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Isolation and characterization of *Penicillium decumbens* from the rhizosphere soil of allelopathic rice as mycoherbicide against Barnyard grass

J.Y. Li[1], Q. Zhang, L. Li, W.W. Hu, Y. Chen, S.X. Lin, H.B. He[1]*
Fujian Provincial Key Laboratory of Agroecological Processing and Safety Monitoring, College of Life Sciences,
Fujian Agriculture and Forestry University, Fuzhou 350002, China.
E-mail: alexhhb@163.com

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ABSTRACT

To screen for mycoherbicides, we isolated 33 fungi from the rhizosphere soil of allelopathic rice variety PI312777, using the plate dilution method. One fungal culture with strong weed suppression potential and no adverse effects on the growth of rice, was identified as *Penicillium decumbens* strain ZQ001. The optimal culture conditions for this fungal strain were determined. In pot culture with soil, the fungal culture broth added at 100 µL /g soil concentration, reduced the barnyard grass growth significantly. HPLC analysis of the culture broth showed that it contained protocatechuic, *p*-hydroxybenzoic acid, vanillic acid, salicylic acid and cinnamic acid. Our results suggest that *P decumbens* strain ZQ001 inhibited the barnyard grass through the production of phenolics) and thus could be a potential mycoherbicide.

Key words: Allelopathic rice, barnyard grass, mycoherbicide, *Penicillium decumbens*, phenolic acids, rhizosphere soil, soil fungus, weed suppression

INTRODUCTION

Weeds are major constraint in rice crop production and numerous herbicides are used for their control. Use of herbicides is not environmental friendly and resistance of weeds to herbicides is increasing (4). Hence, there is now worldwide interest in developing environmental friendly weed control techniques to reduce the use of chemical herbicides. Microbial herbicides, are effective against target weeds and their inclusion in the integrated weed management, has also been examined (10,30).

Studies on the application of fungal herbicides began in China in 1960s, when the preparation Lubao No. 1, a culture suspension of *Colletotrichum gloeosporioides f.* sp. *Cuscutaeda*, [a fungal pathogen that causes anthractone disease in plants (1,23,29)], was used to control dodder (*Cuscuae australis*) (12). Since then, there are numerous investigations in this area. Yang *et al*. (33) reported that *Colletotrichum graminicola* KA001 had potential as mycoherbicide to control the barnyard grass (*Echinochloa crus-galli*) in rice fields. *C. graminicola* isolated from the *Echinochlo*a spp. significantly decreased the growth of *Echinochlo*a spp. (22). *Curvularia lunata* strain B6 isolated from the diseased leaves of barnyard grass proved highly pathogenic to barnyard grass seedlings under both controlled conditions and in field tests (17).

Screening of naturally occurring microorganisms for weed suppression to reduce the use of chemical herbicides is an alternative way to protect the environment and ensure sustainable agricultural development. The microbial herbicides are of two types. (i).

*Correspondence author, [1]Key Laboratory of Crop Ecology and Molecular physiology

Herbicidal preparation, which can be directly used to control the pathogens (2,6,17,22,33). (ii). Microbial-derived herbicides preparation which includes secondary metabolites or phytotoxins (9,25,30,36). The use of former would be risky in agricultural system because some organism may be harmful to human health and to beneficial organisms and insects (34). Hence there is need to evaluate the ecological safety and environmental fate before application. For this reason, it is better to pay more attention to the microbial secondary metabolites as new herbicides.

In recent years, the diverse chemicals and biological activities of plant root exudates and secondary metabolites of rhizosphere soil microorganisms, are getting more attention for use as biological agents for weed control (5,35). Dilday *et al*. (8) first reported the allelopathic activity of rice plants to its weeds. Using the inhibitory-circle method, it was found that the allelopathic rice accessions PI312777 and Taichung Native 1, had a weed-free radius of > 10-12 cms. In our earlier work (17,18), we had found that the growth of barnyard grass was effectively suppressed, when planted at distance of 12 cms from the seedlings of allelopathic rice PI312777. This suggested that this rice strain had an allelopathic effect on the barnyard grass. However, whether soil microorganisms are involved in this was not investigated. Screening rice rhizosphere soil organisms for weed suppression by their metabolites is reliable method to identify the mycoherbicide candidates for weeds control. In this study, we screened fungi from the rhizosphere soil of allelopathic rice accession PI312777 and evaluated their ability to suppress barnyard grass growth.

