

# Microbial Consortia: an Engineering Tool to Mitigate the Clubroot Incidence on Chinese Cabbage by Reshaping the Rhizosphere Microbiome

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## Research Article

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# Abstract

Clubroot disease caused by *Plasmodiophora brassicae* is a serious threat to Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) production, which results in extensive yield losses. At present, clubroot control mainly depends on pesticides that have food safety concerns, and the application of sole biocontrol agents cannot successfully control the disease. In this study, we investigated the biocontrol effect of *Bacillus cereus* BT-23, *Lysobacter antibioticus* 13-6, and *Lysobacter capsici* ZST1-2 as sole strains, intra-/inter-genus co-culture, and microbial consortia on clubroot disease, plant growth, and rhizosphere bacterial diversity in a field experiment. This study showed that the application of microbial consortia efficiently controls the incidence of clubroot disease with a biocontrol effect of about 65.78% by decreasing the soil acidity and enhancing the yield (2909.8 Kg/666.67 m<sup>2</sup>). Alleviation of soil acidity results in the abundance and improved activity of beneficial microorganisms in the rhizosphere soil of Chinese cabbage. High throughput sequencing results demonstrated that bacterial phyla Proteobacteria, Bacteroidetes, and Firmicutes were present in high relative abundance in the rhizosphere soil of Chinese cabbage. The application of microbial consortia recovers the imbalance of indigenous micro-ecology and alters the diversity and structure of rhizosphere bacterial communities. Therefore, we conclude that microbial consortia can reduce the clubroot incidence on Chinese cabbage by reshaping the rhizosphere microbiome and decreasing the soil acidity. This study suggested microbial consortia as a new engineering tool to control devastating soilborne disease in commercial crops.

## 1. Introduction

Clubroot disease caused by soilborne pathogen *Plasmodiophora brassicae* Woron is an emerging threat to the *Brassicaceae* family crop production worldwide, including China (Wei et al., 2021). *P. brassicae* has a broad host range that infects more than 300 species of cruciferous plants and is widely distributed in more than 60 countries, resulting in 10–15% yield reduction losses around the globe (Ren et al., 2016). Incidence of clubroot disease is reported all over China with an average yield loss are range between 20–30%. However, the regions of Chongqing, Hubei, Sichuan, Hunan, Yunnan, and Zhejiang are severely affected by this disease and under serious threat of yield reduction (Chai et al., 2014, Hu et al., 2021).

*P. brassicae* have a very complex life cycle consisting of different zoosporic stages, the formation of plasmodia inside host cells, and resting spores (Kageyama and Asano, 2009). Infected soil acts as a primary source of infection, and pathogen survives in the soil for a longer time (up to 20 years) in the form of resting spores (HWANG et al., 2012). During the growing season, the zoospores germinate from the resting spores enter the host plants through wounds and root hairs, which cause cell swelling and results in "gall or club" formation on the roots, multiply in the xylem vessels that inhibit the water and nutrients uptake ability from the soil (Peng et al., 2015). Infected plants generally show symptoms of yellowing, stunting growth, and wilting, leading to the death of the whole plant (Peng et al., 2015). Once the pathogen completes the life cycle within a host plant, millions of zoospores are released from galls to rhizosphere soil and spread from plant-plant, within the field, and field-filed through irrigation water, mechanical operations, or water erosion (Chai et al., 2014).

The prevention and control of cruciferous clubroot have become a major concern due to the wide host range and longtime persistence in the soil as a lethal infection. To date, the important methods to control this disease are the application of fungicides such as benzimidazoles, chlorothalonil, cyclophosphamide, and quinozene (Chai et al., 2014), resistant cultivars (Diederichsen et al., 2009), crop rotation (YANG et al., 2020), and liming (Murakami et al., 2002). Many studies have reported that the excessive use of agrochemicals develops resistance in the pathogen, is environmentally unfriendly, and have human health concerns (Botero et al., 2019, Hu et al., 2021), and crop lose their resistance against the pathogen due to rapid mutation ability and strong pathogenicity of *P. brassicae* (Strelkov et al., 2018). It is suggested that methods have limitations and only alleviate the incidence of clubroot disease but cannot completely control it. So, there is an urgent need to develop durable, efficient, and environmentally friendly control measures to mitigate this devastating disease.

Biological control through potent endophytes and rhizobacteria provides an effective and environmentally friendly alternative control measure to mitigate soilborne diseases (Ahmed et al., 2022). It is reported that biocontrol agents (BCAs) suppress soilborne diseases through the mechanism of antibiosis, niche exclusion, nutrient acquisition, induction of resistance, plant growth promotion, and production of antimicrobial compounds (Xu et al., 2011, Mendes et al., 2013, Nguvo and Gao, 2019). *Bacillus subtilis* XF-1 can produce antimicrobial compounds and significantly suppress the incidence of clubroot disease up to 76.92% by improving the soil microbial diversity (Liu et al., 2018). The application of *Streptomyces alfalfae* XY25<sup>T</sup> improves soil health, regulates rhizospheric bacterial and fungal communities, enhances plant growth, and mitigates clubroot disease up to 69.4% (Hu et al., 2021). *B. subtilis* QST713 can reduce clubroot incidence in canola crops up to 86% through induction of host resistance and antibiosis (Lahlali et al., 2013).

The plant rhizosphere acts as a hot spot habit for the diversity of microorganisms and is considered one of the most complex ecosystems on the earth (Raaijmakers et al., 2009). Many studies are reported that soil health and rhizospheric microbial diversity plays an important role in maintaining plant health (Daval et al., 2020, Cai et al., 2021), and an imbalance of rhizospheric microbial diversity results in the development of soilborne diseases (Wei et al., 2019, Zhang et al., 2020). Improper use of agrochemicals such as fertilizers, pesticides, herbicides, and root exudates leads to changes in the soil microenvironment, which results in the imbalance of soil micro-ecological environment, affect soil health, and also influence the survival and growth of the pathogen in the soil (Sudini et al., 2011, Xue et al., 2018). Therefore, healthy soil and balanced microbial diversity are considered key factors for healthy crop production and disease suppression (Janvier et al., 2007, Zhang et al., 2020).

