

Article

Pseudomonas chlororaphis YBTa14 as a Multifunctional Biocontrol Agent: Simultaneous Growth Enhancement and Systemic Resistance Induction in *Vitis vinifera* Against Downy Mildew

Baoyan Li ^{1,†}, Qihua Sun ^{2,†}, Jie Shi ¹ , Wei Zhang ¹, Huafei Zhou ¹, Yingzi Wang ¹, Peisong Wang ¹, Meiling Tang ¹, Yuanpeng Du ³, Baoyou Liu ^{1,4,*}  and Jizhuang Wu ^{1,*}

¹ Yantai Academy of Agricultural Sciences, Yantai 265500, China; byli1314@163.com (B.L.);

zhibaoshijie@163.com (J.S.); 13805359930@163.com (W.Z.); zhhf2010@126.com (H.Z.);

ytnkyzbs@yt.shandong.cn (Y.W.); ytnkywps@163.com (P.W.); tmling1999@163.com (M.T.)

² Rushan City Agricultural and Rural Affairs Service Center, Weihai 264500, China; swzxzhk@wh.shandong.cn

³ State Key Laboratory of Crop Biology, Collaborative Innovation Center of Fruit & Vegetable Quality and Efficient Production, College of Horticulture Science and Engineering, Shandong Agricultural University, Taian 271018, China; duyuanpeng001@163.com

⁴ College of Life Sciences, Yantai University, 30 Qingquan Road, Yantai 264005, China

* Correspondence: baoyou1022@163.com (B.L.); wujizhuang@163.com (J.W.)

† These authors contributed equally to the work.

Abstract

Biological control serves as a crucial strategy for crop disease management. The biocontrol potential and plant growth-promoting effects of the strain YBTa14 were investigated. Genetic sequencing confirmed YBTa14 as *Pseudomonas chlororaphis*, which exhibited broad-spectrum antifungal activity against multiple pathogens affecting grapevine, apple, cherry, and wheat. YBTa14 significantly enhanced the growth of wheat and grapevine, specifically increasing wheat seed germination rates and improving root and coleoptile development. In grapevine plant, significant increases in root length, stem length, and fresh weight were observed. The strain demonstrated robust adaptability and stable antagonism under varying sodium chloride (NaCl) concentrations, pH levels, and temperatures. YBTa14 modulated plant hormone levels, elevating the content of indole-3-acetic acid (IAA), gibberellins (GA), and cytokinins (CTK). Furthermore, it effectively stimulated the production of key plant defense enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Pretreatment of grape leaves with YBTa14 triggered plant cell defense response and upregulated the expression of defense-related genes *PR1* (pathogenesis-related protein 1) and *PAL1* (phenylalanine ammonia-lyase 1), thereby mitigating the severity of downy mildew disease and inducing systemic resistance. These findings demonstrate that YBTa14 is a highly promising candidate for development as a multifunctional agricultural biocontrol agent.

Keywords: biocontrol agent; *Pseudomonas chlororaphis*; antagonistic activity; promote growth; downy mildew; *Plasmopara viticola*



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1. Introduction

Grape disease outbreaks have emerged as a key limiting factor for sustainable viticulture [1–3]. Among these, grape downy mildew (*Plasmopara viticola*) stands out due to its

severe damage potential and difficulty in disease prevention and control [3,4], exhibiting highly epidemic characteristics under suitable environmental conditions. By infecting critical organs such as leaves, shoots, inflorescences, and young fruits, it not only significantly impairs photosynthetic capacity but also causes inflorescence abortion and fruitlet drop, resulting in a devastating impact on grape yield [3,5,6].

Currently, chemical prevention and control remains the primary approach for managing this disease [5,6]. However, the series of issues arising from this method have become increasingly prominent: the continuous enhancement of pathogen resistance [7,8], the escalating risk of ecological pollution due to pesticide residues, and growing food safety concerns urgently need to be addressed. It is particularly noteworthy that grapes, as a fruit consumed fresh and as a raw material for winemaking, impose higher demands on product safety and quality stability. Against this backdrop, the development of efficient and eco-friendly microbial biocontrol agents has emerged as a promising breakthrough for achieving green control of grape diseases. This represents a current research focus and developmental direction in the field of plant protection [9].

Pseudomonas spp., as important plant growth-promoting rhizobacteria (PGPR), have become one of the most promising microbial groups for application in the field of biological control, owing to their broad-spectrum antimicrobial activity, environmental adaptability, and multiple growth-promoting mechanisms [10,11]. Bacteria within this genus can not only directly inhibit pathogens by producing antimicrobial metabolites but also promote plant growth through pathways such as inducing systemic resistance (ISR) and regulating plant hormones (e.g., IAA, GA) [12]. For example, Jiao et al. [13] isolated a *Pseudomonas viridis* subsp. Citri pa40, which could effectively prevent the growth of *Rhizoctonia cereali*, the pathogen of wheat sharp eyespot; Rovera et al. [14] demonstrated that *Pseudomonas viridis* subsp. Citrina Sr1 effectively suppresses charcoal rot in soybean caused by *Macrophomina phaseolina* and significantly increases stem length, root length, stem dry weight, and root dry weight; Hu et al. [15] isolated a high-yield PCN producing strain pcho10 from wheat field, which could control wheat scab *Fusarium graminearum*.