MATERIALS AND METHODS

Lettuce seeds were purchased from Fuzhou Yongrong seed Co. Seeds of commercial rice accession ‘II Youhang 2’ were purchased from Fujian Nongjia Seed Industry Co. Ltd. and barnyard grass (*Echinochloa crus-galli*) seeds were collected from the experimental fields of our University. A strong allelopathic rice accession ‘PI312777’, identified by Dilday *et al*. (8), was used to isolate the fungi from its rhizosphere. The variety ‘II Youhang 2’ is the commercial variety. All herbicidal tests were performed with this commercial rice variety

Fungal isolation

Six pre-germinated seeds of allelopathic rice PI312777 were sown in soil in the nursery at Experimental Station, Fujian Agriculture and Forestry University, Fuzhou city, China (26° 5'15" N and 119° 13'46" E) in July, 2013. The field was irrigated as needed to grow the rice seedlings. When the rice seedlings were at 5-leaf-stage, which is the most allelopathic active stage (18), the rice plants were carefully taken out from the nursery soil. The soil adhering to the roots was scraped off and collected and one composite sample was prepared. Standard microbiological procedures were used to isolate and purify the fungi from the collected rhizosphere soil (3). Briefly, 10 g fresh rhizosphere soil was mixed with 90 mL sterilized water and shaken at 180 rpm for 30 min. The aqueous suspension was diluted 10-folds (10^{-2}, 10^{-3} and 10^{-4} g soil /mL water) with sterile water and 0.5 mL of each dilution was plated on Martin’s Rose Bengal (MRB) agar medium (containing chloramphenicol 1 g/L and gentamycin 2 mL/L to suppress the growth of bacteria) in a 15 cm Petri dish and incubated in culture chamber at 28 °C in dark. Each dilution had 3 replicates. After 4-days, the isolated colonies were selected and purified thrice by repeat

transfer on fresh MRB agar, thrice in succession. By this, 33 fungal colonies were selected and stored on Potato Dextrose Agar (PDA) medium slants at 4 °C.

Each of the 33 isolates were then cultured in 250 mL conical flasks containing 100 mL of sterile Potato Dextrose Broth (PDB) and shaken at 120 rpm for 7 days. The resulting fermentation broths were filtered through double filter papers and then a sterile 0.22-µm membrane filter. The filtrates were tested for their weed suppressive activity by subsequent laboratory bioassays

Bioassay tests for weed suppression

The weed suppression capability of the above fermentation broths was evaluated in laboratory bioassay as per He *et al*. (11). A preliminary screening was done using the germinated lettuce seeds, as the receptor. Five germinated lettuce seeds were placed in a tissue culture dish and 5 mL of fermentation broths was added; 5 mL distilled water was used as control. The dishes with seeds were then incubated in a light incubator (28 °C, 12 h light). Each treatment was replicated thrice. The root length and shoot length of the lettuce seedlings were measured after 3 days and degree of inhibition) was determined as % inhibition (% I) as under:

$$\% I = [(Control - Treatment)/Control] \times 100.$$

Where, % I > 0 indicates growth inhibition; % I < 0 indicates growth promotion.

Fungal isolates that reduced both root length and shoot length of lettuce by 50.0% were selected for further evaluation of their culture fluids on barnyard grass as the receptor. The bioassays were conducted in the same way as described above. Isolates with high inhibition rates on barnyard grass growth were then used to determine their effects on rice growth using rice accession (II You Hang 2) as the receptor.

Identification of the fungal isolate

Characterization of the fungal isolate selected was done by microscopic examination of the mycelia and conidia as described by Wei (32) and Visagie *et al*. (31). From microscopic examination the isolate was identified as a species of *Penicillum.*