In our previous study, we evaluated the biocontrol effect of inter-genus (*Bacillus cereus* BT-23 + *Lysobacter antibioticus* 13 – 6) and intra-genus (*L. capsici* ZST1-2 + *L. antibioticus* 13 – 6) bacterial co-culture to suppress clubroot disease of Chinese cabbage in greenhouse experiment through the metabolomic approach (Wei et al., 2021). Results showed that inter-genus bacterial co-culture produced more secondary metabolites and significantly suppressed clubroot disease incidence than intra-genus bacterial co-culture. However, their potential effect on rhizospheric bacterial community diversity is still

unknown. In this study, we enhance our knowledge to decipher the impact of these biocontrol agents on soil health and bacterial community diversity as single, inter-/intra-genus co-culture, and microbial consortia. This study aimed to provide theoretical and experimental knowledge to explore the relationship between rhizospheric bacterial community diversity and *P. brassicae* to mitigate clubroot by engineering the rhizosphere microbiome.

## 2. Materials And Methods

### 2.1. Biocontrol bacterial strains, growth medium, and culture conditions

The potent patented biocontrol bacterial strains *Bacillus cereus* BT-23, *Lysobacter antibioticus* 13 – 6, and *Lysoabcter capsici* ZST1-2 used in this study were preserved in our laboratory. Before the start of each experiment, bacterial strains were grown on King's B (KB) medium plate (Peptone 20 g•L<sup>-1</sup>, KH<sub>2</sub>PO<sub>4</sub> 1.5 g•L<sup>-1</sup>, MgSO<sub>4</sub>·7H<sub>2</sub>O 1.5 g•L<sup>-1</sup>, Glycerol 10 ml•L<sup>-1</sup>, Agar 20 g•L<sup>-1</sup>, and pH 7.0) at 28 °C for 48 h (Wei et al., 2021). The pure culture of bacterial strains was stored in 50% (v/v) glycerol solution at -80 °C for future use.

### 2.2. Assembly of microbial consortia

A single colony was picked from the pure culture, transferred into 500 ml of KB broth, and cultured overnight at 28 °C and 160 rpm, to obtain three simplified probiotic complexes as follows: (i) monoculture (*B. cereus* BT-23, *L. antibioticus* 13 – 6, *L. capsici* ZST1-2), (ii) inter-/intra-genus co-culture (*B. cereus* BT-23 + *L. antibioticus* 13 – 6, and *L. antibioticus* 13 – 6 + *L. capsici* ZST1-2), and (iii) microbial consortia (*B. cereus* BT-23 + *L. antibioticus* 13 – 6 + *L. capsici* ZST1-2). The optical density of culture mediums was adjusted to the same degree OD<sub>600 nm</sub> = 0.5 using a spectrophotometer (Wei et al., 2021).

### 2.3. Experiment site, experimental conditions, and design descriptions

A field experiment was conducted to investigate the biocontrol potential of potent strains on clubroot of Chinese cabbage using Chinese cabbage variety 83 – 1 (highly susceptible) during the growing season in April-May 2020 at Dabai County, Panlong District, Kunming City (25° 2' 47.04" N and 102° 42' 33.84" E), Yunnan Province, China. Chinese cabbage has continuously grown in this field for the last 5 years and is highly infected with clubroot pathogen *Plasmodiophora brassicae*. The average annual temperature is about 15.1 °C, and total rainfall is about 1534 mm per year. Seedlings of Chinese cabbage variety 83 – 1 (highly susceptible) and Kangda No. 3 (highly resistant) were provided by the Qingdao International Seed Co., LTD (Shandong, China) and Yunnan Academy of Agricultural Sciences (Yunnan, China), respectively.

The experiment was performed under 9 conditions as follow: untreated susceptible variety 83 – 1 as a disease control group (CK-D), untreated resistant variety Kangda No. 3 as healthy control group (CK-H), single strain *L. antibioticus* 13 – 6 (T1), single strain *L. capsici* ZST1-2 (T2), single strain *B. cereus* BT-23

(T3), intra-genus co-culture of *L. antibioticus* 13 – 6 + *L. capsici* ZST1-2 (T4), inter-genus co-culture of *L. antibioticus* 13 – 6 + *B. cereus* BT-23 (T5), microbial consortia of *B. cereus* BT-23 + *L. antibioticus* 13 – 6 + *L. capsici* ZST1-2 (T6), and commercial bactericide Kejia (T7). Seedlings were transplanted in a plot (1.8 m × 8 m) on ridges with plant-row distance (40 × 40 cm) under a Randomized Complete Block Design. One week after transplantation, treatments T1-T6 and T7 were inoculated thrice 250 ml/plot of bacterial suspension and commercial bactericide Kejia 80–100 ml/hm<sup>2</sup>, respectively, with an interval of 7 days through irrigation water. The whole experiment was conducted in replicates, and each treatment was repeated thrice with 80 plants per replication.

## 2.4. Assessment of biocontrol effect, plant growth promotion, and soil pH

The biocontrol and plant growth promotion potential of monoculture, inter-/intra-genus co-culture, and microbial consortia bacterial strains were investigated after 2 months of transplantation at the end of the experiment in early June 2020. Randomly 30 plants/plots were uprooted to determine the biocontrol potential of biocontrol strains, whereas plant growth promotion ability was assessed as the fresh weight of plants without roots in terms of yield (Kg/666.67 m<sup>2</sup>), and soil pH was checked using a pH meter. The disease index was investigated using a 5-point disease graded scale as described by Wei et al. (2021). Disease incidence (Di), disease index (DI), and control effect (C.E) was calculated using the following formulas:

$$DI (\%) = [(Number\ of\ diseased\ plants \times Disease\ grading\ scale) / (Total\ numbers\ of\ investigated\ plants \times Highest\ disease\ rating\ scale)] \times 100$$

$$Di (\%) = (Number\ of\ diseased\ plants / Total\ number\ of\ investigated\ plants) \times 100$$

$$C.E (\%) = [(Disease\ index\ of\ control - Disease\ index\ of\ treatment) / disease\ index\ of\ control] \times 100$$

## 2.5. Soil samples collection and extraction of DNA

Bulk soil was removed from the roots by gently shaking the plants (10 plants per replicates), and soil attached to the root surface was collected as rhizosphere soil samples (3 replicates/treatment). Total soil DNA was extracted from 0.5 g of soil/sample using PowerSoil® DNA extraction Kit (MO BIO Laboratories, USA). The DNA quality was quantified at OD<sub>260/280 nm</sub> 1.7 – 1.9 using a NanoDrop spectrophotometer (ND2000, Thermo Scientific) (Zhang et al., 2020) and extracted DNA was stored at – 20 °C for further studies.

