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# Identification of Dominant Microbial Community and Diversity in Continuously Cropped Pepper Fields

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## Abstract

Pepper blight is the most significant soil-borne disease affecting the continuous cropping of peppers. To identify the effect of *Phytophthora capsici* infection on microbial flora, we isolated and counted the microorganisms collected from the rhizosphere soil of *P. capsici*-affected farms that continuously cropped pepper for 3, 6, and 9 years in Liaoning Province, China. The colony and cell morphology, physiological and biochemical characteristics, and 16S rDNA sequence of bacteria and actinomycetes were documented. In addition, colony and microscopic morphology of fungi and the rDNA-ITS sequence were analysed for classification. We observed that healthy and diseased peppers had the largest number of bacteria in the rhizosphere followed by actinomycetes and fungi. After infection, the number of bacteria and actinomycetes decreased with a corresponding increase in the number of fungi, leading to a reduction in the ratio of bacteria/fungi to actinomycetes/fungi. We identified 15 dominant bacterial strains, of which *Bacillus* represented the most abundant genus consisting of 7 strains followed by *Flavobacterium* and *Staphylococcus*. Furthermore, 15 of the 17 actinomycetes strains belonged to the genus *Streptomyces*. Among the six fungal strains, we found *P. infestans*, *Fusarium*, and *Penicillium* consisting of two strains each. This study elucidated the impact of pathogenic *P. capsici* on the composition of soil microbes over time and characterized several cultivatable dominant bacterial groups, which can provide a basis for practical intervention strategies to improve soil conditions for continuous cropping.

**Additional key words:** continuous cropping, dominant microorganism, microbial community, pepper, *Phytophthora capsica*

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**Supplementary Material**

Supplementary materials are available at Horticultural Science and Technology website (https://www.hst-j.org/)

## Introduction

Pepper blight caused by *Phytophthora capsici* devastates pepper production and can spread quickly in a short time (Kim et al., 2002; Zhang et al., 2008; Barchenger et al., 2018). Since its isolation from pepper for the first time in 1918 in New Mexico, United States (Leonian, 1922), nearly 80 pathogenic species have been found residing in soil in various states such as mycelium, sporangium, chlamydospores, and spores (Judelson and Blanco, 2005). Therefore, *P. capsici* not only has the ability to resist rhizosphere stresses, but can also easily activate to infect pepper. New sporangium

and zoospores on pepper spread in the soil by rain or through irrigation water, which could lead to a large infestation in the area at the seedling stage. It may also spread through the air, infecting the plant stem, leaves, flowers, and fruits (Silvar et al., 2006). Recently, large production losses caused by pepper blight have been documented in many countries with particularly devastating effects in the United States, Brazil, India, Mexico, Russia, South Korea, Japan, Bulgaria, France, Italy, Argentina, and Pakistan (Hao et al., 2007; Barchenger et al., 2018; Nawaz et al., 2018; Reyes et al., 2019; Reyes et al., 2019). In China, pepper blight was first discovered in Jiangsu province in the 1950s; more than 10 provinces are reporting serious pepper blight infections, especially in the north (Yao et al., 2016). It is the most severe pathogen in the agricultural sector and causes blackening of the vegetable roots with subsequent wilting and necrosis in the earlier stage and plant death in the later stages, resulting in a >20% loss in harvest yield.

Continuous cropping is common in agricultural production and can lead to a series of issues such as degradation of soil quality, aggravation of diseases and pests, and reductions in crop yield (He et al., 2008; Wu et al., 2015). It is the main reason for the spread of soil-borne diseases due to nutrient imbalances, decreases in enzyme activity, and physical/chemical and microflora disturbances (Li et al., 2016). Soil microbes play key roles in soil micro-ecology such as organic matter decomposition, nutrient transport, purification of environmental pollutants, and mediation of greenhouse gases (Kennedy and Smith, 1995; Berendsen et al., 2012; Zhao et al., 2015). Soil microbial activity and respiration intensity are closely related to soil catalase activity, which is an important indicator of the soil micro-ecological environment. Li et al. (2016) reported that the relative abundance of dominant bacteria increases considerably with long-term consecutive monoculture in the rhizosphere of black pepper, while an increasing trend has been observed in the abundance of pathogenic *Fusarium* fungi paired with a decrease in *Pseudomonas* and *Bacillus* bacteria. Similarly, long-term continuous cropping causes a significant decrease in soil pH and organic matter enzymatic activities and results in bacterial decline in black pepper soil (Xiong et al., 2015). This promotes soil microbial imbalances and reduces the number of beneficial microbes while increasing pathogenic microorganisms, leading to a higher susceptibility of plants to various soil-borne diseases. The plant's ability to counter soil-borne disease and the activity of soil enzymes are closely related to the composition of microbes present in the rhizosphere (Magnuson and Crawford, 1992; Simoes et al., 1997). Therefore, improving the soil environment is critical for countering the detrimental effects of continuous cropping. However, there are few studies on the effect of continuous pepper cropping on the microbial community composition in the rhizosphere over time.

The main methods of prevention and control of pepper blight are the use of resistant pepper varieties and chemical agents. However, the lack of resistant varieties and the problems of using chemical agents limit the prevention and control of pepper blight (Hartman and Wang, 1992; Hai et al., 2013; Barchenger et al., 2018). Therefore, biological control has become an attractive option and has been studied by many researchers, in which screening for bacteria that have the ability to control pepper blight is a key process (Sid et al., 2003; Wang et al., 2019). Many microorganisms with the ability to control pepper blight have been isolated from plants and rhizosphere soil including *Bacillus* sp strains, *Trichoderma harzianum* strains, and *Actinomycetes* sp strains (Anitha et al., 2003; Tran et al., 2008). Twelve isolates have been obtained from the pepper rhizosphere, and one strain, named PS119, has a remarkable ability to suppress the growth of *P. capsici* (Rajkumar et al., 2005). *Aspergillus* sp strains are widely distributed saprophytic fungi that have been used industrially in processes such as alcohol fermentation, biological engineering and transformation, and genetic research to produce antibiotics, organic acids, and enzymes (Berka et al., 1992; Roehr et al., 1992; Aybeke et al., 2014; Park et al., 2017). However, there are limited reports of using *Aspergillus* sp strains to control pepper blight.

To determine the microbial community and diversity in continuously cropped pepper soil, we selected soil that had been subjected to continuous cropping for 3, 6, and 9 years and identified the number of bacteria, fungi, and actinomycetes in the rhizosphere by dilution plating. The morphological, physiological, and biochemical characteristics as well as 16S ribosomal DNA (rDNA) or rDNA internal transcribed spacer (ITS) sequences were examined to study the soil microbial ecology. These findings provide the foundation for optimising the soil micro-environment to control pepper blight.

## Materials and Methods

### Rhizosphere Soil Collection

The experimental soil samples were collected from different long-term pepper greenhouse fields that were consecutively cropped for 3, 6, and 9 years with the same agronomic management and fertilisation regime in Beizhen city, Liaoning province of China. Soil that was less than 5 mm from the root surface was gathered and defined as the rhizosphere. The rhizosphere soil of healthy and diseased pepper plants was collected in the same greenhouse, and six treatments were tested. Three greenhouses were randomly selected for each pepper planting year. The five-point sampling method was used in each greenhouse (Xin et al., 2006), and for each pepper field, five healthy and *P. capsici*-infected pepper plants were randomly collected with intact roots and surrounding soil by shaking (Dong et al., 2013). Each treatment with three replicates included 15 sample sites and 75 rhizosphere soil of pepper. All soil samples were rapidly frozen with liquid nitrogen and stored at −80°C for DNA extraction.

### Culture Media

Luria-Bertani (LB) media was used for bacteria isolation and culture, Potato-Martin substratum was used for fungi isolation and culture, and Gao No. I media was used for actinomycetes isolation and culture. The compositions of the media are shown in Suppl. Table 1s (Fang, 1998; Dong and Cai, 2001).

