words: cellulase, IAA, isolation from fruiting body, mushroom growth promoting bacteria, phosphate-solubilizing

Introduction

Agaricus bisporus, commonly known as the white button mushroom (WBM), is one of the most widely cultivated mushrooms in the world. Although compost grown *A. bisporus* lacks the ability to degrade and consume highly substituted xylan fragments (Jurak *et al.*, 2015), it mainly occupies ecological niches rich in lignocellulose such as forest litter, and grasslands, and thus plays an important role in carbon recycling. *A. bisporus* is the model fungus for the adaptation, persistence and growth in the humic-rich leaf-litter environment (Fermor and Wood, 1981; Burton *et al.*, 1997). Apart from its ecological role, *A. bisporus* has been an important component of the human diet for over 200 years and constitutes the biggest part of the total mushrooms consumed in most western countries. *A. bisporus* was relatively rich in monounsaturated fatty acids and ergosterol (Stojković *et al.*, 2014) and could increase the activity of glutathione peroxidase (Maseko *et al.*, 2014), showing its possible role in nutrient metabolism (Jeong *et al.*, 2010). Because of its importance, the genome of this mushroom has been sequenced recently (Morin *et al.* 2012).

Numerous species of soil bacteria which inhabit soil close to plant roots have been reported to play important roles in nutrient mobilization and plant growth (Kumar *et al.*, 2012). These bacteria are known as plant growth-promoting rhizobacteria (PGPR). Indoleacetic acid production, cellulase production and phosphate-solubilization are three key characteristics of PGPR (Ahmad *et al.*, 2008). The PGPR have been studied in detail, yet relatively little is known about the mushroom growth-promoting bacteria (MGPB). Two *Pseudomonas putida* strains, which were isolated from casing layers of *A. bisporus*, were reported to be potent MGPB and possible inoculants to be applied in mushroom production to increase the yield (Zarenejad *et al.*, 2012). Chao *et al.* screened the mycelial surface of *Pleurotus ostreatus* and found that some fluorescent *Pseudomonas* spp. promoted the formation of primordia and enhanced the development of the basidiome of *P. ostreatus* (Cho *et al.*, 2003). These studies strongly suggest that specific bacteria isolated from mushroom substance may have potential applications in mushroom productions.

The transition from vegetative to reproductive growth stage of *A. bisporus* is stimulated by a number

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of factors, among which bacterial activity was implicated as a major contributor (Eger, 1972; Rainey, 1991). The bacteria inhabit on the casing soil may function *via* secretion of hormone-like compound, phosphate solubilizing and siderophores production (Chen *et al.*, 2013; Ali *et al.*, 2012), and it is possible that the bacteria in casing soil may colonize the hyphae and then enter the fruiting body, making these bacteria as potent MGPB. Most of the reported MGPB were isolated either from mycelium or casing soil, whereas bacteria isolated from the fruiting body were less studied. Although the bacterial community structure contained inside the fruiting body of *Morchella* was examined by using uncultured method like denaturing gradient gel electrophoresis (DGGE) technology (Shen *et al.*, 2008), no pure culture strain had been isolated from mushroom fruiting body. The aim of this study was to evaluate mushroom growth-promoting bacteria resources by isolating microorganisms from the fruiting body of *A. bisporus*, by assessing their potential mushroom growth-promotion and antimicrobial activities, and by analyzing the diversity of the isolates using amplified ribosomal DNA restriction analysis (ARDRA). The diversities and bioactivities of these isolates will be useful to exploit the mushroom growth-promoting bacteria resources.

Experimental

Materials and Methods

Sampling of *A. bisporus*. *A. bisporus* fruiting bodies were collected from five traditional *A. bisporus* production areas in Dayi, Dujiangyan, Chongzhou, Shuangliu and Jintang, SiChuan, China. The fruiting bodies were collected at the first flush. All samples were immediately stored in sterilized valve bags and transported to laboratory.