## 2.6. PCR amplification and analysis of rhizospheric bacterial diversity

PCR was amplified using universal primers 343F (5'-TACGGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTATCCT-3') for the V3-V4 variable region of 16s rRNA to explore the rhizosphere bacterial diversity (Zhang et al., 2020). The PCR products were purified through Nextera XT Index PCR Clean-up Kit

(Illumina Inc.) and sequenced on an Illumina MiSeq platform at OE Biotech Co., Ltd. (Shanghai, China). Raw data from the Illumina sequence were collected in FASTQ format. Chimeras were removed and quality controlled at 20% cutoff level using UCHIME (Version 8.1) and Trimmomatic (Version 0.33), respectively, to generate clean reads (Edgar et al., 2011, Bolger et al., 2014). The clean reads were then clustered into operational taxonomic units (OTUs) at 3% dissimilarity level through UPARSE (Edgar, 2013) and blasted against Ribosomal Database Project (RDP) classifier in SILVA database (<http://www.arb-silva.de>) of bacteria for taxonomic annotation (Quast et al., 2012).

## 2.7. Bioinformatics analysis

Alpha diversity indices (OTUs, Chao 1, Shannon, and Simpson) and beta diversity indices based on Bray-Curtis distance metrics under different treatments were calculated using QIIME v.1.9.1. Principal coordinate analysis (PCoA) based on Bray-Curtis distance metrics was used to visualize the changes in bacterial community structure. The relative abundance bar plots at the phyla and genus levels were generated using R scripts in R v3.5.3. Pearson correlation coefficient for the genus, disease index, yield, pH, and alpha diversity indices was calculated using the HMISC package in R and visualized through a heatmap. Data were statistically analyzed using analysis of variance (ANOVA) at  $p < 0.05$ , and means were compared using Tukey's test at  $p < 0.05$ .

## 3. Results

### 3.1. Effect of microbial consortia on disease incidence and soil pH

We evaluated the effect of bacterial biocontrol strains on clubroot disease and soil pH at the end of experiments (Fig. 1 and Table S1). Results revealed that the Chinese cabbage variety Kangda No. 3 (CK-H) showed resistance against *Plasmodiophora brassicae* with minimum disease incidence (8.89%) and maximum control effect (81.27%). Whereas the application of microbial consortia (T6) suppressed the incidence of clubroot disease, having a control effect of about 65.78% compared with commercial bactericide Kejia (T7); and monocultures (T1-T3) and inter-/intra-genus co-cultures (T4-T5) bacterial biocontrol strains. When the biocontrol strains were applied as monoculture and inter-/intra-genus co-cultures, *L. capsici* ZST1-2 (T2) and inter-genus co-culture *B. cereus* BT-23 + *L. antibioticus* 13 - 6 (T5) showed a maximum control effect of about 58.69% and 58.32%, respectively compared with T1, T3, and T4. However, no significant difference was observed in the control effect of monoculture (T2) and co-culture (T5) compared to the application of commercial bactericide Kejia (T7). The pH of Chinese cabbage rhizosphere soil significantly increased under treatment T6, T7, and healthy control (CK-H) compared with other treatments and disease control (CK-D). Results demonstrated that the application of biocontrol bacteria as microbial consortia has much potential to mitigate the clubroot disease compared with single strain, co-culture, and commercial bactericide.

## 3.2. Plant growth promotion potential of microbial consortia

We investigated the plant growth promotion potential of bacterial biocontrol strains in terms of yield (kg/666.67 m<sup>2</sup>) as single strain, inter-/intra-genus co-culture, and microbial consortia; and compared with the disease control (CK-D), healthy control (CK-H), and commercial bactericide (T7). Results showed that application of microbial consortia (T6) significantly increased the yield (kg/666.67 m<sup>2</sup>) of Chinese cabbage compared with the disease control (CK-D), healthy control (CK-H), commercial bactericide (T7), single strains (T1-T3), and inter-/intra-genus co-cultures (T4-T5) (Fig. 2 and Table S2). The yield (kg/666.67 m<sup>2</sup>) of Chinese cabbage treated with microbial consortia (T6), commercial bactericide (T7), and healthy control (CK-H) increased up to 146.5%, 124.66%, and 135.5% than the disease control (CK-D). When biocontrol agents were applied as monoculture and inter-/intra-genus co-culture, the yield (kg/666.67 m<sup>2</sup>) of Chinese cabbage treated with single strain *L. capsici* ZST1-2 (T2) and co-culture *B. cereus* BT-23 + *L. antibioticus* 13 - 6 (T5) significantly increased compared to other single strains (T1 and T3) and co-culture (T4). The yield (kg/666.67 m<sup>2</sup>) of Chinese cabbage treated with T2 and T5 increased up to 95.66% and 124.28% than the disease control (CK-D).

## 3.3. Effect of microbial consortia on the diversity of bacterial community in rhizosphere soil

Alpha diversity indices (OTUs at > 97%, Chao 1, Shannon, and Simpson indexes) are shown in the table (Table S3). The diversity indexes Chao 1, Shannon, Observed\_species, and PD\_whole\_tree significantly changed under different treatments but have little impact on Goods\_coverage and Simpson indexes. The alpha diversity indices of treatments (T1-T7) and healthy control (CK-H) were found significantly higher than disease control (CK-D), whereas no significant difference was observed between treatments (T1-T7) and healthy control (CK-H). By comparing the alpha diversity indices under different treatments of biocontrol strains, we found that the Chao 1, Shannon, Observed\_species, and PD\_whole\_tree indexes of microbial consortia (T6) and intra-genus co-culture (T4) were higher than the application of monocultures (T1-T3), inter-genus co-culture (T5), and commercial bactericide (T7).

## 3.4. Effect of microbial consortia on the bacterial community structure in rhizosphere soil

Bray-Curtis distance metrics based on principal coordinate analysis (PCoA) showed that the structure of the rhizosphere bacterial community of Chinese cabbage significantly changed under different treatments with the difference of about 30.61% and 25.71% (Fig. 3). By comparing the structure of the rhizosphere bacterial community under different treatments, it was observed that the treatment groups (T1-T7) and the healthy control group (CK-H) were significantly separated from the disease control (CK-D). The treatments (T1-T7) and healthy control (CK-H) were distributed along the first coordinate axis on the left side, and the disease control group (CK-D) was distributed along the second coordinate axis on the right side. The treatments T2, T3, T5, T6, T7, and healthy control (CK-H) are clustered together,

whereas treatment T1 and T4 are clustered together, indicating that the rhizosphere bacterial community structure of these treatments was similar. However, the structure of the rhizosphere bacterial community associated with Chinese cabbage under application of microbial consortia (T6) was significantly different than the disease control (CK-D).

