Evaluation and Genome Analysis of *Bacillus subtilis* YB-04 as a Potential Biocontrol Agent Against *Fusarium* Wilt and Growth Promotion Agent of Cucumber

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Cucumber wilt caused by *Fusarium oxysporum f.sp. cucumerinum* (Foc) is a highly destructive disease that leads to reduced yield in cucumbers. In this study, strain YB-04 was isolated from wheat straw and identified as *Bacillus subtilis*. It displayed strong antagonistic activity against *F. oxysporum f.sp. cucumerinum* in dual culture and exhibited significant biocontrol of cucumber *Fusarium* wilt with a higher control effect than those of previously reported *Bacillus* strains and displayed pronounced growth promotion of cucumber seedlings. *B. subtilis* YB-04 could secrete extracellular protease, amylase, cellulose, and β-1,3-glucanase and be able to produce siderophores and indole acetic acid. Inoculation with *B. subtilis* YB-04 or Foc increased cucumber defense-related enzyme activities for PPO, SOD, CAT, PAL, and LOX. However, the greatest increase was with the combination of *B. subtilis* YB-04 and Foc. Sequencing the genome of *B. subtilis* YB-04 showed that it had genes for the biosynthesis of various secondary metabolites, carbohydrate-active enzymes, and assimilation of nitrogen, phosphorous, and potassium. *B. subtilis* YB-04 appears to be a promising biological control agent against the *Fusarium* wilt of cucumber and promotes cucumber growth by genomic, physiological, and phenotypic analysis.

**Keywords:** *Fusarium oxysporum f.sp. cucumerinum*, biocontrol agent, genome sequencing and assembly, *Bacillus subtilis*, growth promotion

**INTRODUCTION**

Cucumber (*Cucumis sativus* L.) is an important vegetable crop worldwide. Cucumber wilt caused by *Fusarium oxysporum f.sp. cucumerinum* (Foc) is one of the most destructive diseases of cucumber that can lead to severe losses in yield and quality (Zhou et al., 2017). Foc enters root tissues by direct penetration or wounds causing visible symptoms, including necrotic lesions, vascular and root wilt, and ultimately death (Ahn et al., 1998). It can survive up to 20 years in soil (Zhao et al., 2017). Moreover, there are no commercially available cucumber cultivars with resistance...
against Fusarium wilt. Therefore, biological control of cucumber Fusarium wilt using antagonistic microorganisms has been considered to be a promising alternative.

Beneficial microorganisms can be used as biological control agents (BCAs) against different plant diseases. Many of these are species of Bacillus, which are also plant growth-promoting bacteria (PGPB) that can improve plant growth by producing secondary metabolites, such as fengycin, surfactin, bacillaecin, and macrolactin, siderophores, and indole acetic acid (IAA) and secreting hydrolysates to suppress plant pathogens and promote plant growth (Blake et al., 2021). More importantly, Bacillus species have several advantages, such as rapid growth, spore production, safe, non-pathogenic nature, and adaptation to broader environmental conditions (Nayak, 2021). However, there are several problems in the field application of microbial agents, including lack of high-efficiency biocontrol, relatively short shelf life, and variable control effectiveness. Therefore, understanding the growth-promotion and biocontrol mechanisms of beneficial microorganisms can significantly contribute to improved application efficacy of BCAs.

One way to better understand these organisms is through whole genome sequencing allowing for the discovery of genes for the production of bioactive compounds responsible for biocontrol and growth promotion (Bauman et al., 2021). For instance, sequencing the complete genome of the BCA Bacillus velezensis 9912D revealed gene clusters for secondary metabolite synthesis, including several potentially new lantibiotics (Pan et al., 2017). Similarly, the complete genome of Bacillus subtilis 7PJ-16 revealed genes for biosynthesis of antimicrobial metabolites and promoting plant growth traits, indicating its ability to act as a BCA and PGPB (Xu et al., 2019). The genome of B. subtilis 9407 showed that it had genes for the biocontrol mechanism against bacterial fruit blotch, including genes for a newly identified subtilosin A, bacilysin, and bacillaene (Gu et al., 2021).