Isolation of microorganisms. The surface of the fruiting bodies was disinfected in 70% ethanol for 60 sec, followed by 1.0% NaOCl for 5 min, and 70% ethanol for 30 sec, thoroughly washed with sterile distilled water. The surface sterilized fruiting bodies were aseptically cut into 5-mm square and put on the beef extract-peptone medium plates, and incubated at 30°C (Hallmann *et al.*, 2006). Six explants were put on each plate. To confirm the success of sterilization process, aliquots of sterile distilled water from the final rinse were inoculated on beef extract-peptone media. Plates were examined for bacterial growth after 3 days, and then on regularly monitored. Picked colonies were spread on plates and reinoculated until pure cultures were obtained. Pure cultures were grown on freshly prepared beef extract-peptone medium slope medium and kept at 4°C.

Indoleacetic acid production assays. Colorimetric method was used for rapid estimation of Indoleacetic acid (IAA) production (Bric *et al.*, 1991). Fifty microliters of logarithmic-phase culture from beef extract-peptone liquid medium was transferred to 3 ml fresh medium containing 0.5 g l^{-1} tryptophan. The transferred cultures were grown at 140 rpm min^{-1} at 28°C for 36 h to reach logarithmic phase. The assay was done in triplicate for each strain. Fifty microliters culture suspension was absorbed into a white porcelain board and, after adding 100 µl of color reagent (4.5 g l^{-1} $FeCl_3$, 57.6% H_2SO_4), the board was incubated at 25°C for 30 min. Development of pink color indicated positive IAA production. A non-inoculated beef extract-peptone liquid medium with tryptophan was used as a negative control.

To quantify IAA production, 4 ml of color reagent was added to 2 ml of culture supernatant which obtained by centrifugation at 10 000 g min^{-1} for 5 min. The mixture was kept at 25°C for 30 min without light. IAA in each supernatant was measured by reading the optical density at 530 nm. The experiment was done in triplicate. IAA concentration in the supernatant was calculated using an IAA standard curve prepared from pure IAA (Sigma-Aldrich).

Phosphate solubilization assay. Fresh cultures of isolates were inoculated on the standard agar medium (pH 6.8–7.0) containing 0.5% tricalcium phosphate (TCP) as the insoluble P source for testing bacteria capable of releasing inorganic phosphate from TCP (Nautiyal, 1999). Sterile medium served as a control. The plates were incubated at 30°C for 7 days. A clear halo zone formed around colonies indicates phosphate solubilization. Experiments were done in triplicate. The ratio of halo zone to colony diameter (HD/CD value) was used as a measure of phosphate solubilization ability.

Extracellular cellulase assay. Cellulase assay was done as described previously (Teather and Wood, 1982). Five microliters of overnight grown culture was spot inoculated on minimal agar plates (0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.1% KCl, 0.05% yeast extract and 1.5% Bacto agar) with carboxymethylcellulose (CMC) sodium salt as the sole carbon source. The plates were incubated at 37°C for 2 days and flooded with an aqueous solution of Congo red (1 mg ml^{-1}) for 15 min. The Congo red solution was then poured off, and plates containing CMC were flooded with 1 M NaCl for 15 min. Finally, the agar was flooded with 1 M HCl, which changes the dye color to blue and inhibits further enzyme activity. Experiments were done in triplicate. A clear halo zone formed around colonies indicated cellulase production. The ratio of halo zoom to colony diameter (HD/CD value) was used as a measure of cellulase production ability.

Evaluation of antimicrobial activity. Six indicator organisms (*Escherichia coli* [ATCC 35218] and *Sta-*

phylococcus aureus [ATCC 25923], *Curvularia lunata* (Walker) Boedijn [SCAU3697], Rhizoctonia solani Ktihn [SCAU3111], *Fusarium oxysporum* f. sp. vasinfectum [SAUM2312] and *Alternaria solani Sorauer* [SCAU3247], obtained from the Maize research institute of Sichuan Agricultural University), were used to test antimicrobial activity as described previously (Taechowisan *et al*., 2003). Antimicrobial activity was detected by formation of an inhibition zone in 3 days.