### 3.5. Effect of microbial consortia on bacterial community composition at the phylum level

Relative abundance analysis at the phylum level showed that bacterial community composition changed significantly under different treatments. Relative abundance analysis of the top 10 dominant bacterial phyla is shown in Fig. 4. In all rhizosphere soil samples, the dominant bacterial phyla are Proteobacteria, Bacteroidetes, and Firmicutes (average abundance; 57.80%, 18.59%, and 7.68%, respectively), followed by Actinobacteria (7.29%), Gemmatimonades (3.44%), Acidobacteria (2.86%), Cyanobacteria (0.90%), Nitrospirae (0.57%), Verrucomicrobia (0.18%), and others (0.69%). Bacterial phyla such as Bacteroidetes, Firmicutes, and Cyanobacteria were present in high proportion with CK-H and T1-T7 compared with CK-D. Whereas phyla Actinobacteria, Gemmatimonades, Acidobacteria, and Nitrospirae were present in high proportion with CK-D than CK-H and T1-T4. However, Proteobacteria was found in high relative abundance with CK-H, T3, and T4 compared with CK-D (Table S4). In this experiment, *L. antibioticus* 13 – 6 and *L. capsici* ZST1-2 belong to Proteobacteria, while *B. cereus* 1-BT-23 belongs to Firmicutes. Application of *L. antibioticus* 13 – 6, *L. capsici* ZST1-2, and *B. cereus* 1-BT-23 as single strain, co-culture, and microbial consortia enhanced the abundance of Firmicutes in rhizosphere Chinese cabbage compared with CK-D. The abundance of Proteobacteria decreased in the rhizosphere of Chinese cabbage compared to CK-D when treated with *L. antibioticus* 13 – 6 and *L. capsici* ZST1-2, whereas it increased when treated *B. cereus* 1-BT-23.

### 3.6. Effect of microbial consortia on bacterial community composition at the genus level

Bacterial community composition significantly changed at the genus level under different treatments. Relative abundance analysis for the top 15 dominant bacterial genera is shown in Fig. 5. Bacterial genera such as *Sphingomonas*, *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, and *Citrobacter* were found in high relative abundance (average abundance: 9.21%, 6.52%, 5.46%, 2.57%, and 1.58%, respectively) with all rhizosphere soil samples. Genus *Sphingomonas* was present in high relative abundance (14.02%) in the rhizosp

here soil of disease control (CK-D) compared to healthy control (CK-H) and other treatments (T1-T7). Whereas genus *Pseudomonas* was present in high relative abundance in the rhizosphere soil of healthy control (CK-H) and other treatments (T1-T7) than disease control (CK-D) (Table S5). Results suggest that significantly increase and decrease of *Pseudomonas* and *Sphingomonas* in the rhizosphere soil of healthy control (CK-H) and other treatments (T1-T7) play an important role in disease suppression and acceleration. The abundance of genus *Lysobacter* was also increased in the rhizosphere soil of healthy control (CK-H) and other treatments (T1-T7) than disease control (CK-D). Whereas genus *Bacillus* was

present in the same abundance in rhizosphere soil of disease control (CK-D), healthy control (CK-H), and other treatments (T1-T7); and no significant difference was observed between the treatments (Table S5).

### 3.7. Correlation analysis of disease index, yield and pH between bacterial genera and diversity indices

Correlation analysis of disease index, yield, and pH between bacterial genera and diversity indices is shown in Fig. 6. Bacterial genera such as *Lysobacter*, *Streptococcus*, *Ruminococcaceae*, *Dialister*, *Ruminococcus*, *Treponema*, *Lachnoclostridium*, *Prevotellaceae*, *Rikenellaceae*, *Lachnospiraceae*, *Faecalibacterium*, *Faecalibaculum*, *Intestinimonas*, *Odoribacter*, *Romboutsia*, *Ruminiclostridium*, *Bacteroides*, *Coriobacteriaceae*, *Alistipes*, *Eggerthella*, *Escherichia - Shigella*, *Parasutterella*, *Oribacterium*, *Candidatus\_Uzinura*, *Eubacterium\_Coprostanoligenes*, *Solanum\_Torvum*, and *Roseburia* were significantly negatively correlated with the occurrence and severity of clubroot disease, however significantly positively correlated with pH. However, bacterial genera including *Streptococcus*, *Parasutterella*, *Oribacterium* and *Candidatus\_Uzinura* were significantly positively correlated with the yield of Chinese cabbage (Fig. 6A). Diversity indices including Chao 1, PD\_whole, Observed\_species, Shannon, and Simpson were significantly negatively correlated with disease occurrence and severity, whereas positively correlated with yield and pH. However, good\_coverage was significantly positively correlated with disease occurrence and severity, whereas negatively correlated with yield and pH (Fig. 6B).

## 4. Discussion

Clubroot caused *Plasmodiophora brassicae* is one of the most destructive diseases affecting production of Chinese cabbage, all over China. Generally, the disease occurs after 30 days of transplantation, resulting in more than 80% yield losses (He et al., 2019). From the past few decades, the important disease management methods in the form of resistant cultivars, chemical control, and cultural practices have been adopted, but results are not fruitful (Chai et al., 2014, Hu et al., 2021). Therefore, biological control through application of potent endophytes and rhizobacteria is considered a practical approach in the treatment and prevention of clubroot disease (Liu et al., 2018, Hu et al., 2021). The application of sole biocontrol agent cannot efficiently control clubroot disease due to high virulent nature of *P. brassicae* (Wei et al., 2021). Thus, there is an urgent need to develop a long-lasting and environmentally friendly control measures in form of microbial consortia to mitigate this devastating disease.

Our previous study (greenhouse experiment) reported that combined (inter-/intra-genus) application of bacterial biocontrol agent significantly suppressed clubroot disease than single strain (Wei et al., 2021). Based on the results of the greenhouse experiment, we further carried out a field experiment with some modifications to investigate the biocontrol potential of biocontrol strains as monoculture, inter-genus/intra-genus co-culture, and microbial consortia along with the application of commercial bactericide. Results demonstrated that application of inter-genus bacterial co-culture (T5; *Lysobacter antibioticus* 13 - 6 + *Bacillus cereus* BT-23) showed a maximum control effect of about 58.32% compared with intra-genus bacterial co-culture (T4; *L. antibioticus* 13 - 6 + *L. capsici* ZST1-2) and single strain (T1-

T3). However, the control efficacy was increased up to 65.78% when biocontrol agents were applied as microbial consortia (*Bacillus cereus* BT-23 + *Lysobacter antibioticus* 13 – 6 + *L. capsici* ZST1-2). Our results are in accordance with the previous findings (Sarma et al., 2015, Palmieri et al., 2017, Wei et al., 2021), that application of different genera and species as microbial consortia suppressed disease incidence, enhance yield, improves biocontrol efficacy of biocontrol agents, and limit the competition for resources because different microbes occupy diverse niches in the rhizosphere.