Based on 16S rRNA phylogenetic analysis, the genus *Pseudomonas* can be categorized into seven major species: *P. fluorescens*, *P. aeruginosa*, *P. putida*, *P. chlororaphis*, *P. syringae*, *P. pertucinogena*, and *P. stutzeri*. Among these, *P. chlororaphis* has garnered considerable attention as a focal point of research, primarily owing to its remarkable potential as a biological control bacterium. This species boasts a widespread distribution and has been successfully isolated from a variety of habitats. For instance, Strain UFB2 was isolated from soybean field soil [16]. Strain L19 was obtained from saline soil that was contaminated with coal, heavy metals, and petroleum [17]. Additionally, the psychrotolerant strain MCC2693 was recovered from a mountain ecosystem [18].

When endophytes successfully colonize plant tissues, they can effectively activate the plant's systemic resistance against pathogens [19]. The typical features of induced systemic resistance include enhanced biosynthesis of phenolic compounds, altered enzymatic activities of defense-related enzymes (peroxidase/POD, polyphenol oxidase/PPO, phenylalanine ammonia-lyase/PAL, and superoxide dismutase/SOD), as well as the induction and upregulation of multiple defense-related genes [20,21]. *Pseudomonas* bacteria can trigger plant defense responses through the salicylic acid (SA) or jasmonic acid (JA) signaling pathways. For instance, *Pseudomonas fluorescens* WCS417r can induce disease resistance in *Arabidopsis thaliana* via an NPR1-dependent pathway [12].

Although the biocontrol potential of *Pseudomonas* bacteria has been extensively reported, their application still faces a critical bottleneck: it is difficult to achieve both broad-spectrum effectiveness and stability simultaneously. Most strains are only effective against specific diseases and have limited environmental adaptability, easily becoming

inactivated under stress conditions such as high temperature and high salinity. To tackle the above-mentioned problems, *Pseudomonas chlororaphis* YTBa14 was isolated and identified. We systematically evaluated its broad-spectrum antifungal activity, environmental stability, crop growth-promoting efficacy, and hormone-regulation mechanisms. Meanwhile, we analyzed its ability to generate induced systemic resistance. The results demonstrated that YTBa14 possesses a trifunctional characteristic integrating “antagonism, growth promotion, and induced resistance”. This provides a theoretical basis for the development of novel multifunctional microbial agents and is of great significance for the advancement of safe and efficient plant disease prevention and control technologies.

2. Materials and Methods

2.1. Isolation and Screening of Strain YTBa14 Antagonism Assays

In 2019, strain YTBa14 was isolated from the rhizosphere of healthy *Vitis vinifera* plants in a vineyard in Yantai City, Shandong Province, China (37°29' N, 121°16' E). The isolation procedure was performed as follows: healthy root tissues were collected, thoroughly washed with sterile water, and surface-sterilized sequentially with 75% ethanol (1.5 min) and 0.1% HgCl₂ (2.5 min). After 3–5 rinses with sterile water, the sterilized roots were homogenized in sterile water containing quartz sand. The homogenate was allowed to settle for 30 min, and 0.1 mL of supernatant was plated on Nutrient Agar (NA). Following incubation at 30 °C for 24 h, a total of 85 morphologically distinct colonies were initially isolated. These isolates underwent three rounds of subculturing with screening based on colony morphology and growth rate. Through this selection process, strain YTBa14 was ultimately obtained, demonstrating superior characteristics including distinctive colony morphology, rapid growth rate, and excellent stability. Pure cultures of the selected strain were then grown in NA liquid medium at 30 °C with shaking (200 rpm) until reaching an OD₆₀₀ of 1.0.

The initial screening for antagonistic activity was conducted against two major grape pathogens, *Colletotrichum gloeosporioides* (grape anthracnose) and *Coniella vitis* (grape white rot), using dual-culture assays according to Wang et al. [22]. Specifically, a 5-mm agar plug containing pathogenic fungal mycelia was inoculated at the center of a PDA plate. Using the cross-streak method, 2 µL of YTBa14 bacterial suspension (OD₆₀₀ = 1.0) was spotted 2.5 cm away from the plate center. Sterile medium served as the negative control with all treatments performed in triplicate. The plates were sealed and incubated at 28 °C for 7 d and 10 d, respectively. The antagonistic activity was evaluated by measuring the diameter of inhibition zones.

2.2. Antagonistic Activity Evaluation of YTBa14 Against Phytopathogenic Fungi

YTBa14 was subcultured in NA medium at an inoculum ratio of 1% (*v/v*) and incubated at 30 °C with shaking at 200 rpm for 48 h. The culture was then diluted to an OD₆₀₀ of 1.0, followed by centrifugation at 6000× *g* and 4 °C for 15 min. The supernatant was collected, filter-sterilized through a 0.22 µm membrane, and stored as the YTBa14 culture filtrate.