### Soil Microbial Isolation and Purification

Dilution plating was used to isolate bacteria, fungi, and actinomycetes from different long-term consecutively cropped soils. The ratio of microbes between healthy and diseased soil was calculated as follows: $R\ (\%) = (N_1 - N_2)/N_2 \times 100\%$ where $N_1$ represents the amount of bacteria, fungi, and actinomycetes in diseased soil and $N_2$ is the amount in healthy soil.

### Physiological and Biochemical Identification

The physiological and biochemical index, catalase reaction, methyl red (MR) reaction, V-P reaction, starch hydrolysis reaction, cellulose decomposition, nitrate reduction, nitrite reduction, $H_2S$, lipase, tryptophan deaminase enzyme, phenylalanine deaminase, citrate utilization, and salt tolerance were examined for physiological and biochemical identification of bacteria and actinomycetes.

### DNA Extraction and Illumina Sequencing

The DNA extraction kits for bacteria and fungi (Tiangen Biotech, China) were used to extract bacterial and fungal DNA, which was examined by 1.2% agarose gel electrophoresis. The $OD_{260}$ and $OD_{280}$ values were also measured, and $OD_{260/280}$ was calculated to determine the concentration and purity of DNA. We used 2× Taq PCR Master Mix kits (Tiangen Biotech, China) for PCR amplification, after which we sequenced the amplification products. Each reaction contained 12.5 μL master mix, 1.0 μL DNA, 0.5 μL of each primer, and 14 μL sterile distilled water. The PCR cycle was as follows: 94°C denaturation for 3 min, 94°C denaturation for 30 sec, 55°C annealing for 30 sec, and 72°C extension for 1 min for 30 cycles followed by 72°C extension for 5 min. Bacteria and actinomycetes 16S rDNA PCR primers were 5'-AGAGTTTGATCCTGGCTCAG-3' (forward) and 5'-GGTTACCTTGTTACGACTT-3' (reverse). Fungal rDNA-ITS PCR primers were 5'-TCCTCCGCTTATTGATATGC-3' (forward) and 5'-GGAAGTAAAAGTCGTAACAAGG-3' (reverse).

### Sequence Alignment and Cluster Analysis

The obtained sequences were blasted in the NCBI database. Clustalx1.83 was used to construct an alignment, and MEGA 4.0 was used to infer phylogenetic trees. Bacteria and actinomycetes were identified by colony and cell morphology, gram staining, physiological and biochemical tests, and 16S DNA sequence analyses. Fungi were identified by colony and cell morphology and rDNA-ITS sequence analysis.

## Results

### Soil Micro-Ecology

Bacteria including actinomycetes and fungi in the soil drive the soil ecosystem's biochemical cycle (Cao et al., 2011). To understand the effects of continuous cropping of pepper on the rhizosphere soil ecosystem over time, we studied the number of cultivatable bacteria, actinomycetes, and fungi in farms after 3, 6, and 9 years (3a, 6a, and 9a, respectively) of continuous cropping (Table 1). The number of bacteria in the rhizosphere of healthy plants at 3a, 6a, and 9a was significantly higher than in diseased soil and showed a peak at 6a. We did not observe a significant difference in bacteria quantity in healthy plants at 3a, 6a, and 9a or diseased plants at 3a and 9a. However, the number of bacteria in diseased plants at 6a was significantly elevated. In addition, a comparison between the number of rhizosphere bacteria in healthy and diseased plants in the same year showed consistently lower bacteria counts at all time points. We observed the largest decrease in diseased plants at 9a followed by the 3a and 6a time points. Similarly, the number of rhizosphere actinomycetes in healthy plants at 3a, 6a, and 9a was slightly higher than in diseased soil but showed an inverse temporal trend compared with bacteria. Furthermore, the number of rhizosphere actinomycetes was significantly increased at 9a in healthy plants, while we did not detect any significant differences in diseased plants. Actinomycete counts at 3a, 6a, and 9a in diseased plants were 7.67%, 5.42%, and 12.35% lower, respectively, compared with healthy plants. Compared with rhizosphere bacteria, the decrease in actinomycetes in diseased plants was relatively mild. Conversely, the number of fungi in the rhizosphere of diseased plants was significantly higher than in healthy plants with a peak at 6a. We observed increases of 37.82%, 19.75%, and 17.29% at 3a, 6a, and 9a, respectively. In healthy plants, the number of fungi was significantly elevated at 6a compared with 3a, but no difference was found between 6a and 9a. However, in diseased plants, the fungi

**Table 1.** Quantity of soil microbes in the continuously cropped soil of healthy and diseased pepper plants

| Consecutive years | Average number of Bacteria ($10^8$ CFU/mL) | | Average number of Actinomycetes ($10^6$ CFU/mL) | | Average number of Fungi ($10^4$ CFU/mL) | |
|---|---|---|---|---|---|---|
| | Healthy plants | Diseased plants | Healthy plants | Diseased plants | Healthy plants | Diseased plants |
| 3 a | 3.44 ± 0.36 c | 0.64 ± 0.13 b | 3.13 ± 0.33 b | 2.89 ± 0.27 a | 8.09 ± 0.37 b | 11.15 ± 0.45 c |
| R (%) | 537.50 | | 108.31 | | 72.56 | |
| 6 a | 5.55 ± 0.31 a | 1.19 ± 0.16 a | 2.95 ± 0.46 b | 2.79 ± 0.23 a | 12.16 ± 0.50 a | 19. 75 ± 0.82 a |
| R (%) | 466.39 | | 105.74 | | 61.57 | |
| 9 a | 4.49 ± 0.33 b | 0.48 ± 0.10 b | 4.29 ± 0.33 a | 3.76 ± 1.25 a | 11.89 ± 0.40 a | 17.29 ± 0.42 b |
| R (%) | 935.42 | | 114.10 | | 68.77 | |

R: ratio of healthy plants to diseased plants.

The results are given as the mean ± SE.

Different letters indicate a significant difference between treatments by Duncan's post-hoc test at $p < 0.05$ (n = 3).

**Table 2.** Proportion of bacteria, fungi, and actinomycetes in the continuously cropped soil of healthy and diseased pepper plants

| Consecutive years | B/F ($10^4$) | | A/F ($10^2$) | | B/A ($10^2$) | |
|---|---|---|---|---|---|---|
| | Healthy plants | Diseased plants | Healthy plants | Diseased plants | Healthy plants | Diseased plants |
| 3 a | 0.43 ± 0.05 a | 0.06 ± 0.01 a | 0.39 ± 0.06 b | 0.26 ± 0.03 a | 0.92 ± 0.15 a | 4.52 ± 0.53 b |
| R (%) | 716.67 | | 150.00 | | 20.36 | |
| 6 a | 0.46 ± 0.02 a | 0.06 ± 0.01 a | 0.24 ± 0.05 a | 0.14 ± 0.01 b | 0.53 ± 0.10 b | 2.34 ± 0.47 c |
| R (%) | 766.67 | | 171.43 | | 22.65 | |
| 9 a | 0.38 ± 0.04 a | 0.03 ± 0.01 b | 0.36 ± 0.02 b | 0.22 ± 0.07 ab | 0.96 ± 0.16 a | 7.90 ± 1.33 a |
| R (%) | 1266.67 | | 163.64 | | 12.15 | |

R: ratio of healthy plants to diseased plants.

B/F : ratio of bacteria to fungi; A/F: ratio of actinomycetes to fungi; B/A: ratio of bacteria to actinomycetes.

The results are given as the mean ± SE.

Different letters indicate a significant difference between treatments by Duncan's post-hoc test at $p < 0.05$ (n = 3).

count continued to rise significantly at 9a.

The bacteria/fungi ratio reflects the structure and nutritional function of the soil food chain. We found significantly higher bacteria/fungi ratio at 3a, 6a, and 9a in healthy plants compared with diseased plants (Table 2), which decreased by 761.67%, 766.67%, and 1266.67%, respectively. The decrease was highest at 9a, indicating that the growth rate of bacteria was significantly higher than that of fungi. In both healthy and diseased soil, bacteria dominated in absolute numbers followed by actinomycetes and fungi (Tables 1 and 2). Taken together, the number of bacteria and actinomycetes in the rhizosphere of healthy plants was higher than in diseased plants, while the number of fungi was lower. Infection by *P. capsici* had the greatest impact on the number of soil bacteria and the least impact on fungi and actinomycetes. Time point 6a appeared to be the pivotal point at which the microbial composition changed significantly.