Molecular identification of the selected isolate was based on the sequencing of 18S rRNA gene sequence, as follows: A Fungal Genome Extraction Kit (Sangon Biotech, Shanghai, China) was used to purify genomic DNA of the selected fungal isolate, based on the specific primers for fungal identification, upstream primer NS1: 5'-GTAGTCATATGCTTGTCTC-3', downstream primer NS6: 5'-GCATCACAGACCTGTTATTGCCTC -3' to amplify the 18S rRNA of the strains. The size of the target fragment was approximately 1300 bp. The PCR amplification system contained 50 µL, containing 2 × TaKaRa Taq ™ HS Perfect Mix (Takara, Dalian, China) 25 µL, 1 µL of each upstream primer and downstream primer (20 µm/mL), DNA 1 µL and sterile deionized water was added to 50 µL. The reaction conditions were: denaturation at 94 °C for 4 min, cycle parameters were 94 °C for 45 s, 55 °C for 45 s and 72 °C for 60 s. After 35 cycles, extension was performed at 72 °C for 10 min. PCR products (5 µL) were separated on 1.0 % agarose gel and were purified using the Gel Extraction Kit (Omega Bio-Tek, GA, USA) and then ligated into the pBackZero8-T vector with cloning kit (Takara, Dalian, China). In each case, five positive clones were randomly selected and sequenced (Sangon Biotech, Shanghai, China) in both directions using an ABI model 3730 automatic DNA sequencer (ABI, CA, USA). The sequence similarity and multiple sequence alignment of the 18S rRNA gene were analyzed by the BLAST programme provided by the National Center for Biotechnology Information (NCBI). Phylogenetic

analysis of the 18S rRNA gene from the selected isolate with other similar species was performed by Molecular Evolutionary Genetics Analysis 6.05(MEGA 6.05) programme using the neighbour-joining method with the maximum-likelihood model. Bootstrap scores were generated from 1000 replicates. *Penicillium solitum* strain 20-01 (GenBank accession No. JN642222) was used as the out group.

Determining the optimal culture conditions for isolated fungus

(i). **Carbon source:** To select the most optimal carbon source for fungal growth, glucose, sucrose, mannitol and soluble starch were examined. One hundred mL of agar medium (carbon source 10%, NH_4NO_3 0.3%, KH_2PO_4 0.1%, $MgSO_4$ 0.05%, $FeSO_4$ 0.01%, w/v) was prepared in a 250-mL conical flask and sterilized at 121 °C for 20 min. Plates were prepared for each medium and after solidification and cooling, a 0.8-cm fungal disc from a fresh culture slant, was inoculated in the middle of plate. The plates were incubated in an incubator at 28 °C and 2 d after culture, the diameter of the colony was measured.

(ii). **Nitrogen source:** To select the most optimal nitrogen source, agar media containing the soluble starch (10%, w/v) and other salts had different nitrogen sources: peptone, ammonium nitrate ammonium chloride and yeast extract at 0.3% concentration (concentration generally used in conventional media), were prepared and used as above. Colony growth was measured as before.

(iii). **Optimal pH :** To determine the optimal pH for growth, the pH of media containing soluble starch (10%, w/v) as the carbon source and yeast extract (0.3%, w/v) as the nitrogen source and other salts, was adjusted to 5, 6, 7, 8, or 9 with HCl and NaOH and fungal growth was measured as before.

(iv). **Optimal temperature:** To select the optimal growth temperature, agar medium plates with soluble starch (10%, w/v) yeast extract (0.3%, w/v), and other salts pH **7,** were inoculated with the fungus and incubated at 20 °C, 25 °C, 30 °C and 35 °C. Growth was measured as before.

Weed suppression by fungal culture filtrate

These experiments were done at the Agroecological Institute, Fujian Agriculture and Forestry University, Fuzhou, China.The selected fungal culture was grown in 100 ml liquid medium by incubating on a shaker at 180 rpm for 7 d under the optimal culture conditions as described above. The fermentation broth was filtered through a sterile 0.22-µm membrane filter and the filtrate was used to determine its ability to suppress the growth of barnyard grass in pots with soil.

The soil was sandy loam with pH 6.0 ± 0.1 and available NPK were at 41.2 ± 2.76, 132.3 ± 2.37 and 326.5 ± 3.02 mg kg^{-1} respectively (18). Soil (300 g) was placed in plastic pots (12 cm dia × 6 cm height) and 30 mL water was added. Then 5 germinated seeds of barnyard grass were sown in the soil and 30 mL of fungal culture filtrate was gently poured around the seeds. The controls had 30 mL of sterile PDB or 30 mL of water as controls. All the plastic pots were placed in the laboratory under natural light conditions. Night and day time temperatures ranged from 19 to 34°C. Each treatment was replicated thrice. Soil moisture was maintained by spraying water daily. Three days after sowing, the plants were harvested and the roots were washed to remove the culture media. The plant height was measured and the plant dry weights were determined after drying in oven to a constant weight at 80 °C. The degree of growth inhibition was determined by % inhibition over control as mentioned above.