The supernatant's inhibitory activity was evaluated by measuring the mycelial growth inhibition rate (%) against *Colletotrichum gloeosporioides*, *Coniella vitis*, *Rhizoctonia cerealis* (wheat sharp eyespot), *Botryosphaeria dothidea* (apple ring rot), *Alternaria alternata* (apple *Alternaria* leaf spot), and *Phytophthora nicotianae* (cherry stem rot). All fungal pathogens were isolated from host plants exhibiting characteristic disease symptoms and were authenticated through ITS sequence analysis. Specifically, *C. gloeosporioides* and *C. vitis* were isolated from infected grape berries; *R. cerealis* was obtained from wheat specimens with sharp eyespot symptoms; *B. dothidea* and *A. alternata* were derived from diseased apple

fruits, while *P. nicotianae* was isolated from cherry stems showing stem rot symptoms. For cultivation purposes, all pathogens were cultured on Potato Dextrose Agar (PDA) medium (containing 20 g glucose, potato infusion from 200 g potatoes, 15 g agar, and 1000 mL distilled water; pH 7.0), except for *Phytophthora nicotianae*, which was grown on V8 agar medium (containing 200 mL V8 vegetable juice, 15 g Agar, 3 g CaCO₃, distilled water up to 1000 mL). Additionally, the antagonistic effect of YTBta14 against grape downy mildew (*P. viticola*) was assessed using a detached leaf disk assay [23].

The mycelial growth inhibition rate (%) against the pathogens causing white rot, anthracnose, wheat sharp eyespot, apple ring rot, apple Alternaria leaf spot, and cherry stem rot was calculated using the formula $n = [(A - B)/A] \times 100$, where A represents the average colony diameter (mm) in the control plate, and B represents the average colony diameter (mm) in the treatment plate containing YTBta14.

The inhibition rate (%) against grape downy mildew was calculated using the formula $n = (DICK - DIT)/DICK \times 100$, where DICK represents the average disease index in the control group, and DIT represents the average disease index in the treatment group.

Each experiment was independently repeated three times, and all data presented are mean values of three replicates.

2.3. Identification of Strain YTBta14

The YTBta14 strain was inoculated into NA liquid medium and incubated with shaking at 28 °C and 200 rpm for 24 h. Genomic DNA was isolated employing the Tiangen Bacterial Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol. Conserved genes, including 16S rRNA and *rpoD* (Table 1), were selected for PCR amplification and sequencing [24,25]. The resulting PCR products were sent to Sangon Biotech (Shanghai, China) Co., Ltd. for sequencing. Following sequencing, the obtained sequences were aligned and manually corrected using BioEdit software (V 7.05), and concatenated analysis of the two genes (16S rRNA and *rpoD*) was performed [1]. Phylogenetic analysis was conducted using the maximum likelihood method in MEGA version 11.0 software, and a phylogenetic tree was constructed (with 1000 bootstrap replicates) [1].

Table 1. Primer sequences used in PCR.

Gene Name	Primer Sequences	Reference
16S rRNA	5'-AGAGTTTGATCCTGGCTCAG-3'	[24]
	5'-AAGGAGGTGATCCAGCCGCA-3'	
<i>rpoD</i>	5'-CACGGTTGAGCACATCCTCT-3'	[25]
	5'-GGAGAGTACTTCGCGAGTCG-3'	

2.4. Determination of Environmental Adaptability and Antimicrobial Activity of YTBta14

2.4.1. Salt Stress Adaptability Analysis

YTBta14 was inoculated at 1% (*v/v*) into NA liquid medium containing 2%, 4%, 6%, 8%, and 10% (*w/v*) NaCl, respectively. The cultures were incubated at 30 °C with shaking at 200 rpm for 24 h, followed by measurement of OD₆₀₀.

2.4.2. Thermal Stability Determination

YTBta14 bacterial suspension pre-cultured in a shaking incubator at 30 °C and 200 rpm for 24 h was aliquoted and placed in water baths at 20 °C, 40 °C, 60 °C, 80 °C, 100 °C, and 121 °C (autoclave temperature) for 30 min. The treated suspensions were centrifuged at 10,000 rpm for 10 min. The supernatants were filter-sterilized through 0.22 µm microporous membranes to obtain cell-free filtrates. Antimicrobial activity against *P. viticola* was evaluated using the leaf disk assay [23].

2.4.3. pH Stability Determination

YTBTa14 was inoculated into NA medium adjusted to different pH values (2.0, 4.0, 6.0, 6.5, 7.0, 7.5, 8.0, 10.0, and 12.0) and cultured at 30 °C with shaking at 200 rpm for 24 h. Antimicrobial activity against *P. viticola* was then determined using the leaf disk assay.

2.5. Plant Growth-Promoting Effects of YTBTa14

2.5.1. Wheat Seed Germination Assay

YTBTa14 was cultured in NA liquid medium. Cell-free filtrates were prepared from cultures adjusted to OD₆₀₀ = 0.5, 0.1, and 0.05, respectively. For each concentration, 10 mL of filtrate was mixed with 30 mL of 1% carboxymethyl cellulose sodium (CMC) solution to form treatment solutions.

Surface-sterilized wheat seeds (sequential treatment: 75% ethanol for 5 min → sodium hypochlorite for 5 min → 3–5 rinses with sterile water) were immersed in the treatment solutions (50 seeds per concentration, three replicates). After 2 h immersion, seeds were air-dried overnight at room temperature. Seeds were then placed on moist filter paper in Petri dishes and incubated at 25 °C in continuous darkness for 10 d. Germination rate was recorded, and root/shoot lengths were measured. Control group seeds were treated with a mixture of fresh NA medium and CMC solution.