In this study, strain YB-04 was isolated from wheat straw. A number of BCA and PGPB traits were screened for YB-04 in culture. The genome of strain YB-04 was sequenced to identify a number of genes associated with BCA and PGPB traits. Plant growth promotion of cucumber seedlings by soil inoculation of YB-04 was assessed based on chlorophyll content and growth of shoots, roots, stems, and leaves. Biocontrol activity by YB-04 against Fusarium wilt of cucumber was assessed based on disease severity and disease index. Furthermore, the activities of cucumber defense-related enzymes activities, both by YB-04 alone and in combination with Foc inoculation, were examined. The discovery and characterization of B. subtilis YB-04 indicate that it is a promising BCA and PGPB of cucumber.

**MATERIALS AND METHODS**

**Isolation of Strain YB-04 and in vitro Antagonism Test**

Strain YB-04 was isolated from wheat straw and cultured in LB broth by a dilution plate method at 37°C, collected from a field (E113°39', N35°05') at the Henan Academy of Agricultural Sciences in Xinxian, Henan, China in June. Foc was obtained from the College of Plant Protection, Henan Agricultural University. Antagonistic activity against Foc was performed by a dual culture where Foc was grown on PDA at 28°C for 5 days, and then 5 mm agar plugs were excised and transferred to the center of another PDA plate. Strain YB-04 was placed 3 cm away from the edge of the Petri dish, and the growth rate of Foc was measured relative to the control, which was Foc without strain on a plate (Xu et al., 2020).

**Biocontrol Efficiency of Strain YB-04 Against Cucumber Fusarium Wilt and Growth Promotion on Cucumber Seedlings**

Strain YB-04 was cultured in LB broth for 24 h at 37°C with shaking at 180 rpm and harvested by centrifugation (4,000 x g for 5 min), washed once with LB broth, and adjusted to 10^6 CFU/ml based on OD at 595 nm. Foc was grown on PDA at 28°C for 5 days, and then ten 5 mm agar plugs were excised and transferred to 100 ml PDB. The broths were incubated at 28°C in a shaker at 180 rpm for 3 days. The Foc cultures were filtered through 4 layers of sterile gauge, and the filtered spores were adjusted to 10^5 spores/ml using a hemacytometer (XB-K-25, Qiujing, Shanghai, China).

Cucumber seeds of cultivar Chuancui No. 3 were surface-sterilized in 75% ethanol (v/v) for 30 s and then rinsed with sterile water three times. The seeds were air-dried and each seed was planted in a separate pot (10 cm high, 10 cm diameter) filled with a 400 g sterilized mixture of soil. The plants were grown in the greenhouse at 25°C with a 16 h light/8 h dark photoperiod. After 10 days, each pot of cucumber seedlings was treated as follows: (1) drenching with 15 ml of YB-04 suspension; (2) first drenching with 15 ml of YB-04 suspension and 24 h of later drenching with 15 ml Foc spore suspension; (3) first drenching with 15 ml of 0.1% hymexazol and 24 h later drenching with 15 ml Foc spore suspension; (4) drenching with 15 ml of sterile distilled water; or (5) drenching with 15 ml of sterile distilled water and 24 h later drenching with 15 ml of Foc spore suspension. Each treatment was performed using 12 plants with 3 replicates. At 20 and 45 days post inoculation (dpi) with YB-04, chlorophyll content, shoot height and fresh weight, root length and fresh weight, stem thickness, and leaf area were measured, and disease severity and disease index were recorded for plants inoculated with Foc at 45 days post YB-04 inoculation (Chen et al., 2010). In brief, disease severity was assessed using a 0–4 disease scale; 0 = leaf asymptomatic; 1 = leaf wilting below 1/4 of cucumber seedling; 2 = leaf wilting in 1/4 to 1/2 of cucumber seedling; 3 = leaf wilting above 1/2 of cucumber seedling; 4 = the whole plant was wilted and died. The disease index was calculated using DI = [(0 × N0) + (1 × N1) + (2 × N2) + (3 × N3) + (4 × N4)] / T × 100, where N is the number of cucumber seedlings for each disease score and T is the total number of cucumber seedlings. Disease incidence = [N1 + N2 + N3 + N4] / T × 100.