ARDRA analysis of 16S rDNA. Genomic DNA was prepared using standard methods (Ausubel *et al*., 1995). The 16S rRNA gene was amplified by using universal forward primer P1 and the universal reverse primer P6. Primer P1 (5'-AGAGTTTGATCCTGGTCAGA ACGCT-3') corresponds to positions 8–37 and primer P6 (5'-TACGGCTACCTT GTTACGACTTCACCCC-3') corresponds to positions 1479–1506 in the *E. coli* 16S rRNA gene (Yanagi and Yamasato, 1993). The thermocycler program was 94°C for 5 min, followed by 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min for 35 cycles and with a final 10 min extension at 72°C. PCR products were separated by electrophoresis in a 1% agarose gel.

In the ARDRA analysis, the 1.5 kb PCR products (5 µl) were digested at 37°C for 4 h using restriction enzymes *Hha* I, *Hae* III, *Msp* I and *Taq* I. The restriction fragments were separated and visualized by electrophoresis in 2.5% (w/v) agarose gels. Fragments shorter than 80 bp were excluded from the ARDRA analysis because they were very close to the detection threshold. According to the combined ARDRA patterns, isolates were assigned to different rRNA gene types.

Sequencing and phylogenetic analysis. Based on the phenotypic results and 16S rRNA-ARDRA, representative isolates were chosen for 16S rDNA gene sequencing at Shengong Biotechnology Ltd. (Shanghai, China). Sequences from the isolates were compared with GenBank database using the BLASTN. The closest matching sequences were further pairwise aligned by CLUSTAL X (version 2.0) (Larkin *et al*., 2007). A phylogenetic tree was inferred using neighbor-joining method by MEGA6.0 (Tamura *et al*., 2013). The sequences were assigned GeneBank accession number KJ716488-KJ716500 and KM263532-KM263534

Results

Growth promoting potential of the isolates. In total, 55 culturable bacteria were isolated from *A. bisporus* fruiting bodies, among which 9 actinomycetes isolates were found (Table I).

To test whether the isolates have potential as mushroom growth promoting bacteria (MGPB), their abilities to produce IAA and cellulase and to solubilize phosphate were tested. Altogether 41 isolates showed at least one and nine isolates showed all of the three tested abilities (Table I). In total 36 (78.3%) isolates produced IAA at concentrations ranging from 5.34 µg $ml^{-1}$to 19.2 µg ml^{-1}, 19 (41.3%) isolates solubilizes phosphate with HD/CD values from 1.25 to 2.77, and 29 (63.0%) isolates produced cellulose with HD/CD values from 1.43 to 3.14.

Antimicrobial activity. In the antimicrobial activity assay, forty isolates showed activity against one or more pathogens (Table I). Altogether 25 (45.4%) and 23 (41.8%) isolates were active against *S. aureus* and *E. coli*, respectively. The *C. lunata* and *F. oxysporum* f. sp. vasinfectum were inhibited by 27 (49.1%) isolates. However, only 15 (27.3%) and 11 (20%) isolates inhibited the growth of R. solani Ktihn and *A. solani Sorauer*, respectively. The isolates CZ7, DY17, DYA32 and SLA49 showed significant antagonistic activity against the tested pathogen.

ARDRA and phylogenetic analysis. To estimate the diversity of microorganisms in the *A. bisporus* fruiting body, the 16S rRNA genes of the isolates were analyzed by ARDRA. Sixteen different ARDRA patterns were obtained (Table I). From each ARDRA pattern one representative isolate was chosen for16S rRNA gene sequencing. The 16S rRNA genes of the isolates possessed 99–100% similarity with species in the GenBank.

Eleven isolates represented five Gram-positive genera. Five isolates represented five different *Bacillus* species (Fig. 1). In addition to the *Bacillus* spp. isolates, isolates CZ7, DY21 and CZ12 were identified as Gram-positive strains displaying 99% similarity to the type strains of *L. macroides*, *P. barcinonensis* and *P. pnomenusa*, respectively. Three isolates represented *Streptomycetaceae*.

The five Gram-negative isolates were assigned to two genera (Fig. 1). CZ1, CZ8 and CZ13 belonged to the *Alcaligenaceae*. Isolates DJ35 and CZ9 were assigned as *P. fluorescens*.