HPLC analysis of fungal culture broth

The fungal culture broth composition was analysed by SPE-HPLC method modified by Li *et al*. (20) and quantified using the external standards. Eight phenolic acids [protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic, salicylic and cinnamic] were chosen as standards for the calibration curve as they affect the barnyard grass (20). All the phenolics were of analytical grade from Aldrich Chemical Co., USA.

To separate the proteins and starch from the culture broth, 300 ml of 95 % ethanol was added to 100 ml of culture filtrate and was allowed to stand for 12 h at 4 °C. The supernatant was then filtered through a 0.450-µm membrane filter and the filtrate was concentrated under reduced pressure to remove alcohol. Then, NaCl was added to 0.8 % (w/v) and the pH adjusted to 4.0 with phosphoric acid. The liquid was then passed through Cleanert PEP solid phase extraction (SPE) cartridge (Agela, China) and eluted with 8 ml of methanol. The eluent was centrifuged and dried in vacuum, dissolved in 1 ml of methanol and filtered through a 0.22-µm membrane filter. Phenolic acids in this preparation were determined using an Agilent 1206 HPLC (Agilent Technologies, USA) equipped with a C_{18} reversed phase column (ZORBAX SB-C_{18}, 150 mm × 4.6 mm, 5 µm). The mobile phase was methanol (A) and 0.1% phosphoric acid solution (B) with the following gradient elution programme: 0-5 min, A: B = 27: 73, 5-10 min A: B = 27: 73, 10-15 min A: B = 50: 50; the detection wavelength was 280 nm; the flow rate was 1.6 ml/min; the injection volume of the sample was 10 µl; the column temperature was 30 °C. The concentration of the various phenolics in the culture broth were determined from the standard references (20).

Statistical analysis

Effects of the fermentation broth on the growth of rice seedlings were analysed using a one-way analysis of variance (ANOVA) followed by the LSD test via IBM SPSS 20.0 program. Statistical significance of all tests was defined as $P < 0.05$. Data were tested for normality and non-normal data were log-transformed to meet assumptions of normality and homoscedasticity.

RESULTS AND DISCUSSION

Weed-suppression effects

In this study, among the 33 isolates obtained from the rhizosphere soil of the allelopathic rice PI312777, the culture filtrates of 14 showed strong inhibitory effects on early root length and shoot length of lettuce (Table 1). These were selected and further evaluated against barnyard grass as the receptor in laboratory bioassays. Of these 14, isolate No. 24 completely inhibited (100 %) the root length and shoot length of barnyard grass. Isolate No. 33 completely inhibited the (100 %) early root length and shoot length by 64.69 % (Fig. 2). These two isolates were then used to determine their effects on rice growth using rice accession (II You Hang 2) as the receptor. The culture broth of isolate No. 24 did not inhibit the early growth of commercial rice accession (II You Hang 2), but isolate No. 33 had no effect on the early root length but inhibited the early shoot length of rice (Fig. 1). The culture filtrate of isolate No. 24 had a strong weed suppression potential but no adverse effects on commercial rice plant growth. Hence, isolate No. 24 was selected for further studies.

Table 1. Inhibitory effects of fermentation broths of 33-fungal isolates on early growth of lettuce h at 3 days after sowing

Strain No.	% I			Strain No.	% I	
	Root length	Shoot length			Root length	Shoot length
1	86.81±2.23	48.97±10.91		18	31.73±4.16	10.96±3.57
2	-38.52±4.62	48.13±3.06		19$^{\Delta}$	94.15±0.97	75.45±0.26
3$^{\Delta}$	83.81±3.57	75.4±3.06		20	37.18±4.27	21.11±2.68
4	47.98±5.97	31.21±2.19		21$^{\Delta}$	82.16±1.98	66.89±2.14
5	66.27±4.9	33.51±6.77		22	73.77±3.41	35.84±6.34
6	50.88±3.56	39.04±2.51		23	82.46±3.75	30.34±2.85
7$^{\Delta}$	86.43±2.23	54.05±9.62		**24**$^{\Delta}$	**100**	**100**
8$^{\Delta}$	61.1±3.68	56.68±4.86		**25**$^{\Delta}$	**100**	**100**
9	78.01±2.95	23.8±4.15		26	52.19±1.81	-12.77±3.76
10	64.86±3.25	31.79±8.55		**27**$^{\Delta}$	**100**	**100**
11	63.74±5.4	30.68±6.08		**28**$^{\Delta}$	**100**	**100**
12	56.99±0.85	2.29±2.14		29	55.28±5.92	10.86±10.74
13$^{\Delta}$	87.01±1.73	58.45±9.41		**30**$^{\Delta}$	**100**	**100**
14	74.89±4.14	26.87±7.33		**31**$^{\Delta}$	**100**	**100**
15	72.62±1.55	43.39±1.69		32	51.38±3.66	31.35±4.01
16$^{\Delta}$	100	53.66±2.41		33$^{\Delta}$	**100**	**100**
17	82.46±1.29	49.35±4.82				

