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Isolation and Characterization of Phosphate-Solubilizing Bacteria from Mushroom Residues and their Effect on Tomato Plant Growth Promotion

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Abstract

Phosphorus is a major essential macronutrient for plant growth, and most of the phosphorus in soil remains in insoluble form. Highly efficient phosphate-solubilizing bacteria can be used to increase phosphorus in the plant rhizosphere. In this study, 13 isolates were obtained from waste mushroom residues, which were composed of cotton seed hulls, corn cob, biogas residues, and wood flour. NBRIP solid medium was used for isolation according to the dissolved phosphorus halo. Eight isolates produced indole acetic acid (61.5%), and six isolates produced siderophores (46.2%). Three highest phosphate-dissolving bacterial isolates, namely, M01, M04, and M11, were evaluated for their beneficial effects on the early growth of tomato plants (*Solanum lycopersicum* L. Wanza 15). Strains M01, M04, and M11 significantly increased the shoot dry weight by 30.5%, 32.6%, and 26.2%, and root dry weight by 27.1%, 33.1%, and 25.6%, respectively. Based on 16S rRNA gene sequence comparisons and phylogenetic positions, strains M01 and M04 belonged to the genus *Acinetobacter*, and strain M11 belonged to the genus *Ochrobactrum*. The findings suggest that waste mushroom residues are a potential resource of plant growth-promoting bacteria exhibiting satisfactory phosphate-solubilizing for sustainable agriculture.

Key words: 16S rRNA, mushroom residues, phosphate solubilizing bacteria, tomato plant growth

Introduction

In a terrestrial ecosystem, soil microorganisms are important players in the rhizosphere of plants involved in the recycling of nutrients and crucial for long-term soil sustainability (Grönemeyer *et al.*, 2011). After nitrogen, phosphorus (P) is the second major essential macronutrient for plant development in soil, and the lack of P limits plant growth (Nautiyal, 1999; Yu *et al.*, 2011). However, most agricultural soils contain large reserves of total P, which is typically within the range of 0.2–5 g P/kg and with an average of 0.6 g P/kg; moreover, P accumulation partly depends on regular chemical fertilizer application (Fernández *et al.*, 2007). Nevertheless, many soils throughout the world are P-deficient because most of P in nature exists in various organic and inorganic forms; in addition, the concentration of free phosphorus available to plants in fertile soils is generally not higher than 10 μM, even at pH 6.5 at which P is most soluble (Rodríguez and Fraga, 1999; Gyaneshwar *et al.*, 2002).

Although chemical fertilizers are added to the soils, plants can only utilize few amounts of phosphatic fertilizers that are often continuously applied; the remaining amount, which is almost 75–90% of added P fertilizer, is rapidly converted into insoluble complexes, such as calcium phosphate, aluminum phosphate, and iron phosphate in the soil (Gyaneshwar *et al.*, 2002; Vassilev and Vassileva, 2003; Alam and Ladha, 2004). Consequently, chemical fertilizers are frequently applied during crop planting, but its regular use is costly and produces undesirable environmental impacts, such as soil and water contamination. Therefore, P is often regarded a limiting nutrient in agricultural soils (Guiñazú *et al.*, 2010; Yu *et al.*, 2011).

Given the negative environmental impacts of chemical fertilizers and increasing costs, the utilization of phosphate-solubilizing bacteria (PSB) is advantageous for sustainable agricultural practices (Gyaneshwar *et al.*, 2002). PSB could convert these insoluble phosphates into available forms for plant *via* acidification, chelation, exchange reactions, and production of gluconic

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acid (Chung *et al.*, 2005; Gulati *et al.*, 2010). They may also promote plant growth by secreting plant hormones, such as indole acetic acid and cytokinin (Poonguzhali *et al.*, 2008). Currently, many PSB belonging to *Pseudomonas*, *Bacillus*, *Rhizobium*, *Agrobacterium*, *Burkholderia*, *Achromobacter*, *Micrococcus*, *Aerobacter*, *Enterobacter*, *Flavobacterium*, and *Erwinia* have been isolated from soils (Rodríguez and Fraga, 1999). These bacteria can grow in media containing calcium-phosphate complexes as the sole source of P, solubilize and assimilate a large proportion of P, and release P in high amounts. Phosphate is solubilized *via* organic acid synthesis and released by microorganisms (Puente *et al.*, 2004). This reaction, appearing as a halo or clear zone on the plate, is used to assess the P-solubilizing activity of these bacteria. Undoubtedly, the selection of considerably efficient PSB strains as possible inoculants will be a promising way to release large amount of P from soil to improve the current status of extensive chemical fertilizer usage.