A. bisporus is one of the most widely cultivated mushrooms in the world, yet it is still necessary to enhance its production to meet the growing demand. Even though PGPR are widely studied, few studies have systematically analyzed mushroom growth promoting bacteria (MGPB). The microorganisms in casing soil or compost have been found to promote the growth of mushrooms (Cho *et al*., 2003; Zarenejad *et al*., 2012). For example, the transition from vegetative to reproductive growth in *A. bisporus* is stimulated by application of a casing layer, in which some bacteria assist the basidiome initiation (Zarenejad *et al*., 2012). One of the mechanisms by which bacteria stimulate fruiting body formation is thought to remove self-inhibitory substances produced by the vegetative mushroom mycelium (Frey-Klett *et al*., 2011). However, whether the microorganisms inside the fruiting body of *A. bisporus* have potential to promote the growth of *A. bisporus* is still unknown.

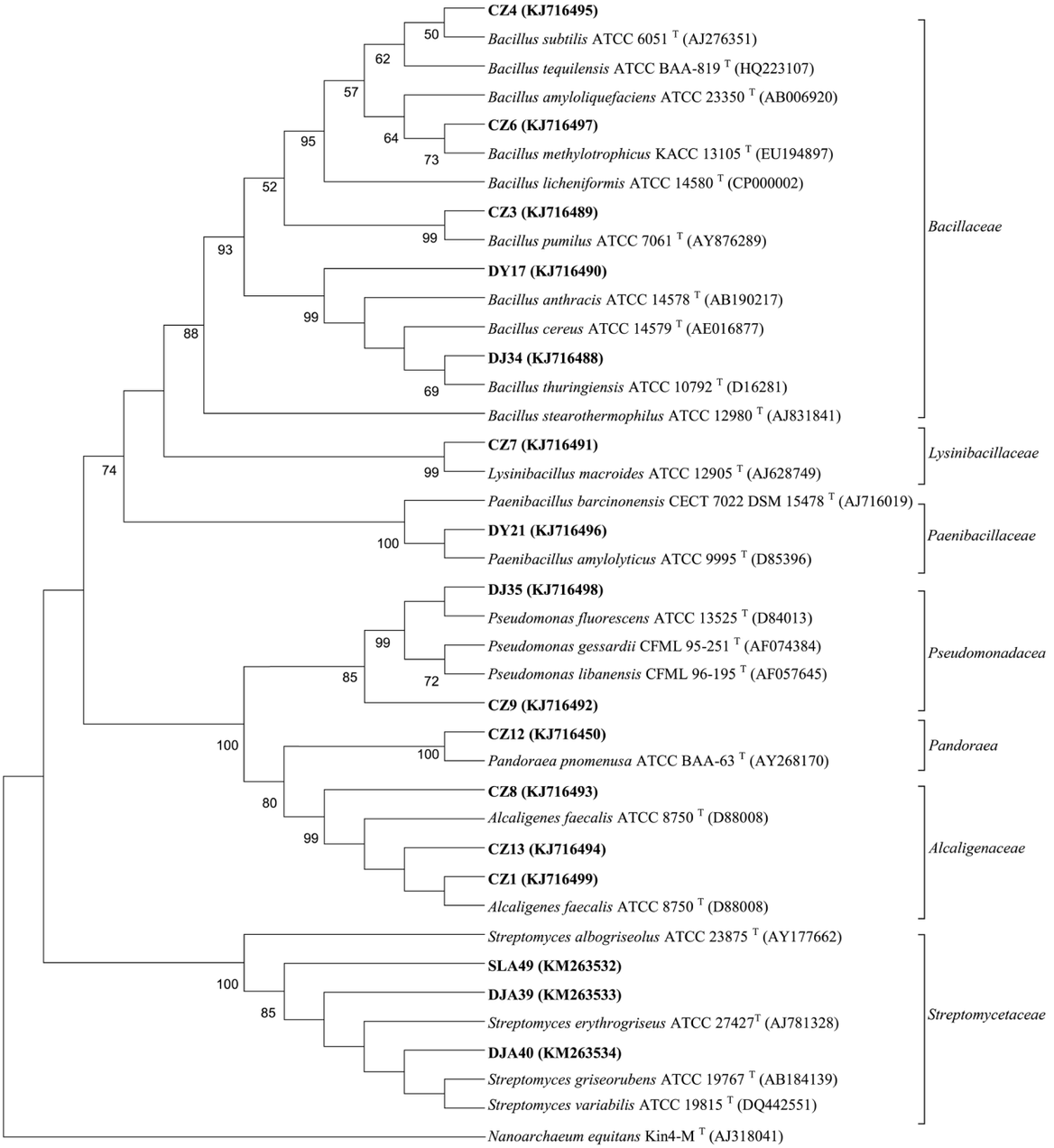

Fig. 1. Neighbor-joining tree based on 16S rRNA sequences of representative isolates (in bold) from the fruiting body of *Agaricus bisporus* and reference strains.

The scale bar corresponds to 0.05 substitutions per nucleotide position. The numbers at nodes indicate the levels of bootstrap support (%) based on 1,000 resampled data sets; GenBank accession numbers in parentheses. Superscript "T" means type stain; *Nanoarchaeum equitans* Kin4-M was used as an outgroup.

The abilities to produce indoleacetic acid (IAA) and cellulase and to solubilize phosphate are key characteristics of plant growth promoting rhizobacteria (PGPR) (Ahmad *et al*., 2008). In our study most of the isolates showed growth promoting abilities. Six, nine and four isolates showed relative strong IAA and cellulase production and phosphate solubilization abilities, respectively. The presence of *P. putida* or *P. fluorescens* in the casing layer is thought to be main contributors to mushroom production of *A. bisporus* (Eger, 1972; Fett *et al*., 1995). The isolate DJ35, assigned as *P. fluorescens*, produced both IAA and cellulase, suggesting it has potential to promote the growth of *A. bisporus*.

A. bisporus can decompose lignocellulosic material (Morin *et al*., 2012), and cellulose is an ideal carbon source for *A. bisporus*. Degradation of the cellulose and

Table I
Geographical origins, rRNA gene type, index of growth promotion factors and antimicrobial activities of endophytes of *A. bisporus*