## Colony Characteristics and Microscopic Morphology of Bacteria, Actinomycetes, and Fungi in the Soil of Continuously Cropped Pepper

Bacteria were isolated from soil by dilution plating, and the morphology of the main colony is shown in Table 3. We performed a Gram stain and observed the microscopic morphology using a microscope at ×100 magnification (Table 4).

**Table 3.** Morphology of bacterial, actinomycete, and fungal colonies in the soil of continuously cropped pepper

| Microbial | No. | Colony morphology |
|---|---|---|
| Bacterial | B15 | Irregular shape, beige, not raised, not shiny, dry, opaque, irregular edges, wrinkled, easy to pick, combined with the medium does not close, some colour on both sides, no pigment production |
| | B16 | Round, white, raised, moist, shiny, opaque, irregular edges, wrinkled, sticky, easy to pick, combined with the medium does not close, some colour on both sides, no pigment production |
| | B22 | Round, white, convex, moist, shiny, neat edge, opaque, easy pick, combined with the medium does not close, some colour on both sides, no pigment production |
| | B23 | Round, yellowish, round, neat edges, raised slightly, moist and shiny, opaque, easy to pick, combined with the medium does not close, some colour on both sides, no pigment production |
| | B32 | Round, yellowish, neat edges, smooth, shiny, waxy, no pigment production |
| | B33 | Small colony, round, white, neat edge, not moist, not shiny, opaque, not raised, easy to pick, some colour on both sides, no pigment production |
| | B40 | Large colonies, round, white, wrinkled, irregular edges, not moist, not shiny, opaque, not raised, easy to pick, some colour on both sides, no pigment production |
| | B41 | Round, yellow, opaque, smooth, neat edge, raised, no pigment production |
| | B43 | Round, white, opaque, moist, shiny, not raised, irregular edges, easy to pick |
| | B44 | Round, white, opaque, not moist, not shiny, wrinkled, not raised, irregular edges, easily provoked, some colour on both sides, no pigment production |
| | B45 | Small colony, round, orange, neat edges, moist and shiny, opaque, easy to pick, some colour on both sides, no pigment production |
| | B47 | White, raised, neat edge, moist, shiny, translucent, viscous, convex colonies, easy to pick, some colour on both sides, no pigment production |
| | B48 | Round, white, opaque, raised slightly, no pigment produced |
| | B51 | Gray, jagged, transparent, easy to pick, some colour on both sides, no pigment production |
| | B52 | Round, white, not shiny, opaque, hard to pick, no pigment production |
| Actinomycete | A3 | Round, neat edges, concentric circles, inside of colony is reddish brown while edge of colony is white, hard and difficult to pick, opaque, grey spore powder, dry, same colour on both sides, no pigment production |
| | A4 | Irregular shape, white, jagged, dry, not shiny, opaque, same colour on both sides, brown pigment production |
| | A6 | Faint yellow colony, pink and white aerial mycelium, opaque, closely combined with the medium, no pigment production |
| | A7 | Inside of the colony is creamy yellow, while the edge of it is white, irregular shape, hard, dry, opaque, same colour on both sides, no pigment production |
| | A9 | Aerial mycelium is white, substrate mycelium is dark blue, brown pigment production |
| | A11 | Colony is white, irregular shape, opaque, dry, same colour on both sides, no pigment production |
| | A12 | Round, creamy yellow, hard, difficult to pick, opaque, spore colour is white to light pink, same colour on both sides, no pigment production |
| | A13 | Round, brown, neat edge, hard and thick, difficult to pick, surface spores white to pale yellow, dry, colonies on the back of the same colour, no pigment production |
| | A14 | Colony is grey, hard and thick, opaque and difficult to pick, reverse side is light brown, no pigment production |
| | A20 | Center of colony is milky white and edge of colony is white, jagged, wrinkled, not shiny, opaque, same colour on both sides, no pigment production |
| | A26 | Round, white, hard thick and difficult to spick, dry, same colour on the both sides, no pigment production |
| | A53 | Irregular shape, white, jagged, dry, not shiny, opaque, same colour on the both sides, no pigment production |
| | A55 | Small colony, center of colony is pink and edge of colony is red, neat edge, hard and difficult to pick, dry, white spore, opaque, reverse side of the colony is red |
| | A60 | Round colony, white, irregular edges, dry, opaque, not shiny, hard to spick, same colour on the both sides, no pigment production |
| | A61 | White colony, hard and difficult to spick, opaque, hard and thick, white spore, dry, same colour on both sides, no pigment production |
| | A62 | Small colony, round, white, neat edge, raised, opaque, not moist, not shiny, same colour on both sides, no pigment production |
| | A63 | Round, grey, neat edges, dense, hard and difficult to pick, opaque, white spore, dry, reverse side of colony is black, brown pigment secretion production |
| Fungi | F17 | The colony is yellow in the beginning and then the colour changes to yellow-green |
| | F18 | The colony is round, white, and fluffy, lots of green conidium can be produced, the resulting colour of the centre of the colony is green, but the outside colony is also white |
| | F21 | The colony is white, mycelia are few and scattered, combined with the media closely, non-pigment production |
| | F27 | The colony is green and fluffy, the colour of the centre of the colony is green, but outside of the colony is milky white |
| | F19 | The colony is white to yellowish, sometimes it is blue, aerial hyphae like light fleece |
| | F66 | The colony is pink or pale purple, aerial mycelium colour changes from white and pink to light pale purple, aerial mycelium like light fleece. |

The isolated actinomycetes with dry, wrinkled, and concentric growth characteristics were selected, inoculated on actinomycete culture medium, and cultured at 28°C for 5 − 7 d. The colony morphology was then noted (Table 3). Glass slides were inserted into the actinomycete culture medium to examine the spore morphology after 7 d (Table 4). Fungi were isolated from soil, inoculated on PDA medium, and incubated at 25°C for 5 − 7 d. The colony morphology was then observed (Table 3), and the mycelia were isolated onto plates and examined using an optical microscope (Table 4).

**Table 4.** Microscopic morphology of bacteria, actinomycetes, and fungi in the soil of continuously cropped pepper

| Microbial | No. | Microscopic morphology |
|---|---|---|
| Bacterial | B15 | $G^+$, rod-shaped bacteria with spores |
| | B16 | $G^+$, oval to rod-shaped bacteria with spores, sporangium enlargement |
| | B22 | $G^+$, rod-shaped bacteria, oval spore, sporangium enlargement |
| | B23 | $G^-$, rod-shaped bacteria, non-spore |
| | B32 | $G^-$, short rod bacteria without spore |
| | B33 | $G^-$, rod-shaped bacteria without spore |
| | B40 | $G^-$, short rod bacteria without spore |
| | B41 | $G^+$, spherical bacteria with spore |
| | B43 | $G^+$, rod-shaped bacteria with spore |
| | B44 | $G^+$, rod-shaped bacteria with spore |
| | B45 | $G^+$, rod-shaped bacteria with spore |
| | B47 | $G^-$, rod-shaped bacteria without spore |
| | B48 | $G^+$, spherical bacteria without spore |
| | B51 | $G^+$, rod-shaped bacteria with spores |
| | B52 | $G^+$, spherical bacteria without spore |
| Actinomycete | A3 | Spore is bent, spherical, or ovoid. |
| | A4 | Sporothrix is slender and branching, the spore is oval. |
| | A6 | Sporothrix is spiral; the spore is cylindrical |
| | A7 | Sporothrix is bent; spore is oblong |
| | A9 | Sporothrix is bent; spore is oval |
| | A11 | Sporothrix is bent; spore is oval |
| | A12 | Sporothrix is bent; spore is oval |
| | A13 | Sporothrix is dense and spiral; spore is oval |
| | A14 | Sporothrix is curved or spiral; spore is oval |
| | A20 | Sporothrix is long with little spirals; spore is spherical to oval |
| | A26 | Sporothrix is dense with big spiral; spore is oval |
| | A53 | Sporothrix is long and curved |
| | A55 | Sporothrix is long and curved |
| | A60 | Sporothrix is bent |
| | A61 | Sporothrix is relatively straight; spore is cylindrical |
| | A62 | Sporothrix is bent |
| | A63 | Sporothrix is long and straight |
| | A3 | Spores is spherical and oval with smooth surface |
| Fungi | F17 | There are many complex branches; part of them produces many long and rough conidiophores, flask-shaped tip produces top-sac or nearly spherical surface produces many small stems (usually double), a small terrier on the generator the spherical surface roughness string conidia |
| | F18 | 1 or 2 times broom branches; conidia are nearly spherical to oval |
| | F21 | Mycelium is filamentous, slender, and without diaphragm; most of them are at a right angle or an acute angle with constriction; sporangiophore is colourless, filamentous; sporangia are ovoid or pear-shaped |
| | F27 | There are many sterigma; conidia are oval |
| | F19 | Microconidia are oval or kidney-shaped; macroconidium is sickle-shaped, both ends are blunt, end spores are bent slightly, and most of macroconidia have three diaphragms. |
| | F66 | Microconidia are oval or kidney-shaped; macroconidium is sickle-shaped; ends of them are sharpened |