2.5.2. Grapevine Plant Growth Promotion Trial

Five one-year-old ‘Cabernet Franc’ were selected. Commencing at the sprouting stage, 50 mL of YTBTa14 supernatant (centrifugation at 6000 × g and 4 °C for 15 min) was applied weekly via root drenching (respective OD₆₀₀ values: 0.5, 0.1 and 0.05 per treatment) for four consecutive weeks. Control plants (n = 5) received equivalent volumes of sterile water. At 24 h after the final treatment, leaf phytohormone content was determined, including IAA, GA, CTK, and abscisic acid (ABA) [26]. Leaf enzymatic activities of SOD, POD, and CAT were measured at designated intervals (1, 3, 5, 7, and 9 d post-final treatment) [27]. The activity of SOD was determined at 560 nm using the nitroblue tetrazolium (NBT) photoreduction method. POD activity was measured by monitoring absorbance changes at 470 nm via the guaiacol method, while CAT activity was assessed based on the decomposition rate of H₂O₂ at 240 nm. All measurements were performed using a UV-Vis spectrophotometer (MAPADA UV-1100B, Shanghai Mapada Instrument Co., Ltd., Shanghai, China). Thirty days after treatment completion (58 d post-initial treatment), root system length, shoot height, and plant fresh weight were quantified.

2.6. Expression of Defense-Related Genes Against *Plasmopara viticola*

After foliar application of YTBTa14 on grape leaves, samples were collected at 6, 12, 24, 48, and 72 hpi to quantify the expression levels of *NPRI*, *PR1*, and *PAL1*. Additionally, two experimental groups were established: one treated with YTBTa14 and the other with sterile water (control). Four days after treatment, *P. viticola* was inoculated onto the leaves in both groups. Samples were then taken at 0, 6, 12, 24, and 48 hpi to evaluate the expression of *PR1* and *PAL1* genes.

Total RNA was extracted from grape leaves at various time points using the Tripure Isolation Reagent kit (Roche Diagnostics, Indianapolis, IN, USA). First-strand cDNA was synthesized from 200 ng RNA using a random hexamer primer and the cDNA synthesis kit (Parstous, Mashhad, Iran), following the manufacturer’s instructions. Gene expression levels were analyzed by qRT-PCR using the primers listed in Table 2.

Quantitative real-time PCR was conducted on a C1000™ Thermal Cycler (BioRad, Hercules, CA, USA) using SYBR Premix Ex (TaKaRa Bio Inc., Kusatsu, Japan) as the fluorescent dye. Real-time PCR reactions (12 µL) contained 6 µL of Master Mix Green High

ROX, 1 µL of cDNA, 0.5 µM of each gene-specific primer (Table 2), and 4 µL of DEPC-treated water. The translation elongation factor 1 alpha (*EF-1α*) gene served as the reference. The thermal cycling protocol comprised an initial step at 45 °C for 5 min, denaturation at 94 °C for 30 s, followed by 40 cycles of 94 °C for 5 s, 60 °C for 15 s, and 72 °C for 10 s. Primer sufficiency was verified, and relative gene expression was calculated in relation to the *EF-1α* gene using the comparative CT approach (Δ CT method). The relative gene expression was calculated using the Δ CT method [1,28]. The formula is as follows:

$$\text{Relative expression} = e^{-\Delta C_t} = e^{-(C_t \text{ target gene} - C_t \text{ reference gene})}$$

Ct values were the means of three biological and three technical replications.

Table 2. Primer sequences used in real-time PCR.

Gene Target	Primer Sequences	Reference
<i>PR1</i>	5'-GGAGTCCATTAGCACTCCTTTG-3' 5'-CATAATTCTGGGCGTAGGCAG-3'	[29]
<i>NPR1</i>	5'-GGAATTCGATGTTGGGTACG-3' 5'-GCAACCTTGTCAAGAATGTCC-3'	[30]
<i>PAL1</i>	5'-CCAGTTCTCAGAGCTTGTTAATGA-3' 5'-ATACATGTTCCCTATCCACCACTT-3'	[31]
<i>EF-1 α</i>	5'-AACCAAAATATCCGGAGTAAA AGA-3' 5'-GAACTGGGTGCTTGATAGGC-3'	[1]

2.7. Analysis of Plant Cell Defense Response Characteristics

Twelve uniformly growing one-year-old ‘Cabernet Franc’ grapevines were randomly allocated into two experimental groups (n = 6 per group). The treatment group was treated with sterile YTBta14 culture filtrate (OD₆₀₀ = 1.0), while the control group was treated with deionized water. For 4 d, a subset of grapevines from each group (n = 3) were treated with a spore suspension of *P. viticola* at a concentration of 1 × 10⁶ spores/mL, followed by inoculation. Samples were collected 6 h after inoculation for the following assays.