Geographical origin	Isolate	GenBank accession number	rRNA gene type[a]	Growth promotion factors[b]			Antimicrobial activity[c]					
				PS HD/CD	IAA (μg/ml)	CP HD/CD	1	2	3	4	5	6
Chongzhou	CZ1	KJ716499	14	1.52	8.62 ± 0.22	1.61	–	–	2.11	–	1.55	0.00
	CZ2		2	1.39	5.85 ± 0.37	1.47	2.09	–	1.62	–	–	–
	CZ3	KJ716489	2	1.42	6.62 ± 0.09	–	1.25	1.58	2.36	2.25	1.46	–
	CZ4	KJ716495	9	1.54	8.23 ± 0.59	1.46	1.56	2.11	2.08	–	2.18	–
	CZ5		3	1.63	5.92 ± 0.28	2.00	2.17	1.42	–	–	–	–
	CZ6	KJ716497	4	1.56	7.62 ± 0.20	1.81	2.10	–	1.57	2.09	2.08	1.00
	CZ7	KJ716491	12	1.32	8.38 ± 0.29	3.14	3.22	3.09	1.67	1.75	2.15	0.81
	CZ8	KJ716493	8	–	12.00 ± 0.61	1.43	1.50	2.25	2.00	–	1.70	
	CZ9	KJ716492	13	–	7.85 ± 0.18	1.67	–	–	1.50	–	2.17	0.00
	CZ10		3	–	10.38 ± 0.81	2.67	–	1.27	1.50	–	2.10	–
	CZ11		2	1.49	16.92 ± 1.01	–	–	1.33	1.31	1.57	–	–
	CZ12	KJ716450	6	1.60	6.31 ± 0.37	–	2.18	2.11	–	–	–	–
	CZ13	KJ716494	3	–	7.77 ± 0.66	–	–	2.27	2.09	1.40	1.64	–
	CZ14		3	1.25	–	–	–	1.69	–	–	–	–
	CZ15		4	2.26	–	–	–	–	–	–	–	–
Dayi	DY16		5	1.52	7.46 ± 0.81	–	1.42	–	2.33	1.55	1.67	0.93
	DY17	KJ716490	5	–	8.23 ± 1.12	1.53	2.11	2.30	1.73	2.22	2.33	0.95
	DY18		4	1.63	8.54 ± 0.65	2.71	2.63	1.38	1.42	–	1.45	–
	DY19		4	2.02	9.54 ± 0.34	1.45	–	–	–	–	–	–
	DY20		3	–	6.08 ± 0.31	1.69	1.25	–	1.45	–	–	–
	DY21	KJ716496	10	–	–	2.11	1.18	–	3.18	–	–	–
	DY22		1	–	9.46 ± 0.40	2.00	–	–	–	–	–	–
Dayi	DY23		7	–	6.38 ± 0.52	1.38	–	–	–	–	–	–
	DY24		3	–	–	2.67	–	1.60	–	–	–	–
	DY25		1	–	7.62 ± 0.43	1.50	–	–	–	–	–	–
	DY26		2	–	6.92 ± 0.12	1.64	–	–	–	–	–	–
	DY27		3	–	9.08 ± 0.57	2.00	1.14	–	–	1.50	1.64	–
	DY28		5	1.34	5.54 ± 0.45	–	–	–	–	–	–	–
	DY29		6	–	5.84 ± 0.14	–	–	1.58	–	–	1.31	–
	DYA30		2	nd	nd	nd	2.20	–	1.55	1.45	3.22	–
	DYA31		1	nd	nd	nd	–	–	1.62	1.69	1.64	1.03
	DYA32		2	nd	nd	nd	3.44	3.56	1.83	1.92	2.20	0.87
Dujiangyan	DJ33		1	–	–	1.62	–	2.08	2.45	1.58	1.62	
	DJ34	KJ716488	7	–	5.34 ± 0.22	1.56	–		2.20	1.62	1.78	0.91
	DJ35	KJ716498	11	–	6.38 ± 0.42	2.00	–	2.09	3.44	–	–	–
	DJ36		1	–	–	–	–	1.67	–	–	–	–
	DJ37		3	–	8.23 ±0.32	–	–	–	–	–	–	–
	DJ38		2	–	–	–	–	–	1.54	–	1.38	–
	DJA39	KM263533	16	nd	nd	nd	3.22	3.11	–	–	1.42	–
Dujiangyan Shuangliu	DJA40	KM263534	1	nd	nd	nd	3.00	2.17	–	–	1.92	–
	DJA41		2	nd	nd	nd	3.11	2.30	–	–	1.90	–
	DJA42		5	nd	nd	nd	–	1.62	–	–	–	–
	SL43		1	2.02	12.92 ± 0.65	–	–	–	–	–	–	–
	SL44		2	2.05	7.92 ± 0.52	–	–	1.55	–	–	–	–
	SL45		6	–	19.23 ± 0.43	1.71	–	–	–	–	–	–
	SL46		1	–	–	1.42	1.62	–	–	–	1.62	–

Table I continued

Geographical origin	Isolate	GenBank accession number	rRNA gene type[a]	Growth promotion factors[b]			Antimicrobial activity[c]					
				PS HD/CD	IAA (μg/ml)	CP HD/CD	1	2	3	4	5	6
	SL47		1	–	5.92 ± 0.29	1.67	–	–	–	–	–	–
	SL48		1	–	5.46 ± 0.61	1.33	1.64	–	1.64	–	–	–
	SLA49	KM263532	15				2.25	2.38	1.69	2.33	3.30	0.71
Jintang	JT50		1	–	12.3 ± 0.27	1.50	–	–	–	–	–	–
	JT51		4	–	–	–	–	–	–	–	–	–
	JT52		2	2.77	14.38 ± 0.39	1.80	–	–	–	–	–	–
	JT53		1	1.33	13.62 ± 0.43	–	–	1.69	1.21	–	1.91	–
	JT54		2	–	–	–	–	–	–	–	–	–
	JTA55		1	nd	nd	nd	1.77	–	2.18	2.10	2.17	0.97

The isolated actinomycetes are underlined. The isolates with no accession number were identified by ARDRA only.