Currently, China has become the largest mushroom consuming country; accordingly, a large amount of mushroom residues, which are a kind of solid organic wastes, have been produced annually (Li *et al.*, 2015). Thus, the ecological environment of the planting area is suffering from large mushroom residues characterized by soil pollution and difficult degradation. Nevertheless, mushroom composting has recently attracted significant attention and been regarded an environmentally friendly and sustainable alternative for the management and recycling of organic wastes (Sæbø and Ferrini, 2006; Liu *et al.*, 2015). For instance, mushroom residues can be fermented for use as growing substrates in vegetable seedling breeding. In addition, mushroom compost is an artificial ecosystem that harbors a complete spectrum of microbial diversity (Johri, 2011); thus, isolating PSB from mushroom compost is significant. To date, rare information is available for the screening of PSB from wasted mushroom residues. Therefore, the present work mainly aimed to isolate and characterize native PSB from waste mushroom residues. Their effect on promoting growth of tomato seedlings were also evaluated under greenhouse condition.

Experimental

Materials and Methods

Collection of mushroom residues. In this study, mushroom residues were collected from the experimental basements in Hefei city (117.27°E, 31.85°N). These residues were composed of cotton seed hulls mixed with corn cob (CC), cotton seed hulls mixed with wood flour (CW), cotton seed hulls mixed with biogas residues (CB), and wood flour (WF). All samples were stored at 4°C until analysis and isolation.

Isolation of phosphate solubilizing bacteria. The serially dilution from 10^{-3} to 10^{-5} was achieved by transferring 1 ml of mushroom residue solution from each preceding attenuation stage to the next. Approximately 0.1 ml volume of the resulting solution was then placed on the National Botanical Research Institute's Phosphate growth medium (NBRIP) contained g/l: glucose, 10.0; $Ca_3(PO_4)_2$, 5.0; $MgCl_2 \cdot 6H_2O$, 5.0; $MgSO_4 \cdot 7H_2O$, 0.25; KCl, 0.2 and $(NH_4)_2SO_4$, 0.1, including 0.5% $Ca_3(PO_4)_2$ as an insoluble P source for selectively screening the bacteria capable of releasing inorganic phosphate from tricalcium phosphate (TCP) (Nautiyal, 1999). Sterile medium served as a control. The PSB were identified by clear halo zones around their colonies after 3 days of incubation at 30°C. Experiments were performed in triplicate. Furthermore, the colonies that had larger solubilization zones were further purified. Thirteen PSB strains thus screened were selected for further analysis. All the isolates were designated as M01-13.

Phenotypic characterization of PSB isolates. Physiological and biochemical characteristics of the bacterial isolates were examined according to the methods described in Bergey's Manual of Determinative Bacteriology Edition 8.0 (Holt *et al.*, 1994). All strains were characterized by Gram staining and light microscopy. The direct observation of isolated colonies was served as the first characterization comprising the color, shape, elevation, margins, diameter, and texture. Such traits as endospore, catalase, and starch hydrolysis were characterized (Yu *et al.*, 2011).

Assay of phosphate solubilizing ability. As the plate assay is not considered a reliable method in determining a strain as phosphate solubilizer (Johri *et al.*, 1999), the pure cultures were further screened in liquid medium containing $Ca_3(PO_4)_2$ at a concentration of $5\,g \cdot l^{-1}$ as insoluble P source. Strains were grown in 30 ml liquid medium shaken ($190 \times g$) at 30 ± 1°C for 20 h. One milliliter culture was then transferred to a 300-ml flask containing 80 ml medium previously. Sterile water-inoculated medium was treated as a control. Three Erlenmeyer flasks for statistical replication were used to incubate in the dark on a gyratory shaker ($190 \times g$) at 30 ± 1°C for 3 days. The supernatant of the medium was used to assess P released into the solution. Phosphorus in the culture was determined by the molybdenium blue method with a spectrophotometer at a wavelength of 700 nm (Watanabe and Olsen, 1965).

Characterization of plant growth promoting traits. Indole acetic acid (IAA) production was measured by the colorimetric method (Gordon and Weber, 1951). The isolates were cultivated in a minimal medium (Park *et al.*, 2011) at 25°C for 7 days in a shaking incubator at $120 \times g$. Bacterial cells were removed from the culture

broth by centrifugation (1.5 ml of bacterial suspension). Supernatants were vigorously mixed at a 1:2 ratio with salkowski's reagent, and incubated in the dark for 30 min at 25°C. The absorbance of the final solution was measured at 530 nm. The concentration of IAA in the culture medium was determined using the standard curve of pure IAA (Sangon Biotech Co., Ltd., Shanghai, China).

Siderophore production was determined using an Fe-deficient mineral salt medium (MSM) (Park *et al*., 2011). The selected strains were inoculated in the MSM and incubated in a shaking incubator at 25°C for 3 days at 174 × g. The cell-free culture supernatants were assayed for siderophore production according to the method former described (Schwyn and Neilands, 1987).

Cellulase production was determined in casein yeast extract agar (contained g/l: casein, 5.0; yeast extract, 2.5; glucose, 1.0; agar, 15.0 dissolved in distilled water) medium amended with 1% carboxymethyl cellulose (Teather and Wood, 1982). After 72 h of incubation at 28°C, the agar was flooded with an aqueous solution of congo red (1 mg/ml) for 15 min. The colonies surrounded by clear halos were considered positive for cellulase production.