## Physiological and Biochemical Properties of Bacteria and Actinomycetes in the Soil of Continuously Cropped Pepper

A set of 15 bacteria and 17 actinomycetes were selected for physiological and biochemical identification and examined by physiological and biochemical indexes, hydrogen peroxide production, MR, V-P, starch and cellulose hydrolysate content, and gelatine liquefaction ability (Table 5).

**Table 5.** Physiological and biochemical properties of bacteria and actinomycetes in the soil of continuously cropped pepper

| No. | Results | Physiological and biochemical index | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Peroxide | MR | V-P | Starch Hydrolysis | Cellulose Hydrolysis | Gelatin Liquefaction | Nitrate Reduction | $H_2S$ | Lipase | NaCl Growth 10% | NaCl Growth 15% | Citric acid Salt use | Phenylalanine Deaminase |
| B15 | *Paenibacillus* sp | + | − | +− | + | + | + | − | − | − | + | + | + | − |
| B16 | *Paenibacillus polymyxa* | + | −+ | + | − | + | + | + | − | − | − | − | − | − |
| B22 | *Bacillus amyloliquefaciens* | + | − | + | + | − | + | − | − | − | + | + | + | − |
| B23 | *Ochrobactrum* sp | + | −+ | − | + | + | + | + | − | − | + | + | − | − |
| B32 | Marseille genus | + | −+ | − | − | + | + | − | − | − | − | + | − | − |
| B33 | *Flavobacterium* sp | + | − | +− | + | + | + | − | − | − | + | + | + | − |
| B40 | *Flavobacterium* sp | − | − | −+ | − | + | + | + | − | + | − | − | − | − |
| B41 | *Agrococcus* sp | + | − | − | − | + | + | − | − | − | − | + | + | − |
| B43 | *Bacillus* sp | + | − | − | + | + | + | + | + | + | − | + | − | − |
| B44 | *Bacillus amyloliquefaciens* | + | − | − | + | + | + | − | − | − | + | + | + | − |
| B45 | *Brachybacterium* sp Mn32 | − | −+ | − | − | + | + | − | − | + | − | − | − | − |
| B47 | *Agrobacterium* sp | − | − | − | − | + | + | + | − | − | − | − | − | − |
| B48 | *Staphylococcus warneri* | + | −+ | − | + | + | + | + | − | + | − | + | + | − |
| B51 | *Bacillus cereus* | − | − | − | + | + | + | − | + | − | − | − | + | − |
| B52 | *Staphylococcus pasteuri* | + | −+ | −+ | − | + | + | − | − | − | + | + | − | − |
| A3 | *Streptomyces xanthophaeus* | − | − | − | + | − | + | − | + | − | − | − | − | − |
| A4 | *S. oeidiscabies* | − | + | − | + | + | + | + | − | − | − | + | + | − |
| A6 | *S. microflavus* | + | − | − | + | − | + | − | + | − | − | + | + | − |
| A7 | *S. fimicarius* | + | − | − | + | − | + | + | + | − | − | + | + | − |
| A9 | *S. coelicoflavus var yongchunensis Liu* | + | − | − | + | + | + | + | − | − | − | + | + | − |
| A11 | *S. fradiae* | + | + | − | + | + | + | + | − | + | − | + | + | − |
| A12 | *S. virginiae* | − | − | − | + | + | + | + | + | − | − | + | + | − |
| A13 | *S. alanosinicus* | − | − | − | + | + | + | + | + | − | − | + | + | − |
| A14 | *S.* sp | + | + | − | + | + | + | + | + | − | − | + | − | − |
| A20 | *S. antibioticus* | + | + | + | − | + | + | + | + | − | − | + | + | − |
| A26 | *S. lavendulae* | + | − | − | + | + | + | − | + | − | − | + | + | − |
| A53 | *S. oeidiscabies* | − | − | − | − | + | + | − | − | − | − | + | + | − |
| A55 | *S.* sp | + | − | − | − | + | + | − | − | − | − | + | + | − |
| A60 | *S.* sp | − | − | − | + | + | + | + | − | − | − | + | + | − |
| A61 | *Amycolatopsis* sp | + | − | − | − | + | + | + | − | − | − | + | − | − |
| A62 | *S. avermitilis* | + | − | − | + | + | + | − | − | − | − | + | − | − |
| A63 | *S.* sp | − | − | − | + | + | + | + | − | − | − | + | + | − |

## Sequencing and Cluster Analysis of Bacteria, Actinomycetes, and Fungi in the Soil of Continuously Cropped Pepper

Identification was performed according to the common bacteria system identification manual. Bacterial, actinomycete, and fungal DNA was extracted and detected by 1.2% agarose gel electrophoresis (Figs. 1A, 2A and 3A). We calculated an $OD_{260/280}$ value of 1.8 to 2.0 and performed 16S rDNA PCR. The results indicated that the size of the bacterial DNA amplification sequences B22 and B15 were about 1000 bp, and the remaining sequences were 1500 bp (Fig. 1B). In comparison, actinomycete and fungi sequences were 2000 bp long (Figs. 2B and 3B). We then sequenced the PCR

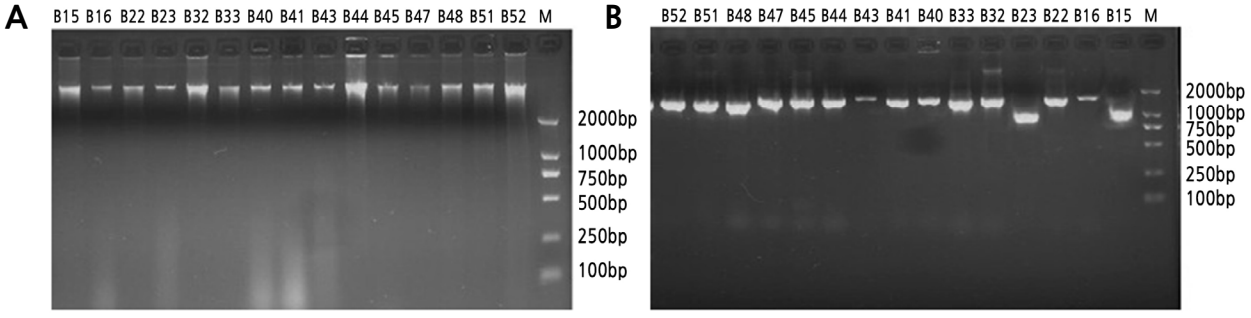


**Fig. 1.** (A) Genomic DNA extraction and (B) PCR amplification of bacteria isolated from the soil of continuously cropped pepper.