[a] rRNA gene types were defined based upon the restriction patterns of ARDRA digested with *Hha* I, *Hae* III, *Msp* I and *Taq* I.

[b] PS: phosphorus solubilization; IAA: Indole-3-acetic acid production; CP: cellulase production; nd: not determined, –,

[c] Indicator strains: 1. *E. coli*, 2. *S. aureus*, 3. *C. lunata* (Walker) Boedijn, 4. *R. solani* Ktihn, 5. *F. oxysporum* f. sp. vasinfectum, 6. *A. solani Sorauer*; (–) no inhibition

hemicellulose in the plant wastes increases the availability of carbon to the cultivated mushroom. Thus, the cellulase producing isolates may promote mushroom growth by degrading cellulose and therefore providing carbon to the host. *Paenibacillus*, which is a predominant endophytic bacterium in colonizing tissue cultures of woody plants (Ulrich *et al*., 2008), are known to produce hormones that stimulate plant growth. In our study, the isolate DY21, assigned as *P. amylolyticus*, showed strong cellulase production ability, which might be one of the mechanisms for *Paenibacillus* to promote growth.

The secondary metabolites produced by microorganisms may confer resistance to pathogenic invasion (Tan and Zou, 2001). The methanolic extract of *A. bisporus* showed relatively high antifungal activity (Stojković *et al*., 2014). In this study, most of isolates exhibited inhibitory activity against the tested pathogens, but the number of strains with antimicrobial activity from different regions were different. Fourteen of the fifteen isolates and half of the isolates from Chongzhou and Jintang, respectively, showed antagonistic activity. The regional difference may be caused by different environmental stress factors. Nine isolates belonging to *Lysinibacillaceae*, *Paenibacillaceae*, *Pseudomonadaceae* and *Streptomycetaceae* genera showed broad antimicrobial activity. Different *B. subtilis* strains produce different antimicrobial compounds (Ongena and Jacques, 2008; Hamdache *et al*., 2011). Our *Bacillus* spp. isolates showed antimicrobial activity against pathogenic bacteria and fungi. Especially, the *B. cereus*-like isolate DY17 inhibited all tested indicator pathogens, making it a good candidate for further studies. Numerous *Streptomyces* strains hosted in medicinal plants produce a wide variety of bioactive metabolites (Li *et al*., 2008). *Streptomyces* spp. are a particularly abundant source of antibiotics and related compounds (Liu *et al*., 2013), and in line with that all our *Streptomyces* isolates showed antimicrobial activity.

In this study, the culturable bacteria isolated from the fruiting body of *A. bisporus* were diverse, representing seven bacterial families. Five of the sixteen sequenced strains were *Bacillus* spp., making *Bacillus* the most dominant genus in *A. bisporus* fruiting body. *Bacillus* spp. and *Pseudomonas* spp. have been isolated from soil samples surrounding *Chroogomphus rutilus* (Wang *et al*., 2011). *Bacillus* spp. that have been isolated from plant rhizospheres and identified as endophytic microorganisms (Forchetti *et al*., 2007) affect the survival of mushroom and decomposition of wheat straw (Bis'ko *et al*., 1995; McDonald *et al*., 1998). *Pseudomonas* spp. are ubiquitous in agricultural soils, and some *Pseudomonas* spp. were found to promote the growth of *P. ostreatus* and *A. bisporus* (Fett *et al*., 1995; Cho *et al*., 2003). Fluorescent *Pseudomonas* spp. accounted for 14–41% of the total bacteria present in casing layer and the populations increased during *A. bisporus* cultivation with positive effects on the mushroom yield (Siyoum *et al*., 2010; Zarenejad *et al*., 2012).

The interaction between bacteria and fungi is complicated. The compost and casing soil layer are the two main possible sources of colonizing bacteria. The timing and means of the fruiting body colonization by bacteria are unknown. However, these microorganisms may coexist with each other. Further studies are needed to test the *in vitro* abilities of these isolates in promoting the growth of *A. bisporus*.

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