Proteolytic activity was determined by plating bacteria onto casein yeast extract agar plates containing with 7% skim milk powder (Kumar *et al.*, 2005). After 72 h incubation at 28°C, a clear zone surrounding the colonies is considered as positive.

Sequencing of 16S rDNA gene. Characterization to the genus level of each selected PSB strain was performed by partial sequencing of the 16S ribosomal DNA gene. Genomic DNA was extracted by the phenol/chloroform method (Sambrook *et al.*, 1989) and amplified using PCR amplification of the 16S rDNA. The primers 27 f (5'-GAGATTTGATTCTGGCTCAG-3') and 1495 r (5'-CTACGGCTACCTTGTTACGA-3') were used for amplification. The 50 µl PCR mixtures contained: 0.5 µM of each primer, 200 µM dNTPs, 3 mM $MgCl_2$, PCR reaction buffer (50 mM KCl, 20 mM Tris-HCl at pH 8.0), 1 U Taq DNA polymerase (Promega, USA), and 2 µl of template DNA. The amplifications were carried out in a thermocycler (AB, USA). The PCR was performed as follows: hot start at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 40 s, and extension at 72°C for 3 min. A final extension step was carried out at 72°C for 7 min. After the reaction, 5 µl of the PCR reaction was analyzed in 1.5% agarose gels containing 1 µg/ml of ethidium bromide and photographed with the gel electrophoresis image system. Sequences of the 16S rDNA nucleotide were determined by Sangon Biotech (Shanghai) Co., Ltd. The sequences obtained in this study were analyzed by BLAST algorithm for comparison of a nucleotide query sequence against public nucleotide sequence database to find the closely related bacteria (Yu *et al*., 2011). The nucleotide sequences of the 16S rDNA were subjected to BLAST analysis with the National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp). Sequences with high similarity scores were downloaded from the NCBI database. A phylogenetic tree was constructed using molecular evolutionary genetics analysis (MEGA 4.0) (Tamura *et al.*, 2007). All sequences were deposited in the GenBank sequence database, and the accession numbers are listed in Table I.

Plant growth promotion assay. Tomato seeds (*Solanum lycopersicum* L. Wanza 15) were surface sterilized according to a previous method (Yegorenkova *et al.*, 2001). The seeds were placed in sterile petri dishes,

Table I
Identification of bacterial isolates based on 16S rDNA partial sequence analysis.

Isolate	Organisms identified	Accession number	Closest type strain in RDP data base	16S rDNA identity (%)
M01	*Acinetobacter* sp.	KT964802	*Acinetobacter* sp. 66A1; GQ178052	99.0
M02	*Klebsiella* sp.	KT964803	*Klebsiella* sp. P058; KC252799	99.0
M03	*Enterobacter* sp.	KT964804	*Enterobacter* sp. 3242O2; KF598876	99.0
M04	*Acinetobacter* sp.	KT964805	*Acinetobacter baumannii*; RSO15; KM502224	99.8
M05	*Acinetobacter* sp.	KT964806	*Acinetobacter baumannii*; RSO15; KM502224	99.8
M06	*Bacillus megaterium*	KT964807	*Bacillus megaterium* DPBS17; EU249559	99.0
M07	*Bacillus* sp.	KT964808	*Bacillus megaterium;* F1; FJ009385	99.0
M08	*Bacillus megaterium*	KT964809	*Bacillus megaterium,* DPBS17; EU249559	99.0
M09	*Paenibacillus taichungensis*	KT964810	*Paenibacillus* sp. H420; KJ943997	98.8
M10	*Paenibacillus taichungensis*	KT964811	*Paenibacillus* sp. L202; KJ944125	99.6
M11	*Ochrobactrum* sp.	KT964812	*Ochrobactrum* sp. SCU-B91; KJ000782	99.0
M12	*Sphingobacterium* sp.	KT964813	*Sphingobacterium* sp. 21; NR_074508	95.0
M13	*Sphingobacterium* sp.	KT964814	*Sphingobacterium* sp. 21; NR_074508	95.0

Note: Accession number, the accession number of the strains deposited in the Genbank (NCBI).

properly watered, and germinated in darkness for 2 days at 25°C.

Germinated seeds were inoculated by immersion in appropriate bacterial suspensions (10^8 cells/ml) for 30 min at 28°C. Control seeds were soaked in distilled water, transferred to glass tubes (Diameter 5 cm × Length 10 cm) containing 80 ml of semi-solid Hoagland medium amended with 1% TCP (Hoagland and Arnon, 1950), and kept in a greenhouse at 25°C with a photoperiod of 16 h light and 8 h dark. The dry weights of shoots and roots were calculated after 15 days.