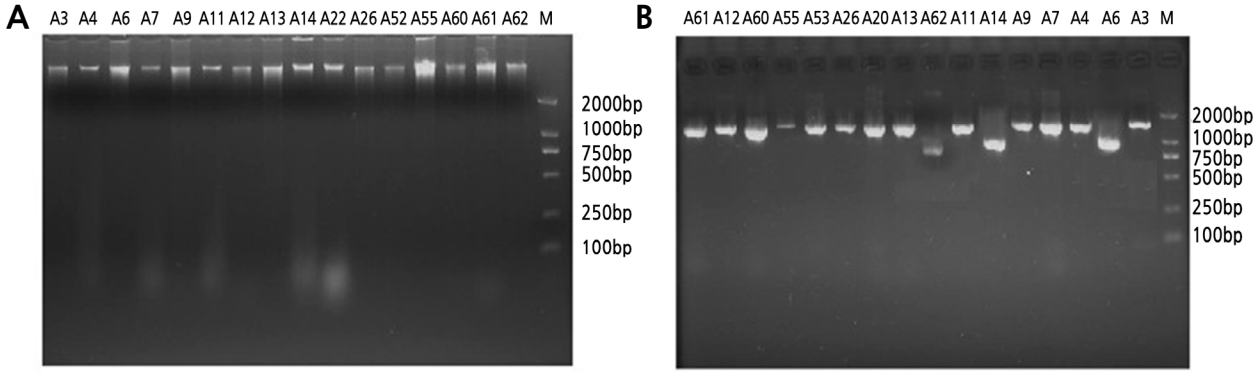


**Fig. 2.** (A) Genomic DNA extraction and (B) PCR amplification of actinomycetes isolated from the soil of continuously cropped pepper.

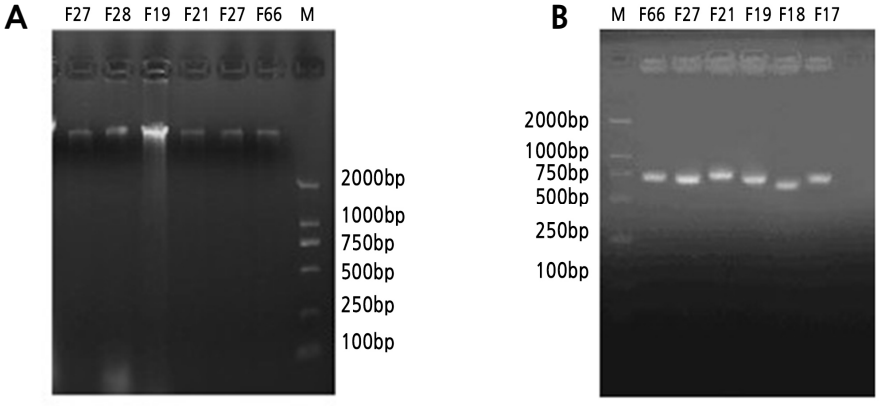


**Fig. 3.** (A) Genomic DNA extraction and (B) PCR amplification of fungi isolated from the soil of continuously cropped pepper.

products and found that the nucleotide fragments of bacteria, actinomycetes, and fungi were 952 − 1466 bp, 948 − 1440 bp, and 500 − 750 bp, respectively (Table 6). The sequences were compared using the NCBI database, and Clustalx1.83 was used to construct an alignment. MEGA 4.0 was employed for cluster analyses of the respective bacteria and actinomycetes.

**Table 6.** Sequencing and cluster analysis of bacteria, actinomycetes, and fungi in the soil of continuously cropped pepper

| Number | Known sequences | Max ident (%) | Taxonomic position |
|---|---|---|---|
| B15 | AB680894.1 *Paenibacillus peoriae* | 99 | *Paenibacillus peoriae* |
| B16 | JN700208.1 *Paenibacillus polymyxa* | 98 | *Paenibacillus polymyxa* |
| B22 | N998403 *Bacillus amyloliquefaciens* | 100 | *Bacillus amyloliquefaciens* |
| B23 | JN256921.1 *Ochrobactrum* sp | 99 | *Ochrobactrum* sp |
| B32 | JF460770.1 *Massilia alkalitolerans* | 98 | *Massilia alkalitolerans* |
| B33 | HE681208.1 *Flavobacterium* sp | 99 | *Flavobacterium* sp |
| B40 | JQ723710.1 HM303244 *Flavobacterium* sp | 99 | *Flavobacterium* sp |
| B41 | JF135709 *Agrococcus* sp | 99 | *Agrococcus* sp |
| B43 | JF496513 *Bacillus thuringiensis* | 100 | *Bacillus thuringiensis* |
| B44 | JN661699.1 *Bacillus amyloliquefaciens* | 98 | *Bacillus amyloliquefaciens* |
| B45 | GU377106.1 *Brevundimonas* sp | 99 | *Brevundimonas* sp |
| B47 | HM151906.1 *Agrobacterium* sp | 99 | *Agrobacterium* sp |
| B48 | JN208111.1 *Staphylococcus warneri* | 98 | *Staphylococcus warneri* |
| B51 | JN700160 *Bacillus cereus* | 99 | *Bacillus cereus* |
| B52 | FR839669 *Staphylococcus pasteuri* | 97 | *Staphylococcus pasteuri* |
| A3 | DQ442560.1 *Streptomyces xanthophaeus* | 99 | *Streptomyces xanthophaeus* |
| A4 | JF923433.1 *Streptomyces acidiscabies* | 100 | *Streptomyces acidiscabies* |
| A6 | FN646655.1 *Streptomyces microflavus* | 99 | *Streptomyces microflavus* |
| A7 | JQ342917.1 *Streptomyces fimicarius* | 99 | *Streptomyces fimicarius* |
| A9 | JQ409522.1 *Streptomyces coelicoflavus* | 99 | *Streptomyces coelicoflavus* |
| A11 | JQ409522.1 *Streptomyces fradiae* | 99 | *Streptomyces fradiae* |
| A12 | FJ481068 *Streptomyces virginiae* | 99 | *Streptomyces virginiae* |
| A13 | HQ426712 *Streptomyces alanosinicus* | 98 | *Streptomyces alanosinicus* |
| A14 | JQ320495.1 *Streptomyces spororaveus* | 99 | *Streptomyces spororaveus* |
| A20 | EF063450 *Streptomyces antibioticus* | 100 | *Streptomyces antibioticus* |
| A26 | FJ517747 *Streptomyces lavendulae* | 100 | *Streptomyces lavendulae* |
| A53 | FJ007427 *Streptomyces acidiscabies* | 99 | *Streptomyces acidiscabies* |
| A55 | HE681150.1 *Streptomyces* sp | 100 | *Streptomyces* sp |
| A60 | JQ234945.1 *Streptomyces* sp | 99 | *Streptomyces* sp |
| A61 | NR_042040 *Amycolatopsis coloradensis* | 99 | *Amycolatopsis coloradensis* |
| A62 | JN392399.1 *Streptomyces avermitilis* | 98 | *Streptomyces avermitilis* |
| A63 | JQ419707.1 *Streptomyces* sp | 98 | *Streptomyces* sp |
| F17 | AB369896.1 *Aspergillus flavus* | 99 | *Aflatoxin* |
| F18 | JN938952.1 *Penicillium expansum* | 99 | *Penicillium expansum* |
| F21 | AF242821.1 *Phytophthora capsici* | 99 | *Phytophthora* |
| F27 | GU566206.1 *Penicillium oxalicum* | 100 | *Penicillium oxalate* |
| F19 | JN390707.2 *F. solani* isolate | 99 | *Fusarium solani* |
| F66 | GU566205.1 *Fusarium oxysporum* | 100 | *Fusarium* |