Control efficacy = (DI of control - DI of treatment) / DI of control × 100%. The chlorophyll content of leaves was measured by using a SPAD-502 Plus chlorophyll content meter (Konica
Determination of Defense Enzyme Activities in the Cucumber Leaves

After 20 days post YB-04 inoculation, leaves were harvested and stored at -80°C. In brief, 0.5 g leaves were ground in liquid nitrogen, and 1 ml of extraction buffer was added. After centrifugation at 8,000 × g for 10 min, the supernatant was removed for enzyme assays. Enzyme activities were measured using assay kits for PPO (Cat. No. BC0195), SOD (Cat. No. BC0175), CAT (Cat. No. BC0205), PAL (Cat. No. BC0215), and LOX (Cat. No. BC0325) following the procedures of the manufacturer (Solarbio, Beijing, China). Absorbance was determined by using a plate reader (Tecan Spark, Tecan, Switzerland).

Detection of Plant Growth-Promoting Bacteria and Biological Control Agents Traits

Protease activity was detected with single colonies of YB-04 grown at 30°C for 5 days on skim milk agar (0.1 g CaCl$_2$, 5.0 g NaCl, 10.0 g skim milk, 10.0 g peptone, and 18.0 g of agar per liter, pH 7.2). Protease activity was observed as clear zones around the colonies (Kazanas, 1968). Amylase activity was detected with single colonies grown at 30°C for 48 h on starch agar (10.0 g soluble starch, 10.0 g tryptone, 5.0 g glucose, 5.0 g NaCl, 5.0 g beef extract, and 18.0 g of agar per liter, pH 7.2). Lugol’s iodine solution (1% iodine in 2% potassium iodide w/v) was added to the starch agar plate, and amylase activity was observed as a colorless halo (Al-Naamani et al., 2015). Cellulose activity was assayed with single colonies grown for 7 days at 30°C on carboxymethylcellulose agar (5.0 g CMC-Na, 0.1 g MgSO$_4$, 7H$_2$O, 0.25 g (NH$_4$)$_2$SO$_4$, 0.25 g K$_2$HPO$_4$, and 18.0 g of agar per liter, pH 5.5). The plates were flooded with 1% (w/v) Congo Red, and then washed with sterilized distilled water, and cellulase activity was detected as a clear zone (Teather and Wood, 1982). The β-Glucanase activity was assayed with single colonies grown at 30°C for 2 days on β-glucan agar (0.05 g glucose, 0.5 g yeast extract, 1 g peptone, 0.5 g NaCl, 0.01 g Congo Red, and 18.0 g of agar per liter, pH 7.0). The β-Glucanase activity was indicated by a clear zone around the colonies (Teather and Wood, 1982). Siderophore production was determined with single colonies grown at 30°C for 2 days in the dark on Chrome Azurol S blue agar (10 ml 20% sucrose solution, 30 ml 10% acid hydrolyzed casein, 1 ml 1 mmol/L CaCl$_2$, 5 ml 0.1 mmol/L phosphate-buffered saline (pH 6.8), 50 ml CAS dyeing solution, and 18 g of agar per liter, pH 7.2). Siderophore production was indicated by a change from blue to orange around the colonies (Schwyn and Neilands, 1987). IAA production was measured with single colonies grown at 30°C for 2 days on L-tryptophan nutrient broth (3 g beef extract, 10 g peptone, 5 g NaCl, 0.5 g L-tryptophan per liter, pH 7.2). After centrifugation at 14,000 × g for 10 min, 1 ml of supernatant was mixed with 2 ml of Salkowski stain, and then kept at room temperature in the dark for 30 min (Glickmann and Dessaux, 1995). All of the above reagents were of analytical grade and produced by China National Pharmaceutical Group Corp., Shanghai, China.