Statistical analysis. All data in the present study were subjected to analysis of variance (ANOVA), and means were separated by the Fisher's protected least significant difference (LSD) test using the SPSS package (version 19.0). Differences obtained at the $P \leq 0.05$ level were considered significant.

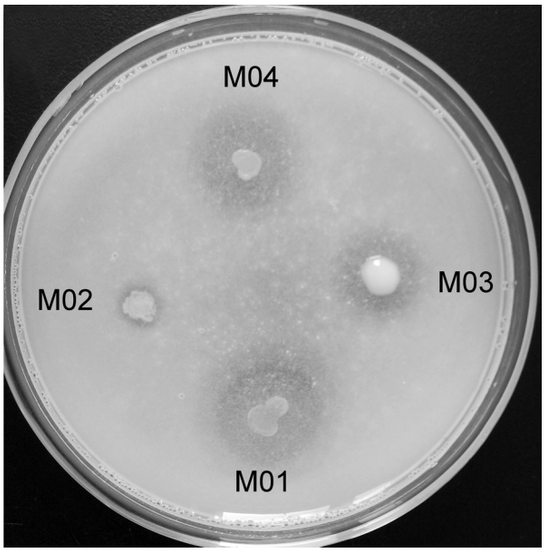

Fig. 1. Dissolved phosphorus halo of isolates on NBRIP solid medium. Strains M01, M02, M03, and M04.

Results

Phenotypic characterization. Thirteen isolates were obtained from mushroom residues by selective NBRIP medium (Fig. 1). Out of 13 isolates, four strains isolated from CC, one strain was separated from each CW and CB, and seven strains were derived from WF (Table II). Most of the strains were Gram-negative, motile rods, and non-endospore forming (Fig. 2). The colonies were milky white and white. The catalase test was positive for twelve isolates. The starch test was positive for ten isolates, and the VP test was positive for four bacterial strains. Nine isolates showed cellulase production and eight ones had proteolytic activity. However, only four strains could utilize of glucose to produce acid (Table III).

Plant growth-promoting trait characterization. Out of 13 tested isolates, eight (61.5%) strains produced IAA within the range of 8.06–62.43 mg/l. Three isolates produced more than 50 mg/l of IAA, and strain M07 showed the highest IAA production (62.43 mg/l). Six (46.2%) isolates produced siderophores, with two strains producing them at high amounts. All the strains can solubilize phosphate ranging within

Table II
Dissolved phosphorus ratio of isolates on NBRIP solid mediumfrom four mushroom residues.

Isolate	Source[a]	Diameter of colonies (cm)	Diameter of hydrolysis circle (cm)	Cultivation time (d)	Dissolved phosphorus ratio[b]
M01	CC	0.6	2.1	4	**0.875**
M02	CC	1.1	2.0	4	0.455
M03	CC	0.7	2.0	4	0.714
M04	CC	0.4	1.8	4	**1.125**
M05	CW	0.6	2.0	4	0.833
M06	CB	0.8	1.3	4	0.406
M07	WF	0.8	1.1	4	0.344
M08	WF	0.4	1.1	4	0.688
M09	WF	0.4	1.0	4	0.625
M10	WF	1.1	1.5	4	0.341
M11	WF	0.5	1.8	4	**0.900**
M12	WF	0.6	1.2	4	0.500
M13	WF	0.4	0.7	4	0.438

[a] CC, Cotton seed hulls + Corn Cob; CW, Cotton seed hulls + Wood flour; CB, Cotton seed hulls + Biogas residue; WF, Wood flour.

[b] Dissolved phosphorus ratio equal to diameter of hydrolysis circle divided by diameter of colonies and cultivation time.

Table III
Selected physiological and biochemical characteristics of phosphate solubilizing strains from waste mushroom residues*.

Isolate	Colony Morphology[a]	Gram stain	Physiological and biochemical characteristics						
			Endos[b]	Cellu[c]	Prote[d]	Catalase	Starch	VP[e]	Fermentation Test (glucose)[f]
M01	RE MW	–	ND	+	+	+	–	–	Acid
M02	RE MW	–	ND	–	–	+	+	–	–
M03	RE MW	–	ND	+	+	+	+	–	Acid
M04	RE MW	–	ND	–	+	+	+	–	–
M05	RE MW	–	ND	+	–	+	–	+	–
M06	RE MW	+	+	+	+	+	+	–	–
M07	IR WH	+	+	+	+	+	+	+	–
M08	RE MW	+	+	+	+	+	+	–	–
M09	RE WH	+	+	+	–	+	+	–	–
M10	RE WH	+	+	–	–	+	+	–	–
M11	RE WH	–	ND	–	–	–	–	–	–
M12	RE WH	–	ND	+	+	+	+	+	Acid
M13	RE WH	–	ND	+	+	+	+	+	Acid

Note: * +, positive; –, negative.
[a] RE, regular; IR, irregular; MW, milky white; WH, white; [b] Endos, endspore; ND, not detected; [c] Cellu, cellulase production; [d] Prote, proteolytic activity; [e] VP (Voges–Proskauer test); [f] Utilization of glucose to produce acid.