Means ± Standard error (SE) from three replications are mean of 5 plants. The early root and shoot length of the lettuce in control (uncultured broth) was 2.37±0.14 cm and 1.27±0.15 cm, respectively. % I = [(Control - Treatment)/Control] × 100 %. Δ : Selected strains that reduced the root length and plant height of lettuce > 50%, their activity was further tested on barnyard grass as the receptor.

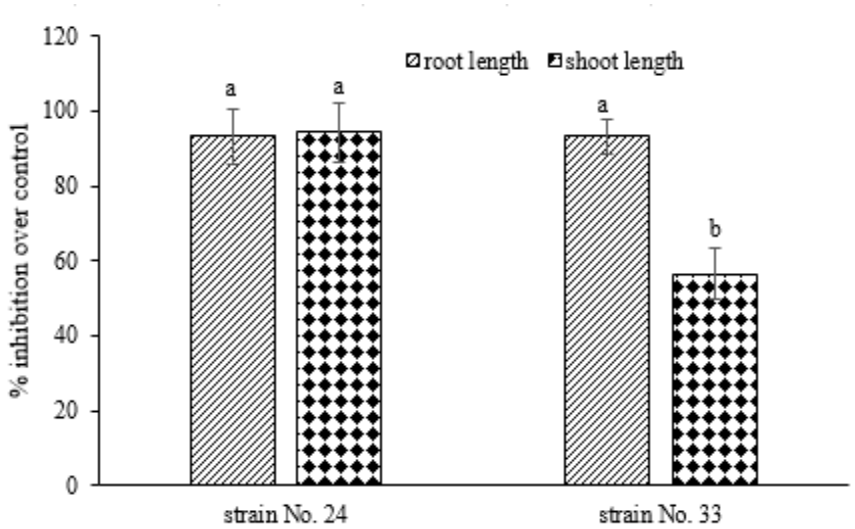

Figure 1. Effects of the fermentation broths of isolate No. 24 and No. 33 on the early growth of rice seedlings (II You Hang 2).Means ±SE from four independent experiments on 5 plants are shown for each determination. % I over control - Treatment/Control× 100 %. A one-way ANOVA is used to analyse the effect (dependent variable: length of rice seedlings; fixed factor: different strains). Columns with different letters indicate significant difference at $p < 0.05$.