Genome Sequencing and Assembly of Strain YB-04

Strain YB-04 was grown in LB broth for 16 h at 37°C by shaking at 180 rpm. Genomic DNA was extracted with a MiniBEST Bacterial Genomic DNA Extraction Kit Ver. 3.0 following the manufacturer’s instructions (Takara, Beijing, China). An approximately 10 kb insert sequencing library was constructed and sequencing was performed using the PacBio Sequel II system (Pacific Biosciences, Menlo Park, CA, United States) by FraserGen (Wuhan, Hubei, China). Sequencing reads were de novo assembled by using HGA4 (Chin et al., 2013) and the Canu (v1.6) (Koren et al., 2017) software. The depth of genome coverage was analyzed by using the align tool (BLASR, v0.4.1) (Chaisson and Tesler, 2012). The assembled complete genome sequence was deposited in NCBI GenBank (Accession number CP072525). A circular map of the genome was constructed using Circos (v0.64) (Krzywinski et al., 2009).

Phylogenetic Relationship of Strain YB-04

The 16S rRNA gene sequences of strain YB-04 and B. velezensis YB-130, B. subtilis H1, B. subtilis 168, B. licheniformis SRCM103583, B. licheniformis ATCC 14580, B. altitudinis CHB19, B. altitudinis GQYP101, B. pumilus SF-4, and B. pumilus ZB201701 were obtained from the genomes (GenBank IDs: CP000560.2, CP054562.1, CP026662.1, NC_000964.3, CP035404.1, CP043559.1, CP040514.1, CP047089.1, and CP029464.1, respectively). A tree of the 16S rRNA gene sequences was constructed with MEGA 7.0 using the Neighbor Joining method (Kumar et al., 2016). Average Nucleotide Identity (ANI) was calculated using an ANI calculator (Yoon et al., 2017).

Analysis of Genes Encoding CAZymes and Gene Clusters Responsible for the Biosynthesis of Secondary Metabolites

Protein-coding genes in the genome of strain YB-04 were aligned with the carbohydrate active enZYme (CAZy) database aligned with the carbohydrate active enZYme (CAZy) database
Isolation of YB-04 and Wilt Disease Biocontrol Activity in vitro and in vivo

Dilution plating from surface-sterilized wheat straw yielded numerous colonies with different colony appearances. Twenty strains were purified and screened for antagonistic activity against Foc in dual culture and reduced wilt severity of cucumber inoculated with Foc in the greenhouse (data not shown). Strain YB-04 was selected based on having the greatest antagonistic activity against Foc in culture (Figure 1) and reduced wilt severity of cucumber seedlings at 20 days after Foc inoculation (Figure 2). Disease incidence, disease index, and control efficacy at 45 dpi revealed that YB-04 significantly reduced wilt symptoms caused by Foc to levels slightly less than the chemical fungicide hymexazol (Table 1).

Growth-Promotion Activity of Strain YB-04
At 20 and 45 dpi with strain YB-04, there was a significant increase in chlorophyll content, height and fresh weight of shoot, root length and fresh weight, stem thickness, and leaf area compared to non-treated cucumber seedlings (Figure 2 and Table 2). At 20 dpi, the greatest increases were observed for the fresh weight of shoots and roots at 115.91 and 334.88%, respectively. At 45 dpi, the greatest increases were observed for shoot height and leaf area at 79.03 and 49.07%, respectively.

At 20 and 45 dpi with strain YB-04 and Foc inoculation, there was also a significant increase in chlorophyll content, height, and fresh weight of shoot, root length and fresh weight, stem thickness, and leaf area compared to that of the Foc inoculated cucumber seedlings (Figure 2 and Table 2). This was also observed with the Foc inoculated seedlings treated with hymexazol. However, strain YB-04 treatment of the Foc-inoculated seedlings resulted in significantly higher chlorophyll content, shoot height and fresh weight, root length and fresh weight, and leaf area than Foc-inoculated seedlings with hymexazol. However, there was no significant difference in the stem thickness of Foc-inoculated seedlings with strain YB-04 or hymexazol at 45 dpi.