17.31–60.87 µg/l. Strains M01, M04, and M11 solubilized phosphate at 54.91, 60.87, and 54.41 µg/ml, respectively (Table IV). In total, five isolates displayed three plant growth-promoting traits.

Phylogenetic analysis. Based on the phylogenetic analysis of the 16S rDNA partial sequence, strains M01, M04, and M05 were identified as *Acinetobacter* sp. Strains M02 and M03 were identified as *Klebsiella* sp. and *Enterobacter* sp., respectively. Moreover, three strains were identified as *Bacillus* sp. Strains M06, M07, and M08 were identified as *B. megaterium*. M09 and M10 were *Paenibacillus taichungensis*, and strain M11 was identified as *Ochrobactrum*. M12 and M13 were both

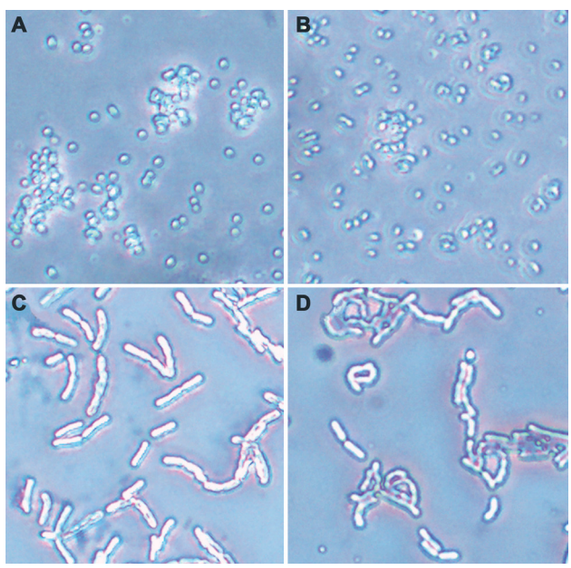

Fig. 2. Single-cell form of isolated strains under optical microscope view. (A) M02, (B) M03, (C) M07, and (D) M10. Bar represents 10 µm.

Table IV
Plant growth-promoting traits of phosphate-solubilizing isolates.

Isolate	Plant growth promoting traits		
	IAA production[a] (mg/l)	Siderophore production[b]	Phosphate solubilization[c] (µg/ml)
M01	–	–	54.91 ± 1.25
M02	11.27 ± 0.85	–	22.94 ± 0.23
M03	13.03 ± 0.79	–	25.66 ± 1.35
M04	10.45 ± 0.65	–	60.87 ± 1.62
M05	–	++++	34.88 ± 1.39
M06	56.32 ± 1.52	+++++	19.45 ± 1.21
M07	62.43 ± 1.52	+++	17.31 ± 1.04
M08	50.87 ±1.10	++++	26.54 ± 1.20
M09	8.06 ± 0.77	++	23.07 ± 0.94
M10	19.82 ± 1.14	++	21.34 ± 2.11
M11	–	–	54.41 ± 1.31
M12	–	–	35.25 ± 0.84
M13	–	–	21.64 ± 1.11

Note: [a] Production of IAA determined in MM liquid medium amended with L-tryptophan after 6 d of growth.
[b] +++++, very high; ++++, high; +++, moderate; ++/+, low; –, not detected.
[c] Amount of phosphorus solubilized into a NBRIP liquid medium.