2.7.1. Reactive Oxygen Species Detection (DAB Staining)

From each grapevine plant, one leaf of uniform maturity was selected (three leaves per treatment), carefully excised at the petiole base (avoiding contact with other leaf regions). The samples were immersed in 1 mg/mL 3,3'-diaminobenzidine (DAB) staining solution. Staining was performed under light at 28 °C for 12 h. Subsequently, the samples were decolorized with 95% ethanol until the tissues became completely transparent. Finally, overall sample photography and localized microscopic observation were conducted.

2.7.2. Cell Necrosis Detection

Cell death was assessed using Trypan Blue staining, an established protocol for visualizing cell death in leaves [32,33]. Leaf samples were collected using the same standardized procedure as detailed in Section 2.7.1. The samples were placed in a staining solution (15 mg Trypan Blue, 10 mL phenol, 10 mL glycerol, 10 mL lactic acid, and 10 mL deionized water) and stained in the dark for 12 h. After removing the staining solution, the samples were transferred to a 2.5 g/mL chloral hydrate solution for decolorization for 12 h. The samples were then suspended in deionized water for microscopic observation.

2.8. Statistical Analysis

Statistical analysis was performed using one-way ANOVA with Duncan’s multiple range test (DMRT) at α = 0.05 significance level via DPS software (v18.10).

2.9. The Flowchart

A schematic representation of the study protocol is provided in Supplementary Figure S1.

3. Results

3.1. Biocontrol Effect of *Pseudomonas chlororaphis* YBTa14 Against Plant Pathogens

The YBTa14 strain exhibited significant broad-spectrum antifungal activity. Compared to the control group, YBTa14 demonstrated pronounced inhibitory effects against major grapevine pathogens. Specifically, the inhibition zone diameters against *C. gloeosporioides* (Figure 1A,B) and *C. vitis* (Figure 1C,D) were significantly larger than those in the control. The diluted YBTa14 culture filtrate exhibited significant inhibitory effects against *C. gloeosporioides*, *C. vitis*, and *P. viticola*, with inhibition rates reaching 62.65%, 52.44%, and 61.69%, respectively (Figure 1E–J). Furthermore, the strain exhibited broad-spectrum inhibitory activity against various other plant pathogenic fungi, including but not limited to *P. nicotianae*, *A. mali*, *B. dothidea*, and *R. cerealis*, all achieving inhibition rates exceeding 40% (Figure 1K–R, Table 3).

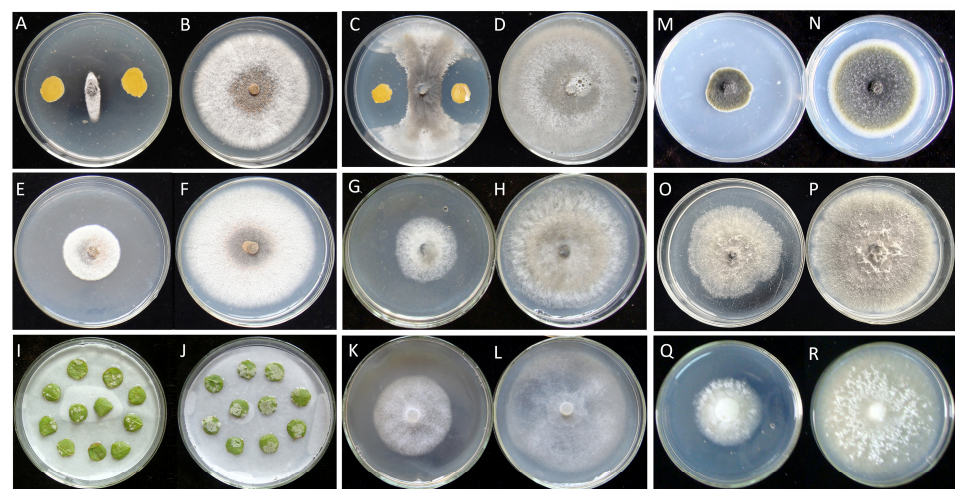


Figure 1. Antagonistic assay of YBTa14 against seven pathogenic fungi. (A) YBTa14+ *C. gloeosporioides*. (B) Control test of A: PDA+*C. gloeosporioides*. (C) YBTa14 + *C. vitis*. (D) Control test of C: PDA+ *C. vitis*. (E) YBTa14 culture filtrate+*C. gloeosporioides*. (F) Control test of E: PDA+*C. gloeosporioides*. (G) YBTa14 culture filtrate+ *C. vitis*. (H) Control test of G: PDA+ *C. vitis*. (I) YBTa14 culture filtrate+ *P. viticola*. (J) Control test of I: NA+ *P. viticola*. (K) YBTa14 culture filtrate+ *P. nicotianae*. (L) Control test of K: V8+ *P. nicotianae*. (M) YBTa14 culture filtrate+ *A. mali*. (N) Control test of M: PDA+ *A. mali*. (O) YBTa14 culture filtrate+ *B. dothidea*. (P) Control test of O: PDA+ *B. dothidea*. (Q) YBTa14 culture filtrate+ *R. cerealis*. (R) Control test of R: PDA+ *R. cerealis*.

Table 3. Inhibitory effect of YBTa14 on seven pathogens in laboratory.