Effect of Strain YB-04 on Activities of Defense-Related Enzymes in Cucumber Seedlings
At 20 dpi with strain YB-04, cucumber seedlings showed significantly higher activities of SOD, CAT, PAL, and LOX, secondary metabolites was analyzed by using antiSMASH5.0 (Blin et al., 2019).

Statistical Analysis
Statistical analysis was performed using SPSS v21.0 by one-way analysis of variance (ANOVA). Means were compared with Duncan’s multiple range tests at a probability of $p \leq 0.05$. 

RESULTS

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At 20 dpi with strain YB-04, cucumber seedlings showed significantly higher activities of SOD, CAT, PAL, and LOX,
but not PPO, compared to non-treated seedlings (Table 3). Inoculation with Foc also significantly increased those enzyme activities, except PPO, compared to seedlings without Foc inoculation. The highest activities were observed with Foc inoculation and strain YB-04 treatment, which was significantly higher for all the enzymes compared to Foc inoculation. However, the activities of PAL and CAT significantly increased but LOX, PPO, and SOD significantly decreased with Foc inoculation and hymexazol treatment compared to Foc inoculation.

**Detection of in vitro Antifungal and Growth-Promoting Traits**

Strain YB-04 could secrete protease (Figure 3A), amylase (Figure 3B), cellulose (Figure 3C), and β-1,3-glucanase (Figure 3D).
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FIGURE 4 | Map of the strain YB-04 genome. The distributions of circles from outwards to inwards are as follows: ring 1 for genome size (black line); ring 2 for the restriction modification system, for forward strand (red) and reverse strand (blue); ring 3 for COG classifications of protein-coding genes on the forward strand and reverse strand; ring 4 for the distribution of tRNAs (brown) and rRNAs (green); ring 5 for GC skew; ring 6 for GC content. (Figure 3D). In addition, it could produce siderophores (Figure 3E) and indole acetic acid (Figure 3F).

Genome Sequencing, Assembly, and Identification of Strain YB-04

A total of 387,797 high-quality sequencing long reads with a mean length of 10,962 bp and an N50 of 13,374 bp were generated from the genomic DNA of strain YB-04 by the Pacbio sequencing platform. Total base pairs were 4,251,215,058 bp with an 882.47X genome coverage. The YB-04 genome consisted of a single circular chromosome of 4,156,177 bp with a GC content of 43.83% (Figure 4). There were 4,325 protein-coding genes covering 88.62% of the genome with an average gene length of 851.6 bp, which included 87 tRNAs, 30 rRNAs (5S, 16S, 23S), and 22 sRNAs. Four gene islands, three CRISPRs, and four prophages were detected (Supplementary Tables 1–3). For the predicted protein encoding genes, 99.70, 90.73, 75.45, 64.30, and 53.87% could be annotated with the NR, Swiss-Prot, COG, GO, and KEGG databases, respectively (Supplementary Table 4).

A phylogenetic tree based on 16S rRNA gene sequences of strain YB-04 and 10 other Bacillus isolates showed that strain YB-04, B. subtilis 168, and B. subtilis H1 clustered (Figure 5). Strain YB-04 and B. subtilis 168 had the maximum ANI value of 98.74%, followed by B. subtilis H1 with 98.65%, which is higher than the cutoff of 95–96% for bacterial species identity. ANI values between strain YB-04 and the 8 other Bacillus species ranged from 71.05 to 77.22% (Figure 6). Therefore, strain YB-04 was identified as B. subtilis.

Genome Analysis of Selected Genes of Bacillus subtilis YB-04

The genome of B. subtilis YB-04 had 111 genes identified as putative CAZymes, namely, 2 auxiliary activities (AAs), 7 polysaccharide lyases (PLs), 15 carbohydrate-binding modules (CBMs), 19 carbohydrate esterases (CEs) 24 glycosyltransferases (GTs), and 51 glycoside hydrolases (GHs) (Figure 7 and
Supplementary Table 5). Six of those were classified as both GHSs and CBMs.