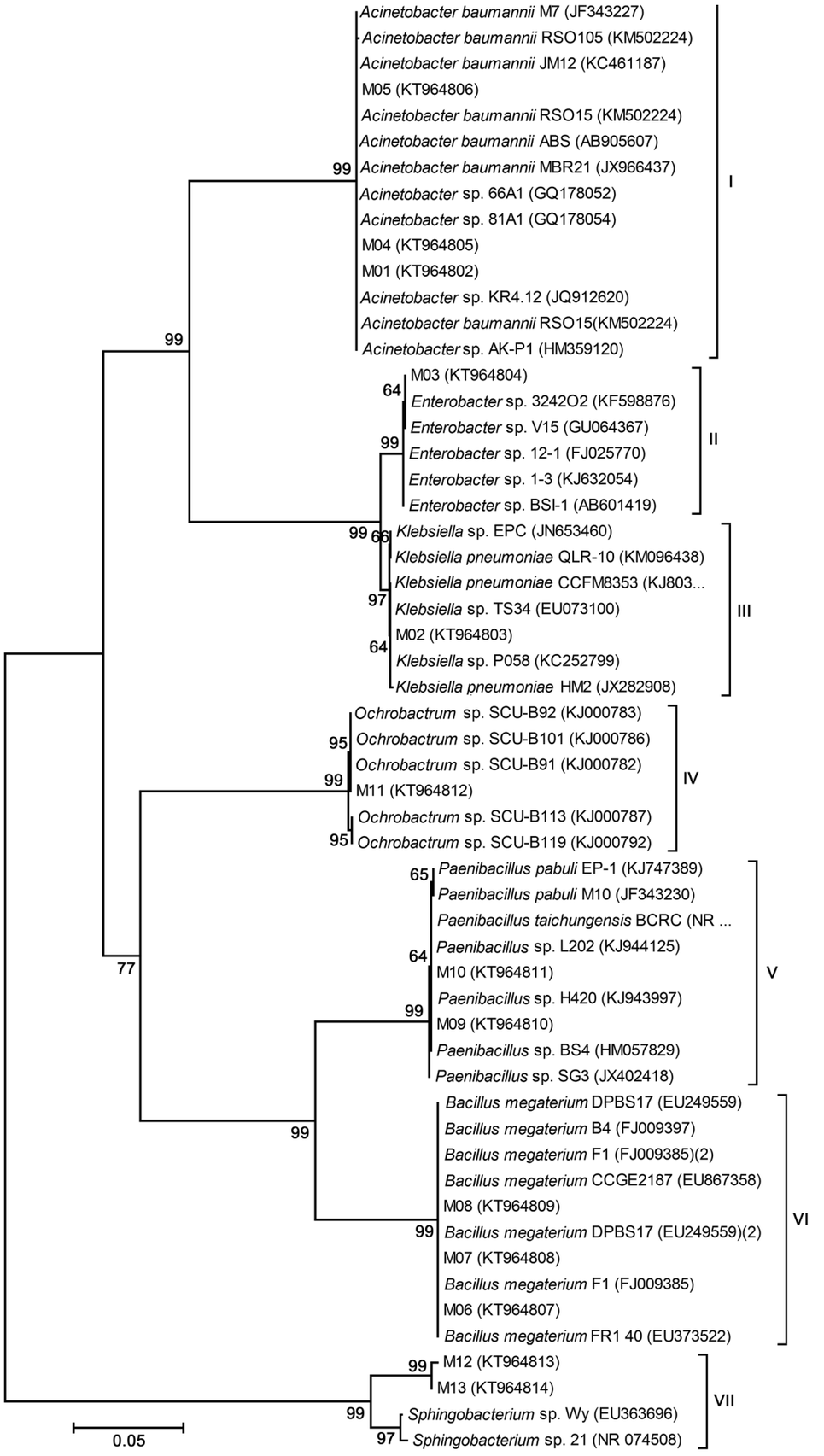
Acinetobacter baumannii M7 (JF343227)
Acinetobacter baumannii RSO105 (KM502224)
Acinetobacter baumannii JM12 (KC461187)
M05 (KT964806)
Acinetobacter baumannii RSO15 (KM502224)
Acinetobacter baumannii ABS (AB905607)
Acinetobacter baumannii MBR21 (JX966437)
Acinetobacter sp. 66A1 (GQ178052)
Acinetobacter sp. 81A1 (GQ178054)
M04 (KT964805)
M01 (KT964802)
Acinetobacter sp. KR4.12 (JQ912620)
Acinetobacter baumannii RSO15(KM502224)
Acinetobacter sp. AK-P1 (HM359120)
I
M03 (KT964804)
Enterobacter sp. 3242O2 (KF598876)
Enterobacter sp. V15 (GU064367)
Enterobacter sp. 12-1 (FJ025770)
Enterobacter sp. 1-3 (KJ632054)
Enterobacter sp. BSI-1 (AB601419)
II
Klebsiella sp. EPC (JN653460)
Klebsiella pneumoniae QLR-10 (KM096438)
Klebsiella pneumoniae CCFM8353 (KJ803...
Klebsiella sp. TS34 (EU073100)
M02 (KT964803)
Klebsiella sp. P058 (KC252799)
Klebsiella pneumoniae HM2 (JX282908)
III
Ochrobactrum sp. SCU-B92 (KJ000783)
Ochrobactrum sp. SCU-B101 (KJ000786)
Ochrobactrum sp. SCU-B91 (KJ000782)
M11 (KT964812)
Ochrobactrum sp. SCU-B113 (KJ000787)
Ochrobactrum sp. SCU-B119 (KJ000792)
IV
Paenibacillus pabuli EP-1 (KJ747389)
Paenibacillus pabuli M10 (JF343230)
Paenibacillus taichungensis BCRC (NR ...
Paenibacillus sp. L202 (KJ944125)
M10 (KT964811)
Paenibacillus sp. H420 (KJ943997)
M09 (KT964810)
Paenibacillus sp. BS4 (HM057829)
Paenibacillus sp. SG3 (JX402418)
V
Bacillus megaterium DPBS17 (EU249559)
Bacillus megaterium B4 (FJ009397)
Bacillus megaterium F1 (FJ009385)(2)
Bacillus megaterium CCGE2187 (EU867358)
M08 (KT964809)
Bacillus megaterium DPBS17 (EU249559)(2)
M07 (KT964808)
Bacillus megaterium F1 (FJ009385)
M06 (KT964807)
Bacillus megaterium FR1 40 (EU373522)
VI
M12 (KT964813)
M13 (KT964814)
Sphingobacterium sp. Wy (EU363696)
Sphingobacterium sp. 21 (NR 074508)
VII
99
99
99
99
66
97
64
99
95
99
95
65
64
77
99
99
99
99
97
0.05

Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence shows the position of isolated strains with the species of each genus downloaded from the NCBI database. Bootstrap percentage values as obtained from 1000 resamplings of the data set are given at the nodes of the tree. Only values higher than 50% are shown. Bar represents 0.05 substitutions per nucleotide position.