We assumed that the control efficacy of microbial consortia might be associated with the changes in the bacterial community structure and diversity. Therefore, we collected rhizosphere soil samples under different treatments to study the bacterial community composition throughout sequencing. Many studies proved that the plant rhizosphere microbiome act as the first line of defense against the soilborne pathogen plays an important role in in maintaining plant health and disease control (Raaijmakers et al., 2009, Topalović et al., 2020, Chiaramonte et al., 2021). In recent years, high throughput sequencing has been used as a practical approach in deciphering the microbial community composition associated with plant rhizosphere and phyllosphere (Liu et al., 2018, Wei et al., 2019). Results of high throughput sequencing proved that application of microbial consortia alters the diversity and structure of rhizosphere bacterial communities associated with rhizosphere soil of Chinese cabbage. Our results are similar to the findings of Zhang et al. (2020) and Hu et al. (2016), who reported that the application of probiotic consortia suppressed the incidence of root rot disease of *Panax notoginseng* and bacterial wilt of tomato by reshaping the rhizosphere microbiome and colonization in the rhizosphere soil and roots, respectively.

Previous studies have reported that different plant components to act as habitat for a specific microbial community (Kaushal et al., 2020) and Proteobacteria is the dominant bacterial phyla in the rhizosphere soil of plants (Delgado-Baquerizo et al., 2018). High throughput sequencing results demonstrated that Proteobacteria was the most dominant phylum with all rhizosphere soil samples with an average abundance of about 57.80%, followed by Bacteroidetes and Firmicutes (average abundance; 18.59% and 7.68%, respectively). Palmieri et al. (2017) reported that a facilitative interaction is present between compatible microbes and enhanced the growth of each other when grown as co-cultured in microbial consortia. In our study, we found that the abundance of Firmicutes increased in the rhizosphere soil of Chinese cabbage under different treatments. Whereas, the abundance of Proteobacteria was decreased in the rhizosphere soil of Chinese cabbage in all treatments except healthy control (CK-H), single strain *B. cereus* 1-BT-23 (T3), and commercial bactericide Kejia (T7). However, in the case of inter-genus co-culture (T5) and microbial consortia (T6), Proteobacteria was found in high relative abundance than single strain (T1) and intra-genus co-culture (T2). This may be due to the fact that a facilitative interaction was present between *B. cereus* 1-BT-23 and *Lysobacter* species while *L. antibioticus* 13 – 6 and *L. capsici* ZST1-2 are maybe antagonists to each other. However, the interaction between *Lysobacter-Lysobacter* species and *Bacillus-Lysobacter* species is unclear and needs further study.

It is reported that soil health, acidity, enzymatic activity, and soil fertility are directly proportional to the rhizosphere microbiome composition. The changes in the rhizosphere microbiome significantly influence these factors, which result in disease development and unhealthy crop production (Zhang et al., 2016,

Xue et al., 2018). The occurrence of soilborne diseases are correlated with soil pH, and an increase in soil pH could enhance the activity of beneficial microorganism in the rhizosphere (Li et al., 2022). This study showed that the application of microbial consortia (T6) significantly alleviates the soil acidity (increase the soil pH value) and improves soil fertility, resulting in maximum yield and less disease development. Our results are similar to the findings of Hu et al. (2021), who reported that the application of *S. alfalfae* XY25<sup>T</sup> suppressed the incidence of clubroot disease of Chinese cabbage by altering the soil fertility and acidity.

## 5. Conclusions

In summary, we conclude that microbial consortia could be used as an engineering tool to reduce clubroot incidence on Chinese cabbage in the continuous cropping soil. After the application of microbial consortia, the bacterial community composition and structure changed in the rhizosphere of Chinese cabbage. Furthermore, the application of microbial consortia reduced soil acidity that inhibits the growth of *Plasmodiophora brassicae* in the rhizosphere soil. This study provides experimental and theoretical knowledge on the importance of microbial consortia to mitigate clubroot of Chinese cabbage. Therefore, it is believed that the construction of microbial consortia is of great importance in biological control due to its plant growth promotion and disease suppressive properties.

## Declarations

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### Author's contribution

GJ and LW conceived and designed the experiments. JZ, ZD, XH, and ZH performed the experiments. JZ, ZD, and WA analyzed the data. JZ, ZD, and WA wrote the manuscript. WA and GJ revised and edit the manuscript. All authors contributed to the final draft of manuscript and agreed to the published version of the manuscript.

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Ethical Statement

All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

## Consent to Participate

All authors have been personally and actively involved in substantive work leading to the manuscript and contributed in the preparation of final draft of the manuscript

## Consent for publication

The manuscript has not been published in whole or in part elsewhere, and is not currently being considered for publication in another journal. All the authors seen the final version of manuscript.

## Availability of data and material

This material has not been previously published elsewhere and have no conflict of interest.