## Discussion

Bacteria play an important role in changing soil fertility and structure and promoting nutrient circulation. In this study, we showed that the number of bacteria and actinomycetes over time in the rhizosphere of healthy and *P. capsici*-infected pepper plants first increased followed by a decrease. Other studies have observed that the bacterial communities in the rhizosphere of *Rehmannia glutinosa* significantly decrease in quantity and diversity with continuous cropping, which impairs the growth of *R. glutinosa* and its underground tubers and reduces its usability as a medicinal ingredient (Zhang et al., 2010). In addition, Wu and Wang (2007) and Qin et al. (2015) observed similar trends in soil bacteria from continuously cropped cucumber and potato (Wu and Wang, 2007; Qin et al., 2015). However, the results of our research showed some discrepancies with these studies, possibly due to the prolonged period of continuous cropping of up to 9 years included in our study. Vegetable farmers increase the application of fertilisers and pesticides at the early stages of discovering *P. capsici* infection, which artificially changes the original nutrients present in the soil followed by adaptations in the number of soil microorganisms and the structure of the flora (Yang et al., 2000; Van Schoor et al., 2009; Meng et al., 2012; He et al., 2014). We showed that after infection, the pepper plant's rhizosphere bacteria decreased significantly, whereas actinomycetes showed no significant changes and the number of fungi increased. These observed changes in bacteria and fungi were consistent with barley root rot (Li et al., 2017), tobacco bacterial wilt (Li et al., 2020), and banana wilt (Deng et al., 2011). An explanation for this phenomenon is that the number of pathogenic fungi in the soil suddenly increases after disease and directly competes with the resident bacteria, actinomycetes, and fungi in the soil for nutrition and space, which leads to a decline in the number of beneficial microorganisms and changes the soil from "bacterial type" to "fungal type."

The stark changes in the ratio of bacteria/fungi and actinomycetes/fungi in this study all appeared in the sixth year. This is possibly due to shifts in the absorption ability and viability of the pepper roots after prolonged continuous cropping, leading to different compositions in root exudates. This may contribute to altering the composition of microorganisms in the soil and the subsequent detrimental effects. Similar observations have been reported previously. Zhu et al. (2019) studied the number of microorganisms in the rhizosphere soil of tobacco after 2, 4, and 6 years of continuous cropping and found that 4 years is the pivotal point when the number of bacteria, actinomycetes, and fungi in the soil change significantly (Zhu et al., 2019). Furthermore, Wu and Wang (2007) reported that the soil's microbial abundance and diversity of continuously cropped cucumber after 7 years is significantly lower than after 2 years (Wu and Wang, 2007). Crop rotation is a means of improving the soil's micro-ecological environment and increases the yield and quality of agricultural products. Dong et al. (2019) found that when garlic and maize are rotated with pepper, the soil's microbial community structure and diversity is improved paired with a reduction in the threat of pathogens (Dong et al., 2019). Zhang et al. (2015) found that cucumber and leafy vegetable rotation increases the number of soil microorganisms and reduces soil salinity (Zhang et al., 2015). Therefore, the data of our study suggest that when peppers are continuously planted for 5 − 6 years, it would be beneficial to rotate them with different crops such as garlic or leafy vegetables (Dong et al., 2019), thus improving the soil's nutrients and increasing enzyme activity and microorganism diversity. These factors would ultimately promote the healthy and sustainable agriculture of peppers.

Seven of the 15 strains of bacteria obtained in this study belonged to the genus *Bacillus thuringiensis*. This is likely because spores are dormant bodies of bacteria, which are among the most stress-resistant and adaptable organisms that can survive adverse external environments such as extreme temperatures, absence of water or light, and presence of

chemicals (Bağcıoğlu et al., 2019). Therefore, *Bacillus* can exist in large quantities when the soil's micro-ecological environment is destroyed by continuous cropping. *Bacillus* also has many ecological functions such as enzyme production, salt tolerance, acid production, and phosphorus solubilisation (Céline et al., 2007). For example, *Paenibacillus polymyxa* secretes polypeptide proteins, enzymes, and plant hormones, which may be used to control plant diseases (Lin et al., 2018) and can promote plant growth by fixing nitrogen and dissolving phosphorus (Khan et al., 2008). Others, such as *Bacillus amyloliquefaciens*, secrete proteins, lipopeptides, and secondary metabolites to inhibit the growth of plant pathogenic bacteria (Yan et al., 2018). *Streptomyces* is the most diverse genus in the phylum of actinomycetes (Dai et al., 2014). It can adapt to different environments through a variety of self-produced secondary metabolites (Zeng et al., 2019; Ma et al., 2019). Therefore, of the 17 actinomycete species isolated in this study, 15 belonged to the genus *Streptomyces*. Additionally, *Streptomyces* can produce a variety of secondary metabolites such as aminoglycosides, nucleosides, polyenes, macrolides, hormones, and tetracyclines, which are important for cell wall and protein synthesis, plants growth, and the creation of an acidic environment that inhibits pathogens (Barakate et al., 2002; Wang et al., 2005; Hamedi and Mohammdipanah, 2015). Therefore, *Streptomyces* may be valuable for biological control of plant disease and promotion of plant growth. Among the six isolated fungi, *Phytophthora*, *Fusarium solani*, and *Fusarium* were the pathogenic microorganisms for *Phytophthora capsica* infection, root rot, and fusarium wilt, respectively, indicating that continuous cropping led to the domination of pathogenic fungi over beneficial bacteria. As a saprophytic fungus, aflatoxin produced by *Aspergillus* is widely distributed in the soil, which does not have a high requirement for growth and is an opportunistic plant pathogen (Horn, 2003). *Penicillium oxalate* promotes disease resistance by inducing plant resistance and phosphorus solubilisation (Fan et al., 2002; Peng et al., 2004; Ali et al., 2006). *Penicillium expansum*, on the other hand, can cause postharvest rot of apples and pears and even human intestinal diseases (Arici et al., 2000; Liu et al., 2010). We found that after continuous cropping, the majority of the bacteria other than *Penicillium* were pathogenic. The dominant bacterial strains isolated in this study, such as *Bacillus*, *Streptomyces*, and *Penicillium*, are beneficial and available microbial resources.

This research was executed using traditional isolation and culture methods. Compared with modern high-throughput molecular sequencing technologies, traditional methods lack versatility and comprehensiveness and can only be applied on cultivatable microorganisms in the soil, thus limiting the scope of the research (Yuan et al., 2014). However, modern molecular biology technology does not aim to obtain living microbial cells that can be cultured, making it difficult to accurately design and efficiently utilise these microorganisms to fine-tune microbial processes in the soil. In this aspect, the traditional pure culture technology can isolate and obtain pure microorganisms that may be directly or indirectly used for research, medicine, industry, and agricultural production through expansion and cultivation or transformation into new strains (Guo et al., 2006). In conclusion, our study provides a basis for human intervention to beneficially alter the soil microbial flora after continuous cropping to break the current limitations and sustainably guide agricultural production.

## Literature Cited

**Ali T, Tang WH, Zhao ZY** (2006) Resistance to Wheat Diseases Induced by Ferment Solution of Penicillium oxalicum (P -o -41). Xinjiang Agric Sci 43:386-390

**Anitha KN, Radhakrishnana NV, Manomohandasa TP** (2003) Screening of antagonistic bacteria for biological control of nursery wilt of black pepper (*Piper nigrum*). Microbiol Res 158:91-97. doi:10.1078/0944-5013-00179

**Arici M** (2000) Patulin production of Penicillium isolates from fermented olives in a synthetic medium. Ernaehrung 24:257-259

**Aybeke M, Sen B, Okten S** (2014) Aspergillus alliaceus, a new potential biological control of the root parasitic weed Orobanche. J Basic Microbiol 54:93-101. doi:10.1002/jobm.201300080

**Bağcıoğlu M, Fricker M, Johler S, Ehling-Schulz M** (2019) Detection and Identification of Bacillus cereus, Bacillus cytotoxicus, Bacillus thuringiensis, Bacillus mycoides and Bacillus weihenstephanensis via Machine Learning Based FTIR Spectroscopy. Front Microbiol 10:902. doi:10.3389/fmicb.2019.00902

**Barakate M, Ouhdouch Y, Oufdou K, Beaulieu, C** (2002) Characterization on of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. World J Microbiol Biotechnol 18:49-54. doi:10.1023/A:1013966407890