There were 13 gene clusters predicted to be responsible for the biosynthesis of secondary metabolites. At 100% similarity, there was each matching gene clusters for bacillaene, fengycin, bacillibactin, subtilin, subtilosin A, and bacilysin synthesis. There was also one gene cluster with 82% similarity to that for surfactin synthesis. There were 6 biosynthetic gene clusters with no similarity in the antiSMASH database that appeared to be novel biosynthetic gene clusters of secondary metabolites. Based on their matches to the antiSMASH database, these were one gene cluster each for types of lanthipeptide-class-i, Type III PKS, tRNA-dependent cyclodipeptide synthases, other unspecified ribosomally synthesized, post-translationally modified peptide products, and two gene clusters, each encoding for terpenes (Table 4).

The genome of *B. subtilis* YB-04 contained predicted genes for an ATP-dependent phosphate uptake system *Pst*ABCS, *phoPR* operon for regulating *Pho* regulon in response to phosphate limitation, and alkaline phosphatase genes of *phoA* and *phoD* for phosphorus acquisition (Table 5). It also contained the *nasABCDEF* gene cluster for nitrite transport and reduction. Additionally, there were the potassium uptake system *ktrABCD*,...
B. subtilis cucumber seedlings. Previously, it was also a PGPB with pronounced growth promotion on hymexazol, which is used to control the disease in China. Reduced Fusarium than those achieved by Fusarium wilt of cucumber by (Chen et al., 2010; Li et al., 2012; Samaras et al., 2020). MBI600, and B. subtilis Foc microbial products. Biocontrol traits will help in developing them into successful screen for greater efficiency, shelf life, and consistency. As plant growth is considered to be promising (Radhakrishnan et al., 2017; Koskey et al., 2021), new strains are needed to screen for greater efficiency, shelf life, and consistency. As part of that, an in-depth analysis of growth promotion and biocontrol traits will help in developing them into successful microbial products.

In this study, B. subtilis YB-04 was found to be a BCA with antagonistic activity against Foc in dual culture and significantly reduced Fusarium wilt caused by Foc at levels comparable to hymexazol, which is used to control the disease in China. It was also a PGPB with pronounced growth promotion on cucumber seedlings. Previously, B. subtilis B579, B. subtilis MB1600, and B. subtilis B068150 were also shown to significantly reduce cucumber Fusarium wilt and promote cucumber growth (Chen et al., 2010; Li et al., 2012; Samaras et al., 2020). Compared to those bacteria, the percentage reduction in the Fusarium wilt of cucumber by B. subtilis YB-04 was greater than those achieved by B. subtilis B068150, B. subtilis B579, or B. subtilis MB1600. The percentage of increased growth-promotion based on the shoot and root fresh weight and plant height by B. subtilis YB-04 was greater than those achieved by B. subtilis B579 or B. subtilis MB1600. Thus, B. subtilis YB-04 appears to be more effective as a BCA and PGPB than some of the previously described B. subtilis tested on cucumber.

To act as a BCA against plant pathogenic fungi, bacteria possess a number of mechanisms including synthesis of hydrolytic enzymes, production of antibiotics, and induction of systemic resistance (Morales-Cedeño et al., 2021; Saeed et al., 2021; Xu et al., 2021). In this study, B. subtilis YB-04 had all of those mechanisms. Hydrolytic enzyme activities including protease, amylase, cellulose, and β-1, 3-glucanase were present in pure cultures, and they can break down chitin, glucans, and other polymers in fungal cell walls, thus inhibiting the growth of fungal pathogens (Naglot et al., 2015; Li et al., 2019). Other B. subtilis BCAs with similar enzymes include B. subtilis BCC6327, B. subtilis ZIM3, and B. subtilis LR1 (Thakaew and Niamsup, 2013; Banerjee et al., 2017; Dai et al., 2020). Furthermore, a large number of CAZyme genes were found in the genome of B. subtilis YB-04, also suggesting that it has a strong capability to be antagonistic against fungal plant pathogens based on the potential degradation and use of fungal polymers as nutrient sources (Banani et al., 2015; Chen et al., 2018; Sui et al., 2020). In addition, gene clusters were found to be responsible for the biosynthesis of known secondary metabolites, including bacillicene, fengycin, bacillibactin, subtilin, subtilosin A, bacilysin, and surfactin, indicating antibiotic production by B. subtilis YB-04, which is common in Bacillus species (Xu et al., 2019; Su et al., 2020). Other B. subtilis BCAs found to produce antibiotics or have genes for antibiotic production included B. subtilis BSD-2 for lantibipetide (Liu et al., 2016). Surfactin and fengycin can also be elicitors of induced systemic resistance in plants (Romero et al., 2007; Ongena et al., 2010). Many studies have reported that plant defense enzymes play important roles in disease resistance (Prasannath, 2017; Ji et al., 2020; Xu et al., 2021). Induction of the activities of defense-related enzymes in leaves following soil inoculation with B. subtilis YB-04 and Foc indicates a form of systemic resistance. Defense-related enzymes activities for PPO, SOD, CAT, PAL, and LOX could be induced by inoculation with B. subtilis YB-04 or Foc alone and the greatest increase was with the combination of B. subtilis YB-04 and Foc. Other B. subtilis BCAs causing host induction of defense-related enzyme activities include B. subtilis B579, B. subtilis SL-44, and B. subtilis CBB05 (Chen et al., 2010; Chandrasekaran and Chun, 2016; Wu et al., 2019).