Plant Pathogens	Radius (cm)/Disease Index (100%)		Inhibition Rate (%)
	YBTa14	CK	
<i>Colletotrichum gloeosporioides</i>	3.10 ± 0.26	8.3 ± 0.26	62.65 ± 2.84
<i>Coniella vitis</i>	3.90 ± 0.17	8.2 ± 0.10	52.44 ± 1.65
<i>Plasmopara viticola</i>	22.17 ± 0.32	58.4 ± 2.29	61.69 ± 1.97
<i>Botryosphaeria dothidea</i>	5.20 ± 0.26	8.8 ± 0.17	40.91 ± 3.57
<i>Alternaria mali</i>	2.70 ± 0.26	6.7 ± 0.20	59.70 ± 4.28
<i>Phytophthora nicotianae</i>	4.90 ± 0.30	8.3 ± 0.10	40.96 ± 3.30
<i>Rhizoctonia cerealis</i>	4.50 ± 0.30	8.5 ± 0.17	47.06 ± 3.73

Note: The data in the table are the average values of three replicates.

3.2. YBTa14 Was Identified as *Pseudomonas chlororaphis*

The 16S rDNA and *rpoD* gene sequences of strain YBTa14 were obtained by PCR amplification. Phylogenetic analysis based on the concatenated sequences revealed that this strain clustered within a clade comprising several *Pseudomonas chlororaphis* strains (Figure 2A). So, the strain YBTa14 was identified as *Pseudomonas chlororaphis*.

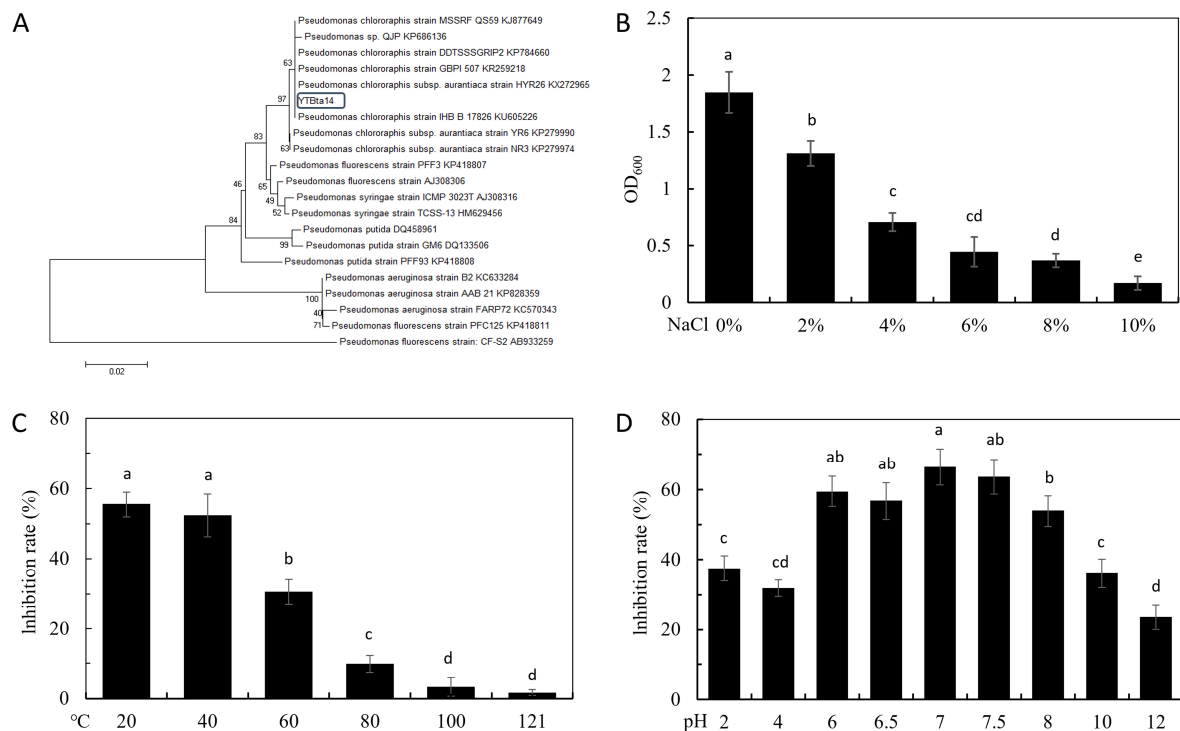


Figure 2. Identification and stability analysis of YBTa14. (A) Phylogenetic tree of strain YBTa14 based on partial 16S rDNA and *rpoD* gene sequences. (B) OD₆₀₀ of YBTa14 under different NaCl concentrations at 1 dpi. (C) Temperature-dependent inhibitory activity of YBTa14 fermentation broth against *P. viticola*. (D) pH-dependent inhibitory activity of YBTa14 fermentation broth against *P. viticola*. Numbers at branching points indicate bootstrap value derived from 1000 replicates. Lowercase letters indicate statistically significant differences ($p < 0.05$) among treatments.