Sphingobacterium sp. (Table I). On the basis of the neighbor-joining method, a total of 13 strains were clustered into seven genus groups, and the three major groups were *Acinetobacter*, *Bacillus*, and *Paenibacillus* (Fig. 3).

Plant growth promotion. Three isolates displaying sufficient phosphorus production were chosen to determine their beneficial effects on tomato growth under greenhouse conditions. Tomato shoots grew better than controls when inoculated with the three isolates. All three strain M01, M04 and M11 significantly promoted the shoot dry weight by 30.5%, 32.6% and 26.2%, and root dry weight by 27.1%, 33.1% and 25.6%, respectively, compared to those of the control (Fig. 4).

Discussion

Phosphorus in soil is important for plant development, and the lack of P limits plant growth. Although chemical fertilizers are added to the soils, plants can only utilize low amounts of phosphatic fertilizers. In this case, the selection of highly efficient PSB will practically increase phosphorus in plant rhizosphere. Various PSB have been isolated from different plant roots (Yu *et al.*, 2011; Afshan *et al.*, 2015). Hence, PSB can be regarded as one kind of plant growth-promoting rhizobacteria, which are widely considered as alternatives to common biofertilizers (Vessey, 2003; Hafeez *et al.*, 2006; Adesemoye *et al.*, 2009). In addition, the presence of P-solubilizing microbial population in soils will be considered a positive indicator of utilizing the microbes as biofertilizers for crop production (Afshan *et al.*, 2015). Mushroom waste residues are an artificial ecosystem that harbors a complete spectrum of various bacteria (Johri, 2011). In the present study, a total of 13 isolates were obtained from mushroom residues. Selective NBRIP medium was used for isolation according to their phosphate-solubilizing ability. Our results confirmed that different PSB could be isolated from mushroom resides.

Currently, numerous attempts have been made to isolate the effective plant growth-promoting bacteria according to different criteria, such as auxin and siderophore production or nitrogen-fixing activities (Ding *et al.*, 2005; Chopade *et al.*, 2009; Park *et al.*, 2011; Majeed *et al.*, 2015; Ullah and Bano, 2015). The beneficial effect of PSB in maintaining adequate levels of mineral nutrients, particularly P, in crop production had also been previously reported (Afshan *et al.*, 2015). In the present study, phosphate-dissolving ability was considered a criterion for isolating highly effective PSB strains from waste mushroom residues according to the dissolved phosphorus halo. Out of 13 isolates, seven strains were derived from WF, thereby indicating that wood flour favored the growth of PSB. Considering the isolation of PSB from mushroom resi-

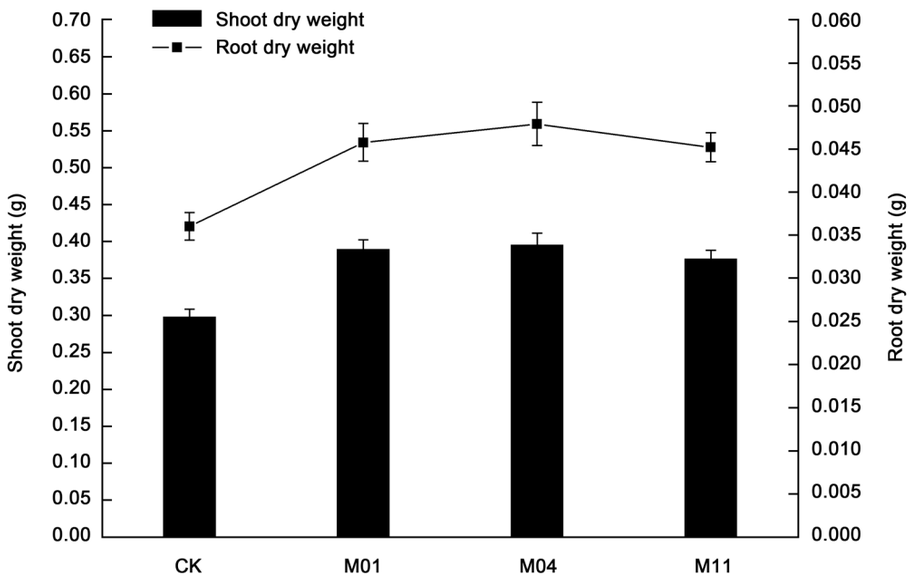

Fig. 4. Growth promotion effects of phosphate-solubilizing isolates on tomato shoot and root dry weights. Significant differences tested according to Fisher's protected LSD at $^{*}p \leq 0.05$. Values are the means ± standard deviations of three experiments.

dues, the amount of isolates in the current study was lower than that reported by Yu *et al.* (2011), but higher than in other reports (Afshan *et al.*, 2015). However, bacteria are considerably diverse because of different crops and soil types. To our knowledge, only few reports have identified the bacterial species in mushroom compost.