**Barchenger DW, Lamour KH, Bosland PW** (2018) Challenges and strategies for breeding resistance in capsicum annuum to the multifarious pathogen, *Phytophthora capsica*. Front Plant Sci 9:628. doi:10.3389/fpls.2018.00628

**Berendsen R, Pieterse C, Bakker P** (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478-486. doi:10.1016/j.tplants.2012.04.001

**Berka RM, Dunn-coleman N, Ward M** (1992) Industrial enzymes from *Aspergillus* Species. Biotechnology 23:155-202

**Cao ZP, Li DP, Han XM** (2011) The fungal to bacterial ratio in soil food webs, and its measurement. Acta Ecologica Sinica 31:4741-4748

**Céline J, François V, Claude A, Véronique EH, Thierry M, Christian S** (2007) Soil health through soil disease suppression: Which strategy from descriptors to indicators? Soil Biol Biochem 39:1-23. doi:10.1016/j.soilbio.2006.07.001

**Dai FP, Li SW** (2014) Progress on the Secondary Metabolites and Applications of Streptomyces. Biotechnology Bulletin 3:30-35. doi:10.13560/j.cnki.biotech.bull.1985.2014.03.004

**Deng X, Li QF, Hou XW, Hong K** (2011) The Ecological Characteristics of Culturable Microbes Isolated from Infected Soil by Fusarium wilt of Banana. China J Trop Crops 32:283-288

**Dong XZ, Cai MY** (2001) Common bacterial system identification manual. Science Press, Beijing, China

**Dong Y, Yang ZX, Dong K, Tang L, Zheng Y, Hu GB** (2013) Effects of nitrogen application rate on faba bean fusarium wilt and rhizospheric microbial metabolic functional diversity. J Appl Ecol 24:1101-1108

**Dong YF, Lv XZ, Zhang ZK, He HJ, Yu JQ, Zhou YH** (2019) Effects of different cultivation patterns on soil microbial community and enzyme activity in continuous cropped pepper field. Acta Agric Zhejiangensis 31:1485-1492. doi:10.3969/j.issn.1004-1524.2019.09.12

**Fan BQ, Jin JY, Ge C** (2002) Isolation of Pennicillium oxalicum and its effect on solubilizition of insoluble phosphate under different conditions. Sci Agric Sin 35:525-530

**Fang ZD** (1988) Plant Disease Research Methods (Third Edition), China Agriculture Press, Beijing, China

**Guo B, Wu XL, Qian Y** (2006) Approaches for increasing the culturability of microorganisms. Acta Microbiol Sin 46:504-507. doi:10.13343/j.cnki.wsxb.2006.03.036

**Hai THT, Kim JH, Cho MC, Chae SY, Lee HE** (2013) Identification and development of molecular markers linked to Phytophthora root rot resistance in pepper (*Capsicum annuum* L.). Eur J Plant Pathol 135:289-297. doi:10.1007/s10658-012-0085-3

**Hamedi J, Mohammdipanah F** (2015) Biotechnological application and taxonomical distribution of plant growth promoting action bacteria. J Ind Microbiol Biotechnol 42:159. doi:10.1007/s10295-014-1537-x

**Hao ZP, Wang Q, Christie P, Li XL** (2007) Allelopathic potential of watermelon tissues and root exudates. Sci Hortic 112:315-320. doi:10.1016/j.scienta.2006.12.030

**Hartman GL, Wang TC** (1992) Phytophthora blight of pepper: screening for disease resistance. Tropical Pest Management 38:319-322. doi:10.1080/09670879209371717

**He JZ, Zheng Y, Chen CR, He YQ, Zhang LM** (2008) Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture-dependent and culture-independent approaches. J Soil Sediment 8:349-358. doi:10.1007/s11368-008-0025-1

**He YH, Zhou GY, Wang SJ, L H** (2014) Fungal diversity in *Cunninghamia lanceolata* plantation soil. Acta Ecologica sinica 34:2725-2736

**Horn BW** (2003) Ecology and population biology of aflatoxingenic fungi in soil. J Toxicol Toxin Rev 22:351-379. doi:10.1081/TXR-120024098

**Judelson HS, Blanco FA** (2005) The spores of Phytophthora: weapons of the plant destroyer. Nat Rev Microbiol 3:47-58. doi:10.1038/nrmicro1064

**Kennedy AC, Smith KL** (1995) Microbial diversity and sustainability of agricultural soils. Plant Soil 23:69-79. doi:10.1007/BF02183056

**Khan Z, Kim SG, Jeon YH, Khan HU, Son SH, Kim YH** (2008) A plant growth promoting rhizobacterium, Paeribacillus polymyxa strain GBR-1, suppresses root-knot nematode. Bioresour Technol 99:3016-3023. doi:10.1016/j.biortech.2007.06.031

**Kim CH, Kim YK** (2002) Present status of soilborne disease incidence and scheme for its integrated management in Korea, Research in Plant Disease 8:146-161. doi:10.5423/RPD.2002.8.3.146

**Leonian LH** (1922) Stem and fruit blight of peppers caused by Phytophthora capsici sp. nov. Phytopathology 12:401-408

**Li XP, Li JH, Qi YH, Guo W, Li X, Li MQ** (2017) Effects of naked barley root rot on rhizosphere soil microorganisms and enzyme activity Acta Ecologica Sinica 37:5640-5649. doi:10.5846/stxb201605311049

**Li YY, Wang L, Peng WX, Li XH** (2020) Analysis of the Changing Trend of Microbial Community in Rhizosphere Soil. Chin Tob Sci

41:73-78. doi:10.13496/j.issn.1007-5119.2020.05.010

**Li ZG, Zu C, Wang C, Yang JF, Yu H, Wu HS** (2016) Different responses of rhizosphere and non-rhizosphere soil microbial communities to consecutive *Piper nigrum* L. monoculture. Sci Rep 6:35825. doi:10.1038/srep35825

**Lin MZ, Jin MF, Xu K, He LC, Cheng DL** (2018) Phosphate-solubilizing bacteria improve the phytoremediation efficiency of Wedelia trilobata for Cu-contaminated soil. Int J Phytoremediation 20:813-822. doi:10.1080/15226514.2018.1438351

**Liu Y, Zhang HY, Han T** (2010) The Screening and Identification of Antagonistic Bacteria Against Penicillium expansum and the Study of Inhibitive Effects of Fermentation Broth. Chinese Agricultural Science Bulletin 26:8-13

**Ma R, Zhang F, Wang CQ, Yang XY, Xiao W, Wang KL** (2019) Species Diversity and Biological Activity of Actinobacteria in Hehua Hot Spring of Yunnan Province. J Dali Univ 4:69-74. doi:10.3969/j.issn.2096-2266.2019.12.012

**Magnuson TS, Crawford DL** (1992) Comparison of extracellular peroxidase- and esterase-deficient mutants of Streptomyces viridosporus T7A. Appl Environ Microbiol 58:1070-2. doi:10.1128/aem.58.3.1070-1072.1992

**Meng PP, Liu X, Qiu HZ, Zhang WM, Zhang CH, Wang D, Zhang JL, Shen QR** (2012) Fungal population structure and its biological effect in rhizosphere soil of continuously cropped potato. J Appl Ecol 23:3079-3086. doi:10.13287/j.1001-9332.2012.0464

**Nawaz K, Shahid AA, Subhani MN, Iftikhar S, Anwar W** (2018) First report of leaf spot caused by Phytophthora capsici on chili pepper (*Capsicum frutescens* L.) in Pakistan. J Plant Pathol 100:127-127. doi:10.1007/s42161-018-0018-x

**Park HS, Jun SC, Han KH, Hong SB, Yu JH** (2017) Diversity, Application, and Synthetic Biology of Industrially Important Aspergillus Fungi. Adv Appl Microbiol 100:161-202. doi:10.1016/bs.aambs.2017.03.001

**Peng XW, Zhang HX, Bai ZH** (2004) Induced resistance to Cladospoirum cucumerinum in cucumber by pectinaces extract of Penicillium oxalicum. Acta Phytopathologic Sinic 34:69-74. doi:10.13926/j.cnki.apps.2004.01.012