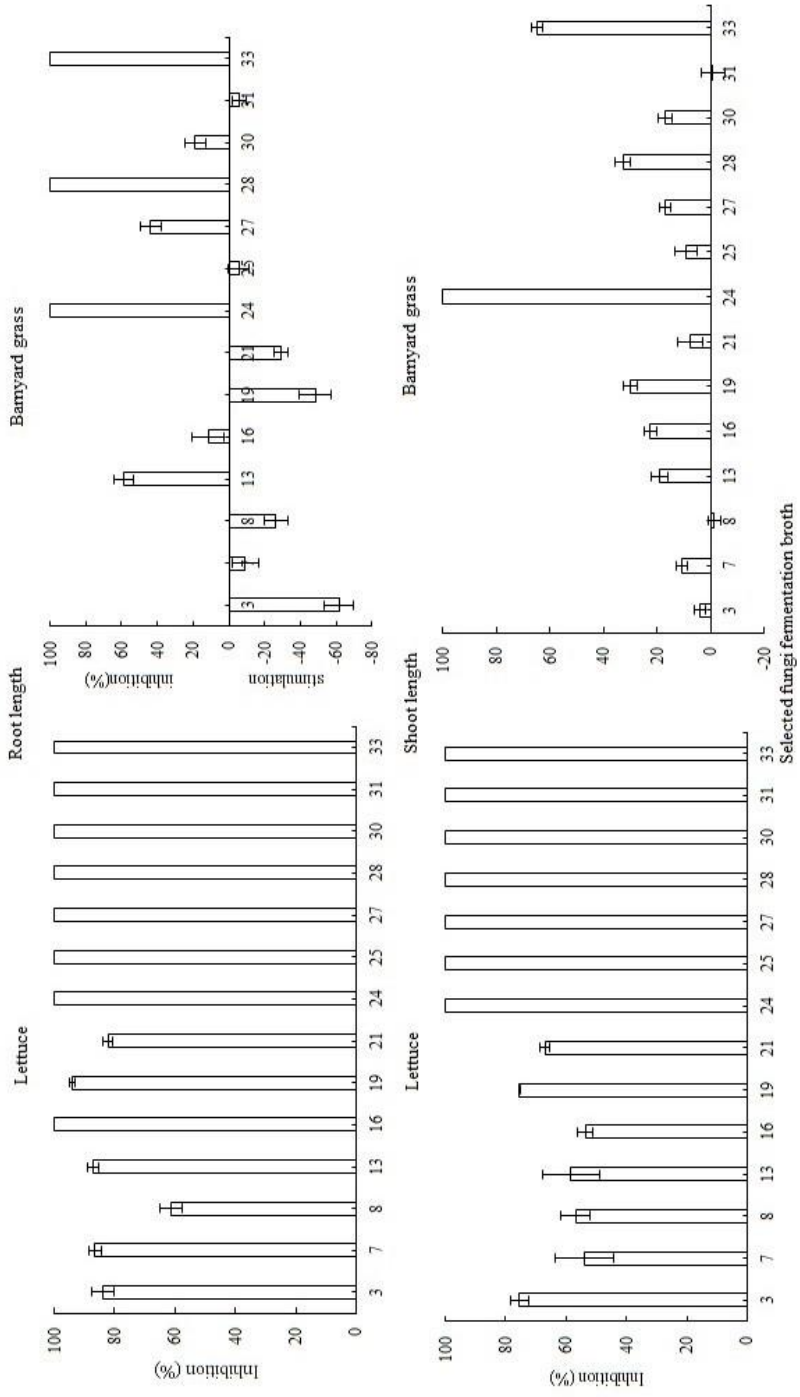

Figure 2. Inhibitory/stimulatory effects of fermentation broth of selected fungi on the lettuce and barnyard grass seedling growth at 3 days after sowing. Means ±SE from four independent experiments on 5 plants are shown for each determination. Inhibition (%)> 0 indicates growth inhibition; Inhibition (%) < 0 indicates growth promotion. promotion.

The colonies of isolate no. 24 on PDA medium were dark green, with a white edge. The surface was dry, powdery and dense without concentric rings. The texture was felt-like, the bottom was yellowish white and there were no surface exudates. Under the microscope, the septate hyphae bore conidiophores with conidia (Fig. 3). This isolate no. 24 was tentatively identified as a species of *Penicillum* based on the description by Wei (32) and Visagie *et al*. (31).

Further, the 18S nucleotide sequence of the isolate no. 24 was performed by BLAST homology alignments online at NCBI (NCBI ID: KF857287). Nine individuals were screened and from the comparision results, the sequence of the isolated strain had the closest relationship with the strains of *Penicillium decumbens* (99% similarity). Moreover, it belongs to the same clade as the *Penicillium decumbens* strain ML-017, *Penicillium decumbens* strain C5 and *Penicillium expansum* strain F3 according to the phylogenetic analysis (Fig. 4). These results strongly suggested that the isolate no. 24 is a strain of *Penicillium decumbens* and was named as *Penicillium decumbens* strain ZQ001.

Optimization of culture conditions for *P. decumbens* ZQ001

The diameter of colonies on various media was used as the basis to evaluate the growth and the optimal culture conditions for the strain ZQ001 as under: carbon source: soluble starch 10%; nitrogen source: Yeast extract 0.3%; optimum pH 7 and the optimum temperature 30 °C. Under the optimum conditions, the colony diameter could reach 6.75 cm in 4 days (Table 2).

Table 2. Optimization of culture conditions for *P. decumbens* ZQ001

Factor	Colony diameter (cm)				
Carbon	Carbon-free 2.52±0.06b	Soluble starch 4.3±0.05a	Glucose 2.13±0.07c	Sucrose 1.8±0.13d	Mannose 2.5±0.11b
Nitrogen	Nitrogen-free 0.60±0.10c	Yeast extract 4.60±0.16a	Peptone 4.27±0.09b	NH_4Cl 1.36±0.03c	NH_4NO_3 1.65±0.03c
pH	5 5.18±0.08b	6 5.24±0.04b	7 5.93±0.02a	8 5.57±0.02ab	9 3.23±0.17c
Temperature (°C)	20 1.40±0.06d	25 2.50±0.06c	30 6.75±0.02a	35 4.40±0.03b	

Means ± standard error (SE) from three replications for each determination are shown. Data in a row followed by the same letter are not significantly different at $p < 0.05$.