All the growth parameters of cucumber seedlings measured in this study were increased with B. subtilis YB-04 treatment. Importantly, growth parameters were all increased much more in infected seedlings with Foc treated with B. subtilis YB-04 than those infected with Foc and treated with hymexazol. This indicates that B. subtilis YB-04 can improve plant growth while providing disease control, which would be an advantage over using hymexazol that did not promote growth. This was similar to Pseudomonas aeruginosa CQ-40 that controlled tomato gray mold caused by Botrytis cinerea and promoted the growth of tomato seedlings, whereas pyrimethanil only controlled the disease with a prevention effect of up to 64.71% (Wang et al., 2020). To act as a PGPB, bacteria have a variety of mechanisms including the production of enzymes and siderophores for nutrient acquisition and phytohormones to promote growth, and enzymes to reduce the negative effects of various abiotic stresses.
TABLE 4 | List of the putative gene clusters encoding for secondary metabolites by antiSMASH in the B. subtilis YB-04 genome.

| Clusters | Types | Genomic locations | Most similar known clusters | Similarity |
|----------|-------|-------------------|-----------------------------|------------|
| Cluster 1 | NRPS | 349,833–413,272 | Surfactin | 82% |
| Cluster 2 | Terpene | 1,124,835–1,145,348 | Bacillaene | 100% |
| Cluster 3 | Lanthipeptide-class-i | 1,699,549–1,725,648 | | |
| Cluster 4 | transAT-PKS,PKS-like,T3PKS,transAT-PKS-like,NRPS | 1,747,915–1,862,664 | Bacillibactin | 100% |
| Cluster 5 | NRPS, betalactone | 1,920,534–2,002,654 | Fengycin | 100% |
| Cluster 6 | Terpene | 2,073,306–2,095,204 | | |
| Cluster 7 | T3PKS | 2,142,940–2,184,037 | | |
| Cluster 8 | NRPS | 3,179,833–3,229,574 | Subtilin | 100% |
| Cluster 9 | Lanthipeptide-class-i | 3,377,840–3,404,065 | | |
| Cluster 10 | CDPS | 3,523,308–3,544,054 | | |
| Cluster 11 | Sactipeptide | 3,768,784–3,790,395 | Subtilosin A | 100% |
| Cluster 12 | Other | 3,797,486–3,838,904 | Bacilysin | 100% |
| Cluster 13 | RiPP-like | 4,040,385–4,053,116 | | |

TABLE 5 | Genes responsible for nitrogen, phosphorous, and potassium assimilation identified in the strain YB-04 genome.