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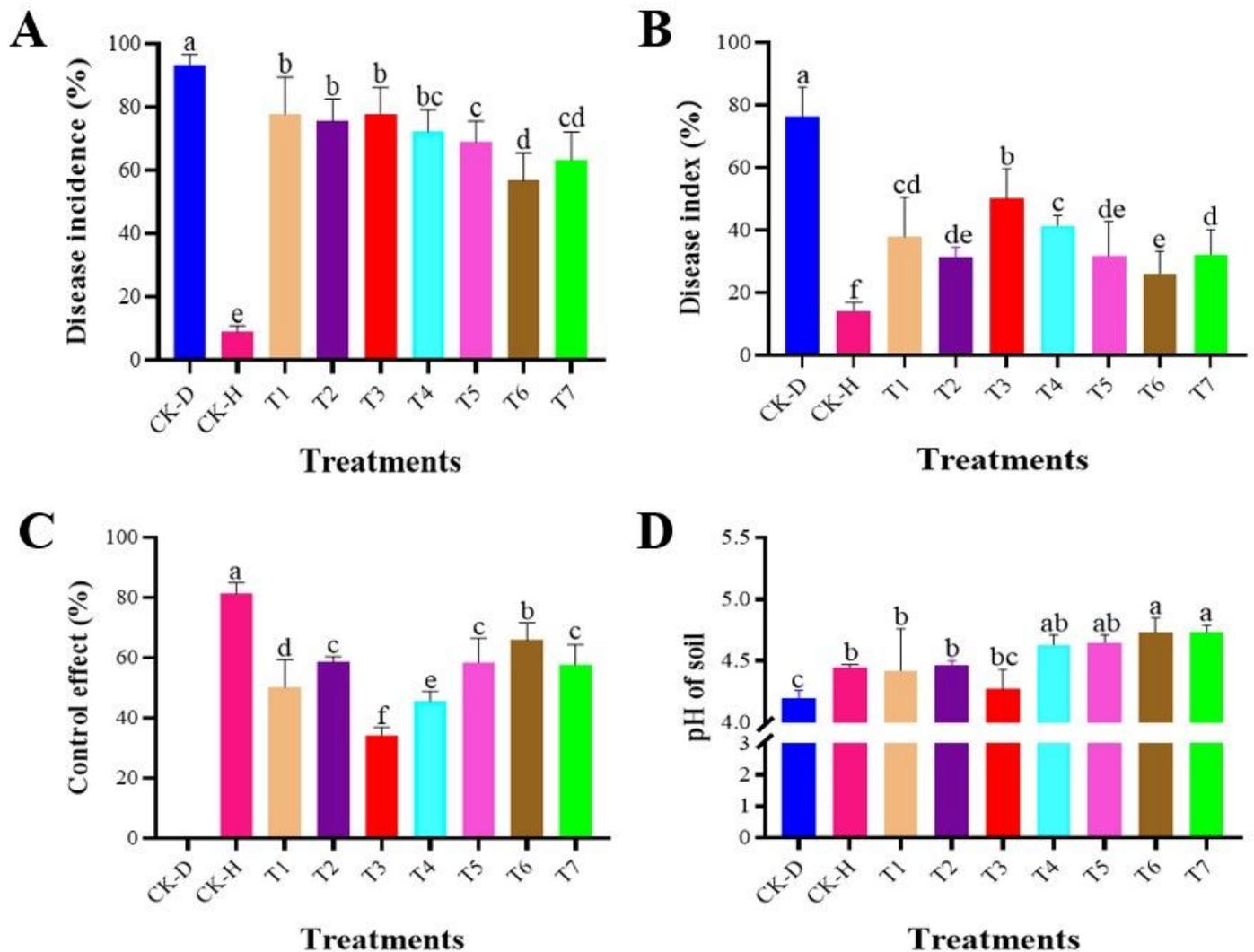
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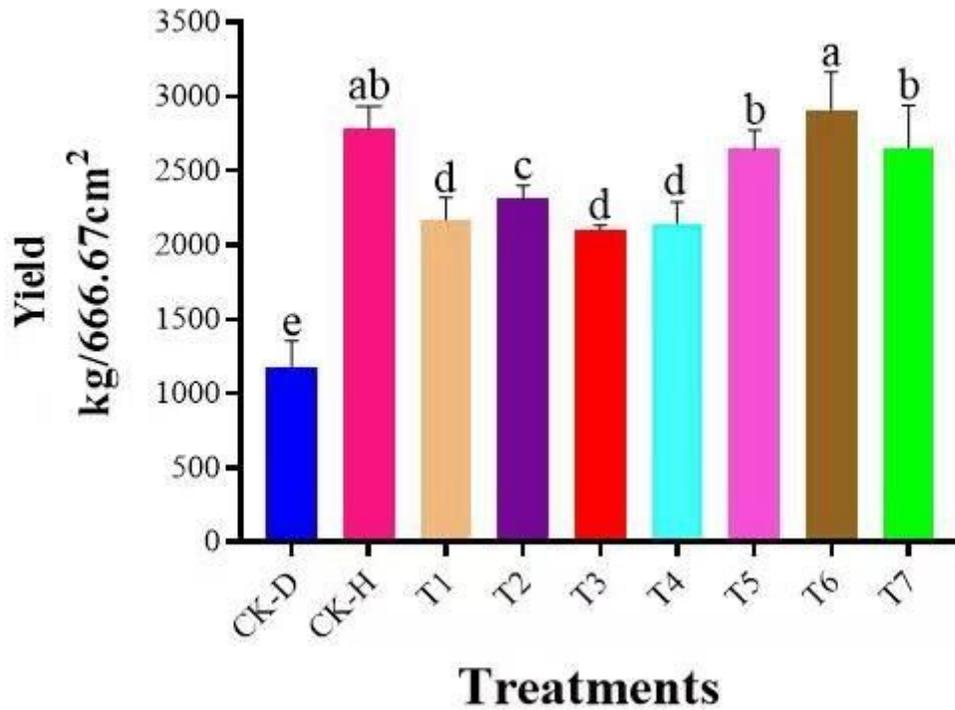
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## Figures