IAA production by bacteria isolated from different crops, such as wheat and rice, had already been reported (Park *et al.*, 2005; Afshan *et al.*, 2015). In the current study, about 61.5% of the isolates produced IAA within the range of 8.06–62.43 mg/l, which indicated a substantial variability among isolates for IAA production. Furthermore, approximately 46.2% of strains produced siderophores, and all the strains solubilized tricalcium phosphate. The amount of IAA detected in the present study is close to that reported by Afshan *et al.* (2015), but lower than that reported earlier (Park *et al.*, 2005). These differences may be attributed to the different sources and substantial variability among bacteria. IAA production is also an indicator of plant growth-promoting rhizobacteria (Chopade *et al.*, 2009), thus indicating that the mushroom residues under investigation have bacteria that can enhance plant growth. Previous reports showed that the phosphate solubilization ability of *Pseudomonas* sp. and *Bacillus* sp. is 90 and 60 μg/ml, respectively (Nautiyal, 1999). The same amount of phosphate solubilization abilities were obtained from the mushroom residues in the present study. However, the soluble phosphate released was also lower than that reported by Hafeez *et al.* (2006) and Yu *et al.* (2011). Considering the genus and sources, bacteria may show different phosphate solubilization abilities. In total, about 38.5% of isolates displayed three types of plant growth-promoting traits, hence suggesting the possibility to isolate PSB from mushroom resides.

All the strains displaying phosphate-solubilizing traits were identified based on a 16S rDNA partial sequence. Out of 13 sequenced isolates, 3, 3, and 2 isolates belonged to the cluster of *Acinetobacter* sp., *Bacillus*, and *Paenibacillus* sp., respectively. One isolate belonged to each of the genera *Klebsiella* sp., *Enterobacter* sp., and *Ochrobactrum* sp. Two strains belonged to *Sphingobacterium* sp. The number of isolates belonging to the genera *Acinetobacter* and *Bacillus* was higher than those from other groups, which mean that these two genera are dominant in mushroom waste residues. In addition, *Bacillus* spp. is dominant in root-adhering soil (Laguerre *et al.*, 1994). Based on the neighbor-joining phylogenetic tree constructed from 16S rRNA gene sequences, a total of seven genera were identified in the present study. Therefore, these bacterial strains exhibiting phosphate-solubilizing activity presented large spectrum of microbial diversity, and the present results were consistent with previous findings (Johri, 2011). Notably, strain M11 belonged to genus *Ochrobactrum*, which will help us further understand this genus.

Considering the low solubility of phosphorus in plant rhizosphere soil, three highest phosphorus-dissolving isolates were used to determine their beneficial effects on tomato growth under greenhouse conditions. The inoculation of three isolates M01, M04, and M11 significantly increased the tomato's shoot and root dry weight. This finding indicated that PSB could stimulate early root growth in tomato seedling. Simultaneous growth promotion as the result of PSB inoculations leads to an increase in the yield of maize and other cereals (Ullah and Bano, 2015). The significant increase in plant dry weight caused by the inoculation with PSB strains has also been reported in other plants, such as walnut plant seedlings and wheat (Yu *et al.*, 2011; Afshan *et al.*, 2015). The present results were in agreement with those reported for greenhouse experiments. Hence, inoculation with PSB might have released considerable amount of available P in plant root, which were utilized by plants in the pot experiment. Based on 16S rRNA gene sequence comparisons and phylogenetic positions, isolates M01 and M04 belonged to the genus *Acinetobacter*, and M11 belonged to the genus *Ochrobactrum* sp. To date, few reports have revealed that genus *Ochrobactrum* possesses phosphate-dissolving ability. The use of PSB as inoculants would minimize the negative impact of chemical fertilizers on the environment and promote plant growth. Taken together, the selection of an efficient PSB strain as possible inoculants should be based not only on the laboratory assays and greenhouse trails, but also in field experiments. Further studies should be focused on the practical applications in the field.

Conclusion

A total of 13 strains were isolated in this study. Most of the isolates displayed plant growth-promoting traits, thereby indicating that they have doable function for enhancing plant growth. The present findings suggest that waste mushroom residues are a potential resource of plant growth-promoting bacteria. Furthermore, the obtained isolates exhibited satisfactory phosphate-solubilizing activity for application in sustainable agriculture.

Disclosure statement

The authors declare that they have no competing interests.

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