| Function | Gene | UniProt accession No. | Description | Best hit in YB-04 | Identity |
|----------|------|-----------------------|-------------|-------------------|----------|
| Phosphate assimilation | phoA | P19406 | Alkaline phosphatase 4 | orf00988 | 99.35% |
| | phoR | P23545 | Alkaline phosphatase synthesis sensor protein PhoR | orf03027 | 99.48% |
| | phoP | P13792 | Alkaline phosphatase synthesis transcriptional regulatory protein PhoP | orf03028 | 99.58% |
| | phoD | P42251 | Alkaline phosphatase D | orf00275 | 99.49% |
| Phosphate transport | pstS | P46338 | Phosphate-binding protein | orf02500 | 99.67% |
| | pstC | A0A6M4JLF7 | Phosphate transport system permease protein | orf02499 | 99.35% |
| | pstB1 | P46342 | Phosphate import ATP-binding protein PstB 1 | orf02496 | 99.62% |
| | pstB2 | P46341 | Phosphate import ATP-binding protein PstB 2 | orf02497 | 99.63% |
| | pstA | A0A6M3ZE53 | Phosphate transport system permease protein | orf02498 | 100.00% |
| Nitrate/nitrite assimilation | nasD | P42436 | Nitrite reductase | orf00344 | 99.26% |
| | nasE | P42435 | Assimilatory nitrite reductase [NAD(P)H] small subunit | orf00343 | 100.00% |
| | nasA | P42432 | Nitrate transporter | orf00347 | 99.50% |
| | nasC | P42434 | Assimilatory nitrate reductase catalytic subunit | orf00345 | 98.03% |
| | nasB | P42433 | Assimilatory nitrate reductase electron transfer subunit | orf00346 | 97.54% |
| | nasF | P42437 | Uroporphyrinogen-III C-methyltransferase | orf00342 | 97.30% |
| Potassium assimilation | ktrC | P39760 | Ktr system potassium uptake protein C | orf01561 | 100.00% |
| | ykgA | P39759 | Putative gamma-glutamylcyclotransferase YkgA | orf01560 | 96.75% |
| | ktrD | O31658 | Ktr system potassium uptake protein D | orf01445 | 100.00% |
| | yugO | Q795M8 | Putative potassium channel protein YugO | orf02370 | 100.00% |
| | ktrB | O2081 | Ktr system potassium uptake protein B | orf02341 | 99.10% |
| | ktrA | O2080 | Ktr system potassium uptake protein A | orf02340 | 99.10% |

(Glick, 2012; Saeed et al., 2021). B. subtilis YB-04 produced siderophores that can improve iron uptake and alleviate harmful effects of iron on plants that have been associated with enhanced plant growth (Haas, 2003; Dimkpa et al., 2009). The genome of B. subtilis YB-04 also contained genes responsible for nitrogen, phosphorous, and potassium assimilation. Plant growth and development depend on macronutrients, such as nitrogen, phosphorous, and potassium, that are mostly obtained from the soil and can be made more available to plants by soil microbes that have the ability to solubilize nutrients and transfer them to plants (Glick, 2012; Rana et al., 2020). Finally, B. subtilis YB-04 produced IAA, which may be taken up by the cucumber seedlings stimulating the transcriptional expression of IAA responsive genes and enhancing biomass (Spaepen et al., 2014; Jiang et al., 2020).

In summary, B. subtilis YB-04 appears to be an effective BCA against cucumber Fusarium wilt and an effective PGPB of cucumber seedlings. The BCA mechanisms could include induced systemic host resistance as indicated by greater host defense-related enzyme activities, and direct pathogen inhibition through secretion of extracellular enzymes and antibiotics. The PGPB mechanisms could include nutrient acquisition via siderophores and enzymes for fixing nitrogen and solubilizing potassium and phosphorus, and direct plant growth enhancement through increased amounts of indole acetic acid. Compared to other B. subtilis strains used as cucumber
**Fusarium** wilt BCAs and PGPBs in cucumber, *B. subtilis* YB-04 is a more effective BCA than all those reported thus far and is a more effective PGBP than most reported so far. Thus, it appears to be a very promising novel beneficial *B. subtilis* strain for cucumber production.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

LY and WX conceived the research and designed the experiments. QY, XX, FY, XD, and WX performed the experiments and analyzed the data. WX prepared the manuscript draft. PG, BT, and LY critically revised the manuscript. All authors approved the final version of the manuscript.

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