Environmental adaptability and antagonistic stability are critical indicators for evaluating antagonistic strains as biocontrol agents. This study systematically evaluated the antagonistic stability of *Pseudomonas chlororaphis* YBTa14, providing theoretical support for exploiting endophytic microbial resources in agricultural applications. Analysis of antagonistic stability demonstrated that strain YBTa14 exhibited robust growth across a NaCl concentration range of 0.0–10.0% (w/v) (Figure 2B). Although its inhibitory activity decreased under 40–60 °C, the active metabolites remained stable and retained sustained antimicrobial efficacy (Figure 2C). Notably, the highest antagonistic activity was observed under neutral pH conditions. While exposure to extreme pH values (either highly acidic or alkaline) significantly reduced its inhibitory efficacy against *P. viticola*, the antimicrobial activity was consistently maintained above 20% (Figure 2D).

3.3. The Growth-Promoting Potential of Strain YBTa14 on Crops

The experimental results demonstrated that YBTa14 exhibited significant plant growth-promoting effects on both wheat and grape. Treatment with YBTa14 cell-free filtrate at varying concentrations resulted in significant differences ($p < 0.05$, Figure 3A) in germination vigor and root length among wheat seed treatment groups. The optimal effect was observed with the filtrate derived from a culture at OD₆₀₀ = 0.1, which yielded

the highest germination vigor, root length, and coleoptile growth: the germination rate reached 93.33%, representing a 21% increase compared to the control (71.33%) (Figure 3B). At this concentration, YTBa14 significantly enhanced wheat seedling growth, increasing the primary root length and coleoptile length by 2.33 cm and 2.57 cm, respectively, relative to the control (Figure 3B). Assessments on grapevine plant revealed that treatment with YTBa14 culture supernatant at $OD_{600} = 0.1$ and 0.05 significantly increased shoot length, root length, and fresh weight compared to the control group (Figure 3C,D). The treatment with $OD_{600} = 0.1$ filtrate demonstrated the most pronounced growth-promoting effect, increasing root length, shoot length, root fresh weight, and shoot fresh weight by 91.07%, 103.39%, 38.46%, and 62.50%, respectively, relative to the control. These findings provide a crucial theoretical foundation for subsequent research on growth promotion and disease resistance.

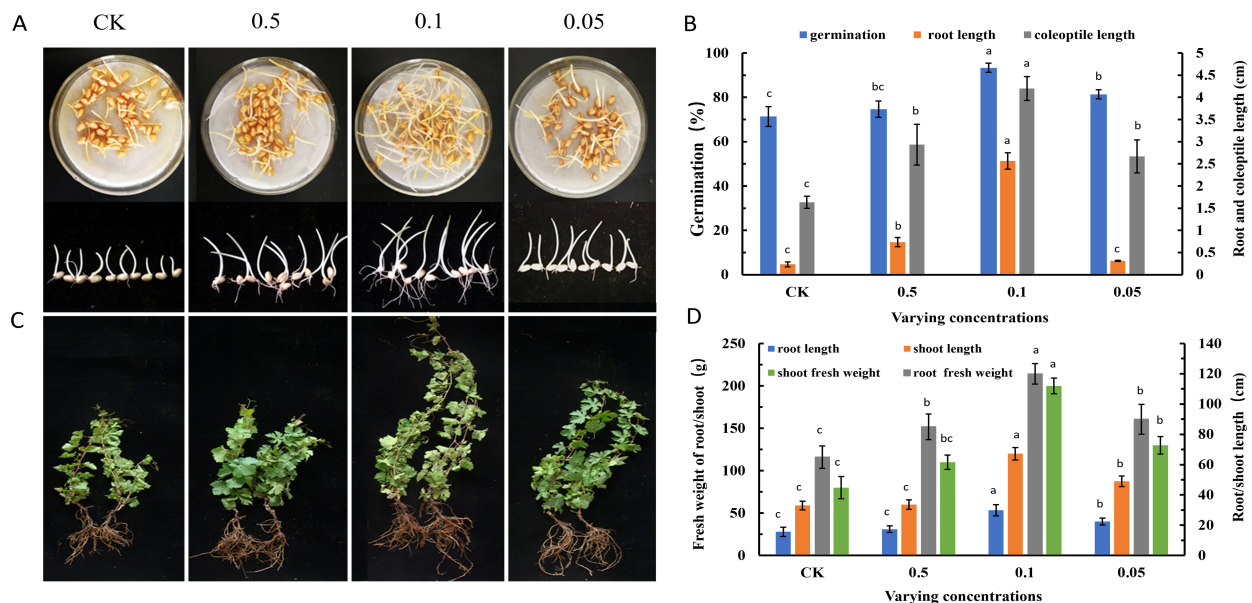


Figure 3. Growth-promoting effects of YTBa14 on wheat and grapevine. (A) Phenotypic presentation of wheat seedlings and roots grown on filter papers for 10 d. (B) Statistical analysis of germination rate, root length, and shoot length in wheat under different treatments. (C) Phenotypic comparison of grape plants treated with NA broth (control, CK) versus various concentrations of YTBa14. (D) Quantitative measurements of grape shoot length, root length, and fresh weight under different treatments. Lowercase letters indicate statistically significant differences ($p < 0.05$) among treatments.