Pot culture of fermentation broth of *P. decumbens* ZQ001

In pot experiments with soil, the fermentation broth of *P decumbens* ZQ001 significantly suppressed the growth of barnyard grass (Fig. 5). Compared with the water control, the plant height and plant dry weight of barnyard grass were reduced by 77.22 % and by 46.39 %, respectively (Table 3). The PDB medium also inhibited the barnyard grass plant height by 22.87 %, which was significantly higher than water control, but there was no significant effect on plant dry weight. The effects of PD broth may be due to some components present in the yeast extract and this needs to be investigated. However, the degree of inhibition by the fermentation broth of *P. decumbens* ZQ001 was very strong.

Hence, we isolated a fungus from the rhizosphere soil of the allelopathic rice PI312777 and identified it as a strain of *Penicillium decumbens* (Fig. 3), whose fermentation broths strongly inhibited the early growth of lettuce and barnyard grass in a laboratory bioassays and also significantly suppressed the barnyard grass growth in pot experiment with field soil (Table 3, Fig. 5). This suggested it to be a potential bioherbicide for barnyard grass.

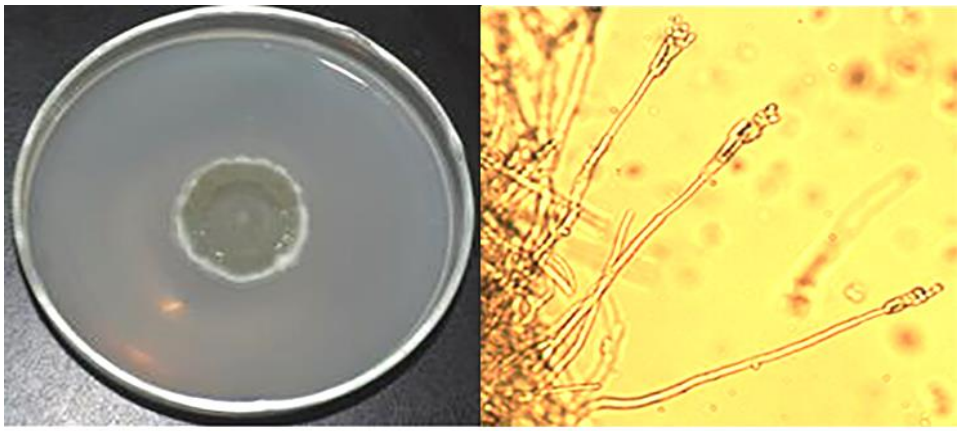

Figure 3. The morphological characteristics of colony and mycelium of Isolate 24.
A: Colony shape. B: Conidiophore and conidia (× 400).

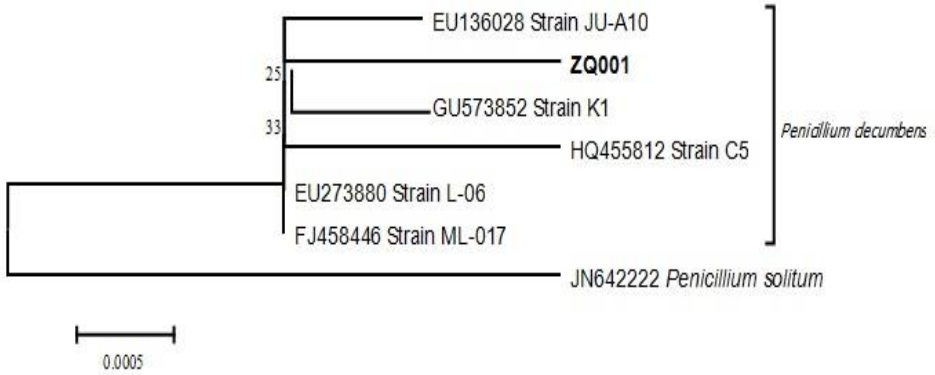

Figure 4. Neighbour-joining the phylogenetic tree based on the 18S rRNA gene sequence of *Penicillium decumbens* ZQ001.