**Qin Y, Ma K, Liu P** (2015) Effect of potato continuous cropping on genetic diversity of soil microorganisms. J China Agric Univ 23:225-232. doi:10.13930/j.cnki.cjea.140755

**Rajkumar M, Lee WH, Lee KJ** (2005) Screening of bacterial antagonists for biological control of *Phytophthora* blight of pepper. J Basic Microbiol 45:55-63. doi:10.1002/jobm.200410445

**Reyes TA, Castro RA, Rodriguez AG, Vazquez MG, Pedraze SME, Lamour K, Larsen J, Fernandez PSP** (2019) Virulence Phenotypes on Chili Pepper for Phytophthora capsici Isolates from Michoacan, Mexico. Hortscience 54:1526-1531. doi:10.21273/HORTSCI13964-19

**Roehr M, Kubicek CP, Kominek J** (1992) Industrial acids and other small molecules. Biotechnology 23:91-131

**Sid A, Ezziyyani M, Egea-Gilabert C, Candela ME** (2003) Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. Biol Plant 47:569-574. doi:10.1023/B:BIOP.0000041063.38176.4a

**Silvar CF, Merino F, Díaz J** (2006) Diversity of Phytophthora capsici in Northwest Spain: Analysis of Virulence, Metalaxyl Response, and Molecular Characterization. Plant Dis 90:1135-1142. doi:10.1094/PD-90-1135

**Simoes DCM, Mc ND, Kristiansen B, Mattey M** (1997) Purification and partial characterization of a 1.57 kDa thermostable esterase from Bacillus *stearothermophilus*. FEMS Microbiol Lett 147:151-156. doi:10.1111/j.1574-6968.1997.tb10235.x

**Tran H, Kruijt M, Raijmakers JM** (2008) Diversity and activity of biosurfactant-producing *Pseudomonas* in the rhizosphere of black pepper in Vietnam. J Appl Microbiol 104:839-851. doi:10.1111/j.1365-2672.2007.03618.x

**Van Schoor L, Denman S, Cook NC** (2009) Characterisation of apple replant disease under South African conditions and potential biological management strategies. Sci Hortic 119:153-162. doi:10.1016/j.scienta.2008.07.032

**Wang GF, Govinden R, Chenia HY, Ma Y, Guo DJ, Ren GD** (2019) Suppression of Phytophthora blight of pepper by biochar amendment is associated with improved soil bacterial properties. Biol Fertil Soils 55:813-824. doi:10.1007/s00374-019-01391-6

**Wang Y, Zong ZF, Cheng LS** (2005) Biological Control of Plant Pathogens with Actinomyces. J Yangling Voca & Tech Col 4:21-23

**Wu FZ, Wang XZ** (2007) Effect of monocropping and rotation on soil microbial community diversity and cucumber yield, quality under protected cultivation. Scientia Agricultura Sinica 40:2274-2280. doi:10.17660/ActaHortic.2007.761.77

**Wu LK, Wang JY, Huang WM, Wu HM, Chen J, Yang YQ, Zhang ZY, Lin WX** (2015) Plant-microbe rhizosphere interactions mediated by Rehmannia glutionosa root exudates under consecutive monoculture. Sci Rep 5:15871.doi:10.1038/srep15871

**Xin JS, Tian YG, Ren R, Huang TP, Shao H, Lu MX, Zheng L** (2006) Soil testing - Part 1: Collection, treatment and storage of soil samples. NY/T 1121.1, Industry Standard-Agriculture, China

**Xiong W, Li ZG, Liu HJ, Xue C, Zhang RF, Wu HS, Li R, Shen QR** (2015) The Effect of Long-Term Continuous Cropping of Black Pepper on Soil Bacterial Communities as Determined by 454 Pyrosequencing. PLoS ONE 10:13. doi:10.1371/journal.pone.0136946

**Yan XN, Ma TY, Du RJ, Jiao SY, Lv J, Cao H, Han P** (2018) Extracellular antibacterial compounds produced by *Bacillus amyloliquefaciens*: Research progress. Chin J Micrecol 30:229-234. doi:10.13381/j.cnki.cjm.201802026

**Yang YH, Yao J, Hua XM** (2000) Effects of pesticide against functional microbial diversity in soil. J Microbiaol 20:23-25

**Yao YP, Li Y, Huang ZL, Yang H, Sun CP, Shu JK, Du RH, Pang XL, Wang Q** (2016) Targeted selection of Trichoderma antagonists for control of pepper Phytophthora blight in China. J Plant Dis Prot 123:215-223. doi:10.1007/s41348-016-0025-0

**Yuan ZH, Wang J, Yang WJ, Wu YY** (2014) Progess in cultivation research on soil Microbes. Acta Pedologica Sinica 51:1183-1191. doi:10.11766/trxb201311270569

**Zeng XY, Dai FP, L Y, Kang XH, Li SW** (2019) Study on culturable Streptomyces and its environmental adaptive characteristics in the soils of three ecotype reeds in Hexi Corridor. J Arid Land Resour Environ 33:164-170. doi:10.13448/j.cnki.jalre.2019.058

**Zhang XL, Xue QH, Guo ZY, Yang B, Zhou YQ, Xu YJ** (2008) Effect of the inoculation with the fungi and actinomycetes against Phytophthora capsici and the colonization capability and the growth and PPO activity of capsicum's leaf and root. J Northwest Sci-Tech Univ Agric For 36:151-158. doi:10.13207/j.cnki.jnwafu.2008.04.029

**Zhang XY, Tian L, Wu P, Gao YM, Li JS** (2015) Changes of soil nutrients and microbial community diversity in responses to different growth environments and cultivation practices in 30 years. Plant Nutr Fert Sci 21:1581-1589. doi:10.11674/zwyf2015.0625

**Zhang ZY, Chen H, Yang YH, Chen T, Lin RY, Chen XJ, Lin WX** (2010) Effects of continuous cropping on bacterial community diversity in rhizosphere soil of Rehmannia glutinosa. J Appl Ecol 21:2843-2848. doi:10.13287/j.1001-9332.2010.0419

**Zhao YZ, Wu LK, Chu LX, Yang YQ, Li ZF, Azeem S, Zhang ZX, Fang CX, Lin WX** (2015) Interaction of Pseudostellaria heterophylla with Fusarium oxysporum f. sp. heterophylla mediated by its root exudates in a consecutive monoculture system. Sci Rep 5:8197. doi:10.1038/srep08197

**Zhu JF, Fan ZQ, Chen QL, Wang JQ, Chen C, Lu AQ, Li HL, Wang Y** (2019) Effects of continuous cropping years on soil microbial flora in the tobacco rhizosphere. J North Agric 47:42-48

**Supplementary Table 1s.** The composition of medium

| Medium | Composition | Type of mediun |
|---|---|---|
| Luria-Bertani | 5.0 $g \cdot L^{-1}$ beef extract, 5.0 $g \cdot L^{-1}$ peptone, 5.0 $g \cdot L^{-1}$ NaCl, and 20.0 $g \cdot L^{-1}$ agar at pH 7.0 | Bacteria |
| Potato-Martin | 1.0 $g \cdot L^{-1}$ $KH_2PO_4$, 0.1 $g \cdot L^{-1}$ chloramphenicol, 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, 3.3 $mL \cdot L^{-1}$ rose bengal, 10.0 $g \cdot L^{-1}$ glucose, 5.0 $g \cdot L^{-1}$ peptone, and 20.0 $g \cdot L^{-1}$ agar | Fungi |
| Gao No. I | 0.5 $g \cdot L^{-1}$ $K_2HPO_4$, 0.5 $g \cdot L^{-1}$ NaCl, 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, 20.0 $g \cdot L^{-1}$ soluble starch, 0.01 $g \cdot L^{-1}$ $FeSO_4 \cdot 7H_2O$, 1.0 $g \cdot L^{-1}$ $KNO_3$, and 20.0 $g \cdot L^{-1}$ agar at pH 7.4 − 7.6 | Actinomycetes |