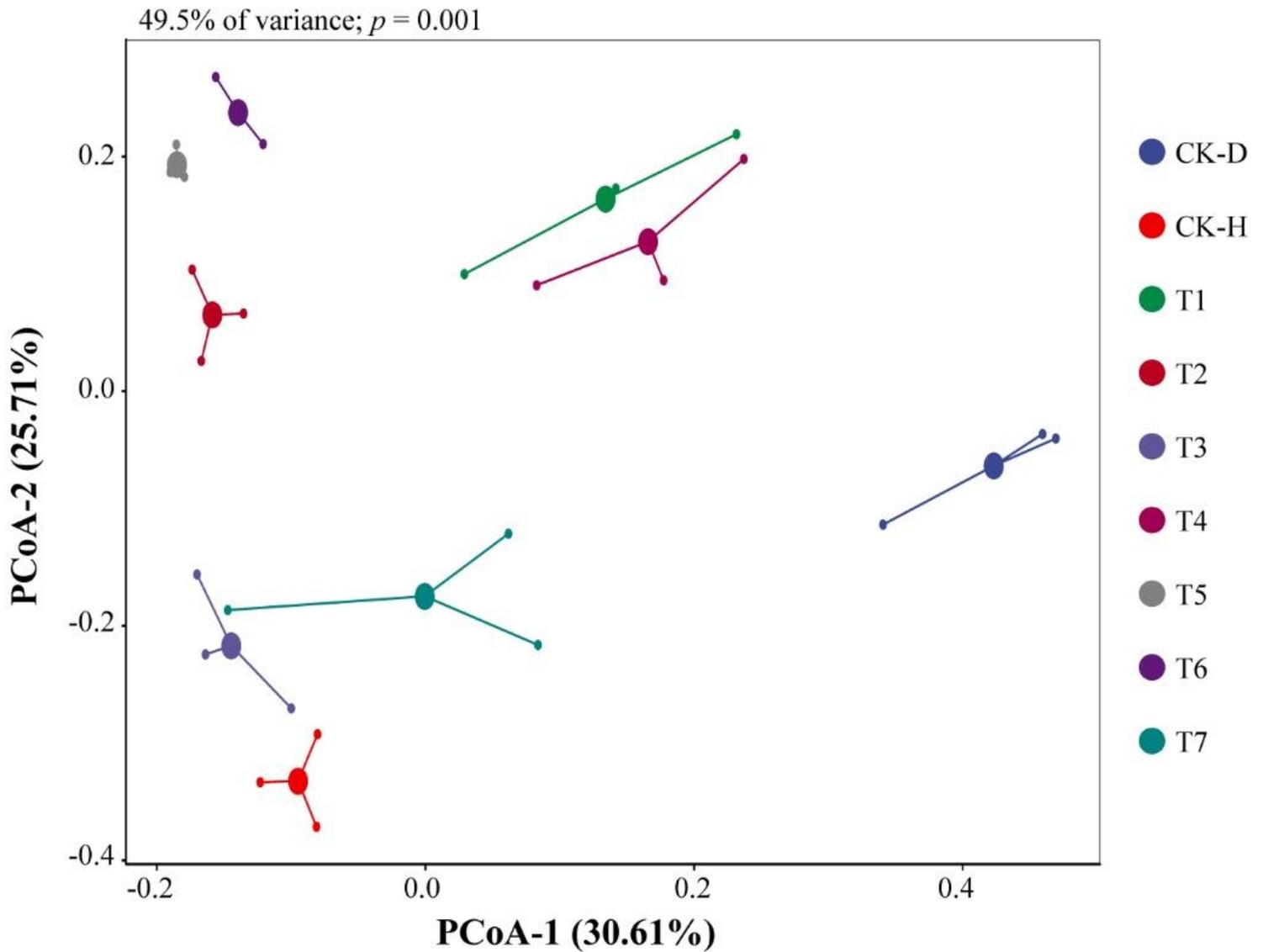
**Figure 1**

Biocontrol potential of candidate's strains against clubroot disease of Chinese cabbage. Disease incidence (**A**), disease index (**B**), control effect (**C**), and pH (**D**). Different small letters on the error bar are shown the significant difference among treatments according to Duncan's multiple range test at  $p < 0.05$ . **Here:** Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial bactericide Kejia (**T7**).



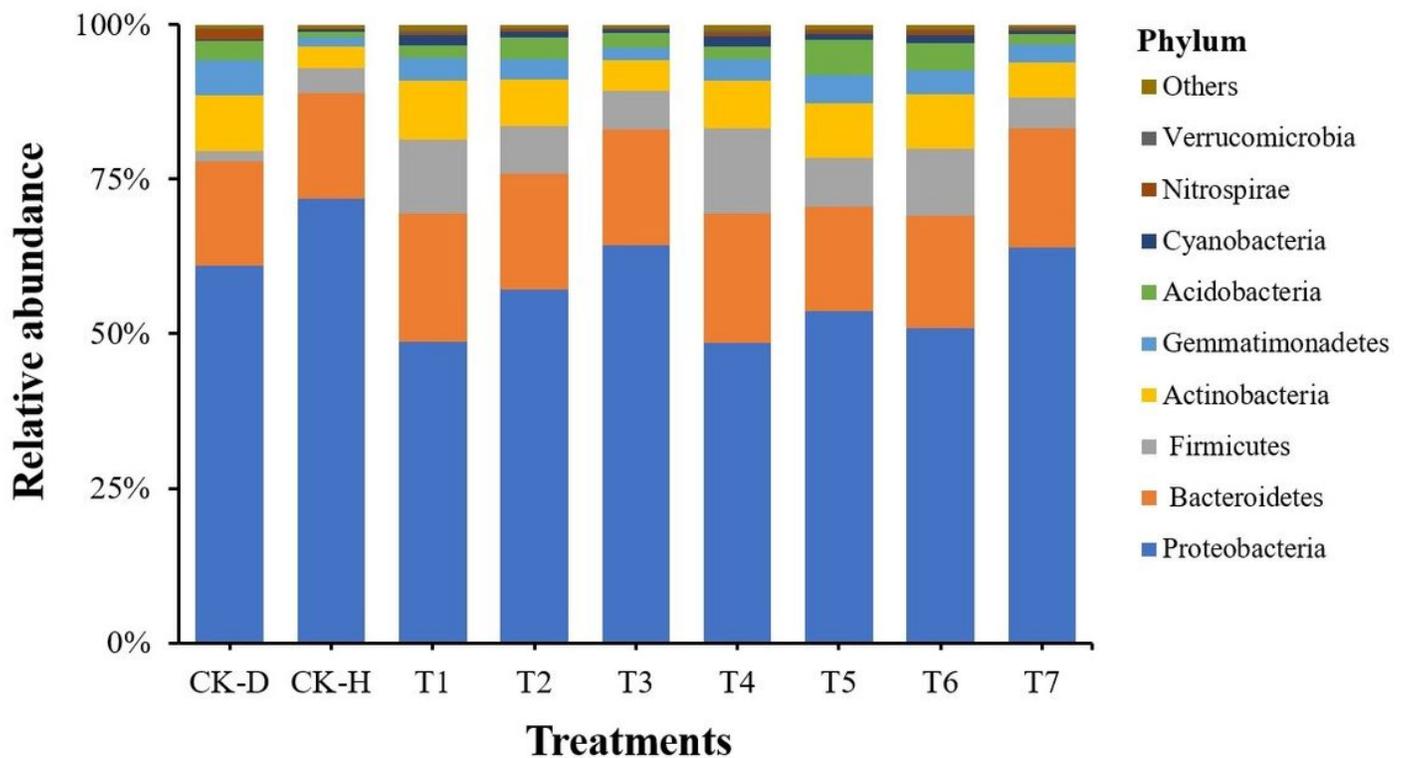
**Figure 2**

Plant growth promotion potential of candidate's strains on Chinese cabbage. Significance difference among treatments is shown by different small letters on the error bar according to Duncan's multiple range test at  $p < 0.05$ . **Here:** Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial bactericide Kejia (**T7**).