Phenolic acids in the fermentation broth of *P. decumbens* ZQ001

Analysis of the culture broth by HPLC showed the presence of cinnamic acid (3.204 mg/ L), salicylic acid (2.884 mg/L), protocatechuic acid (0.075 mg/L), *p*-hydroxybenzoic acid (0.093 mg/L) and vanillic acid (0.402 mg/L) with cinnamic acid and salicicylic acid dominating. The total contents of the 5-phenolic acids were approximately 6.658 mg /L.

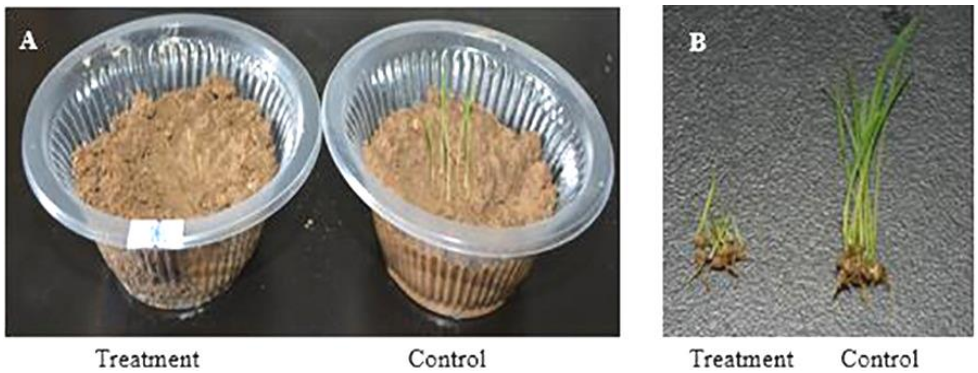

Figure 5. Impact of fermentation broth of *P. decumbens* ZQ001 on the growth of barnyard grass in pot culture with soil
(A : Pot experiment, B : Barnyard grass)

Table 3. Effects of culture broth of *P. decumbens ZQ001* on Barnyard grass inhibition in pot culture with soil

Treatments	Plant height (cm)	Inhibition (%)	Plant dry weight (mg/plant)	Inhibition (%)
Control (water)	3.60±0.07a	-	2.442±0.012a	-
PDB medium	2.78±0.07b	22.87	2.309±0.009a	5.44
Fermentation broth	0.82±0.15c	77.22	1.309±0.002b	46.39

Means ± standard error (SE) from three replications for each determination are shown. % Inhibition = [(Control - Treatment)/Control] × 100 %. Data in a row followed by the same letter are not significantly different at $p < 0.05$.

Many plant secondary metabolites [phenolic acids, flavonoids, terpenoids and steroids] suppress the weeds (11,16,21,26). Although Olofsdotter *et al*. (24) raised doubts about the effects of phenolic acids in allelopathic rice cultivars against weeds, our preliminary research showed that phenolic acids are an essential and principal component in the multi-component systems of rice allelopathy (18,19). The fermentation broths of *P. decumbens* ZQ001 contained a higher concentration of cinnamic acid and salicylic acid and a small amount of protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid, which are widely recognized as rice allelochemicals and found in allelopathic rice culture solutions of PI312777 (20). Therefore, the phenolic acids detected in the fermentation broths of *P. decumbens* ZQ001 may also be involved in weed suppression. Barnyard grass suppression could therefore, be the result of the combined effects of many substances including the chemicals released through the rice roots as well as the chemicals produced by the fungi in the rhizosphere. The present study identified a fungus, which may be involved in controlling the barnyard grass in association with the root exudates of rice plant. However do such cooperative activity persists in the soil, needs further studies. A combined action of rice allelochemicals and secondary metabolites of microorganisms resulting in the formation of the weed inhibitory conditions is possible (8,18). Many

studies have reported that the microbes could mediate (positive or negative) the allelopathic effects of released metabolites of plants (7,14,15,27,28). Our study showed that search for new kind of microbial herbicides from the rhizosphere soils of allelopathic plants, is a potential approach to develop the mycoherbicides.

CONCLUSIONS

We isolated a strain of *P. decumbens* which exerted its influence to suppress the barnyard grass though the production of phenolic acids in its fermentation broth. This strain could be a potential mycoherbicide. Additional field experiments are however needed to verify, whether the fungus produces any phenolics in soil and the application method.

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