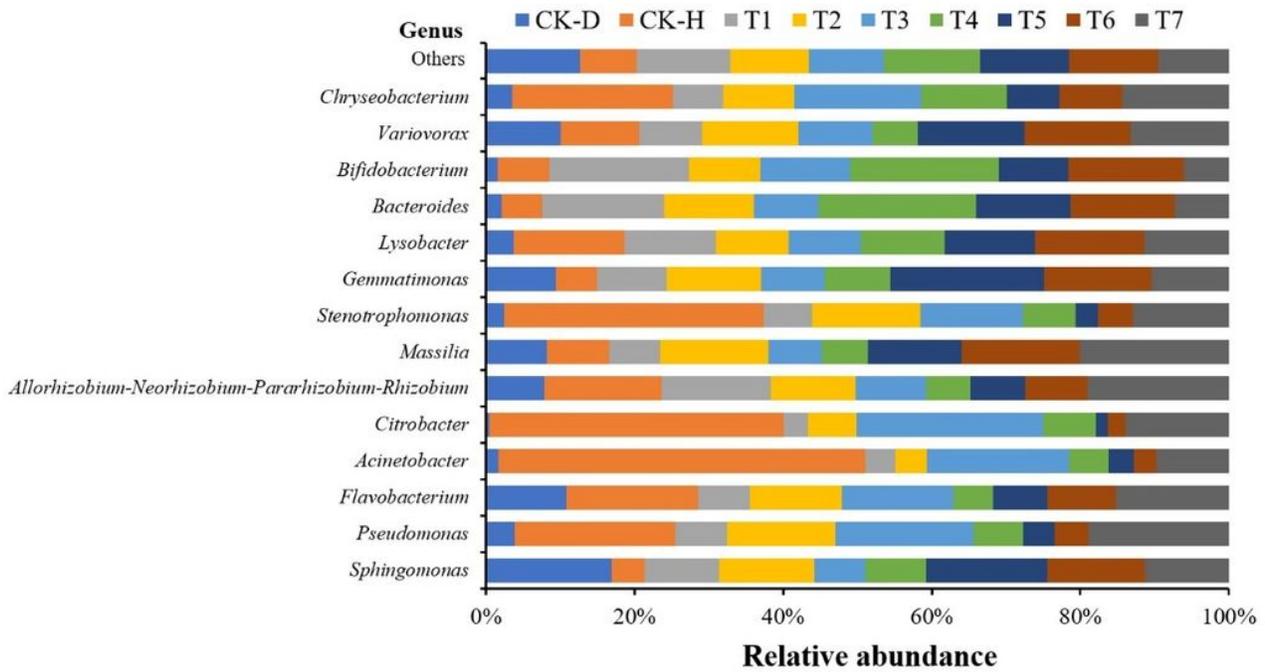
**Figure 3**

Principal component analysis (PCA) was based on the Bray-Curtis dissimilarity metric showing the beta diversity of bacterial community structure under different treatments. **Here:** Disease control (**CK-D**), healthy control (**CK-H**), single strain *Lysobacter antibioticus* 13-6 (**T1**), single strain *L. capsici* ZST1-2 (**T2**), single strain *Bacillus cereus* BT-23 (**T3**), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T4**), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (**T5**), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (**T6**), and commercial bactericide Kejia (**T7**).



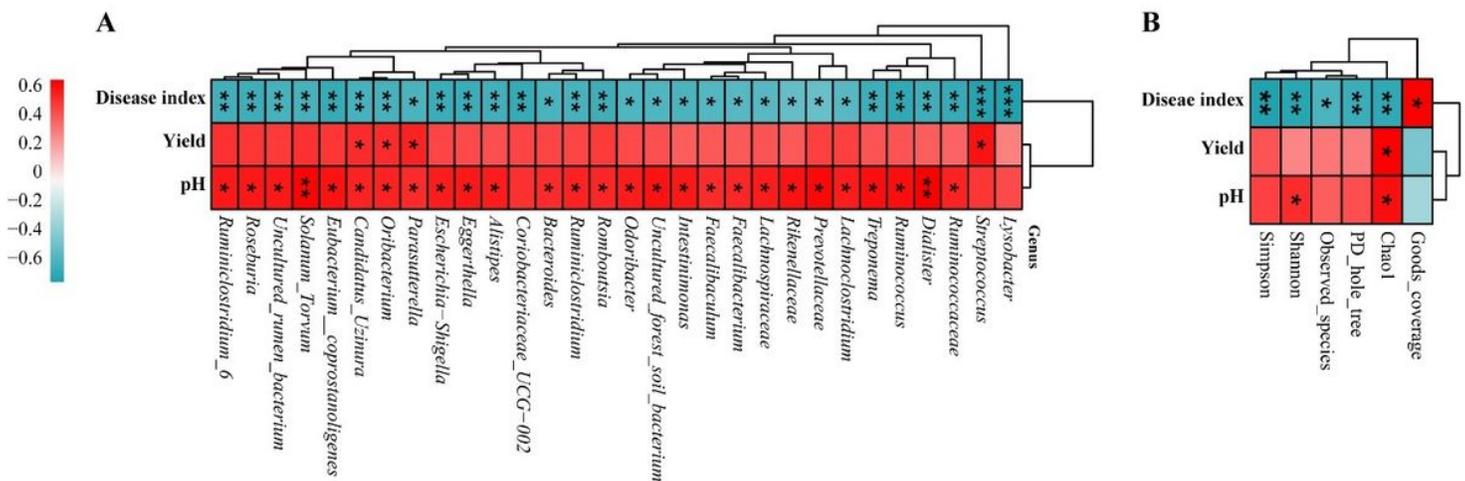
**Figure 4**

Relative abundance bar plots for top 10 bacterial phyla under different treatments. **Here:** Disease control (CK-D), healthy control (CK-H), single strain *Lysobacter antibioticus* 13-6 (T1), single strain *L. capsici* ZST1-2 (T2), single strain *Bacillus cereus* BT-23 (T3), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (T4), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (T5), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (T6), and commercial bactericide Kejia (T7).



**Figure 5**

Relative abundance bar plots for top 15 bacterial genera under different treatments. **Here:** Disease control (CK-D), healthy control (CK-H), single strain *Lysobacter antibioticus* 13-6 (T1), single strain *L. capsici* ZST1-2 (T2), single strain *Bacillus cereus* BT-23 (T3), intra-genus co-culture of *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (T4), inter-genus co-culture of *L. antibioticus* 13-6 + *B. cereus* BT-23 (T5), microbial consortia *B. cereus* BT-23 + *L. antibioticus* 13-6 + *L. capsici* ZST1-2 (T6), and commercial bactericide Kejia (T7).



**Figure 6**

Correlation analysis of disease index, yield and pH between bacterial genera and diversity indices.  
Correlation analysis of disease index, yield and pH between bacterial genera **(A)** and Correlation analysis of disease index, yield and pH between diversity indices **(B)**. Significance difference at according to Tukey's test at  $*p < 0.05$ ,  $**p < 0.01$ , and  $***p < 0.001$ .

## Supplementary Files

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