3.4. Differential Regulation of Plant Hormones Mediates Growth Promotion by *Pseudomonas chlororaphis* YTBa14

Research has demonstrated that plant growth is primarily influenced by microorganisms through direct modulation of physiological and biochemical responses, including endogenous hormone levels and enzyme activities. As illustrated in Figure 4, plant hormone metabolism was significantly regulated by *Pseudomonas chlororaphis* strain YTBa14. Quantitative analysis indicated that the contents of auxin (IAA), gibberellin (GA), and cytokinin (CTK) were substantially elevated in YTBa14-treated plants compared to the control group ($p < 0.05$), with increases of 42.85%, 94.64%, and 36.81% observed, respectively. Although a slight increase in abscisic acid (ABA) content was detected, no significant difference was found relative to the control ($p > 0.05$). These results suggest that plant growth promotion by YTBa14 may potentially be mediated through selective activation of IAA, GA, and CTK biosynthesis pathways.

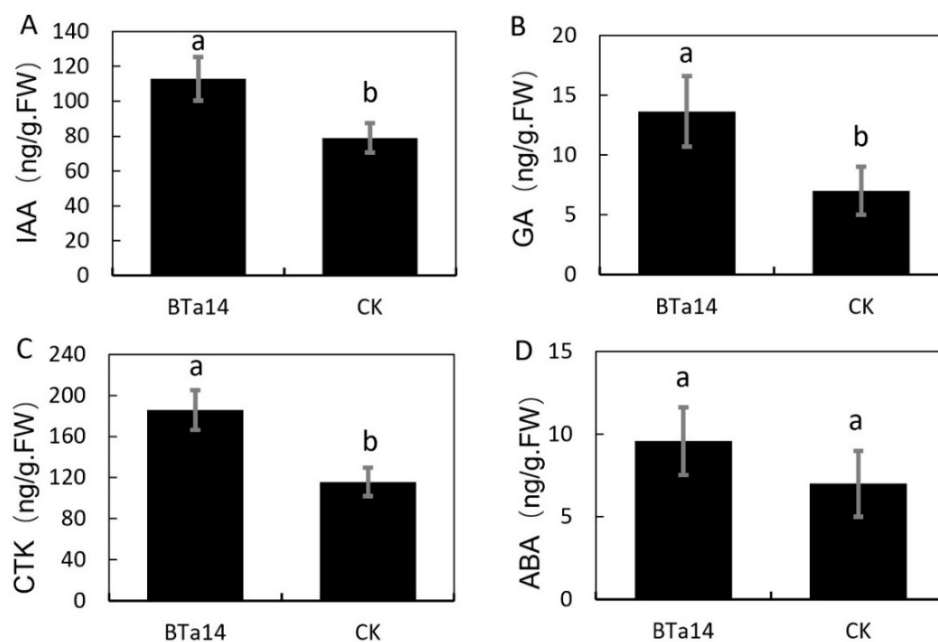


Figure 4. Quantitative analysis of endogenous hormone levels in grapes treated with YTBta14 versus control: (A) IAA, (B) GA, (C) CTK, and (D) ABA. Lowercase letters indicate statistically significant differences ($p < 0.05$) among treatments.

3.5. YTBta14-Mediated Priming Enhances Pathogen Resistance and Accelerates Defense Gene Expression

After 4 d of treatment with *P. chlororaphis* YTBta14 or sterile water, grape leaves were inoculated with *P. viticola*. The YTBta14-pre-treated group exhibited significantly reduced disease severity, whereas the control group (sterile water treatment + pathogen inoculation) developed severe disease symptoms (Figure 5A). This observation was further verified through quantitative assessment of pathogen proliferation in leaves. High levels of *P. viticola* were detected in inoculated grape leaves, while the pathogen was undetectable in non-inoculated controls. YTBta14-pre-treated leaves exhibited only trace pathogen levels following inoculation. PCR analysis demonstrated that YTBta14 pre-treatment significantly suppressed *P. viticola* proliferation compared to pathogen-only controls (Figure 5B).

The rapid induction of *PR* gene expression serves as a canonical molecular hallmark of plant defense responses. YTBta14 treatment directly activated the expression of defense-related genes *PR1* and *NPR1*, both exhibiting sustained and significant upregulation from 6 to 72 h (Figure 5C). Specifically: RT-PCR analysis revealed that both *PR1* and *NPR1* transcript levels reached maximal induction at 48 hpt, showing comparable 3-fold increases relative to untreated controls. Although exhibiting more moderate induction, phenylalanine ammonia-lyase gene *PAL1* still reached maximal expression levels at 24 h and 48 h post-YTBta14 treatment, with values 2-fold greater than control samples (Figure 5C).

Defense-related gene expression was analyzed at 0, 6, 12, 24, and 48 hpi with *P. viticola* alone. Compared to pathogen inoculation alone, YTBta14 pretreatment significantly accelerated the expression of the defense-related genes *PR1* and *PAL1*. As shown in Figure 5D,E, inoculation of untreated grapevine leaves with *P. viticola* resulted in only weak upregulation of *PR1* and *PAL1* at 12 hpi. In contrast, plants pre-treated with sterile YTBta14 fermentation broth exhibited upregulation of these genes even at 0 hpi, followed by an increasing expression trend over time.

These results demonstrate that inoculation with *P. viticola* following YTBta14 treatment triggered enhanced expression of defense-responsive genes, indicating the induction of

systemic resistance in grapevines. Therefore, the priming effect induced by YTBta14 plays a role in enhancing grapevine resistance against *P. viticola*.

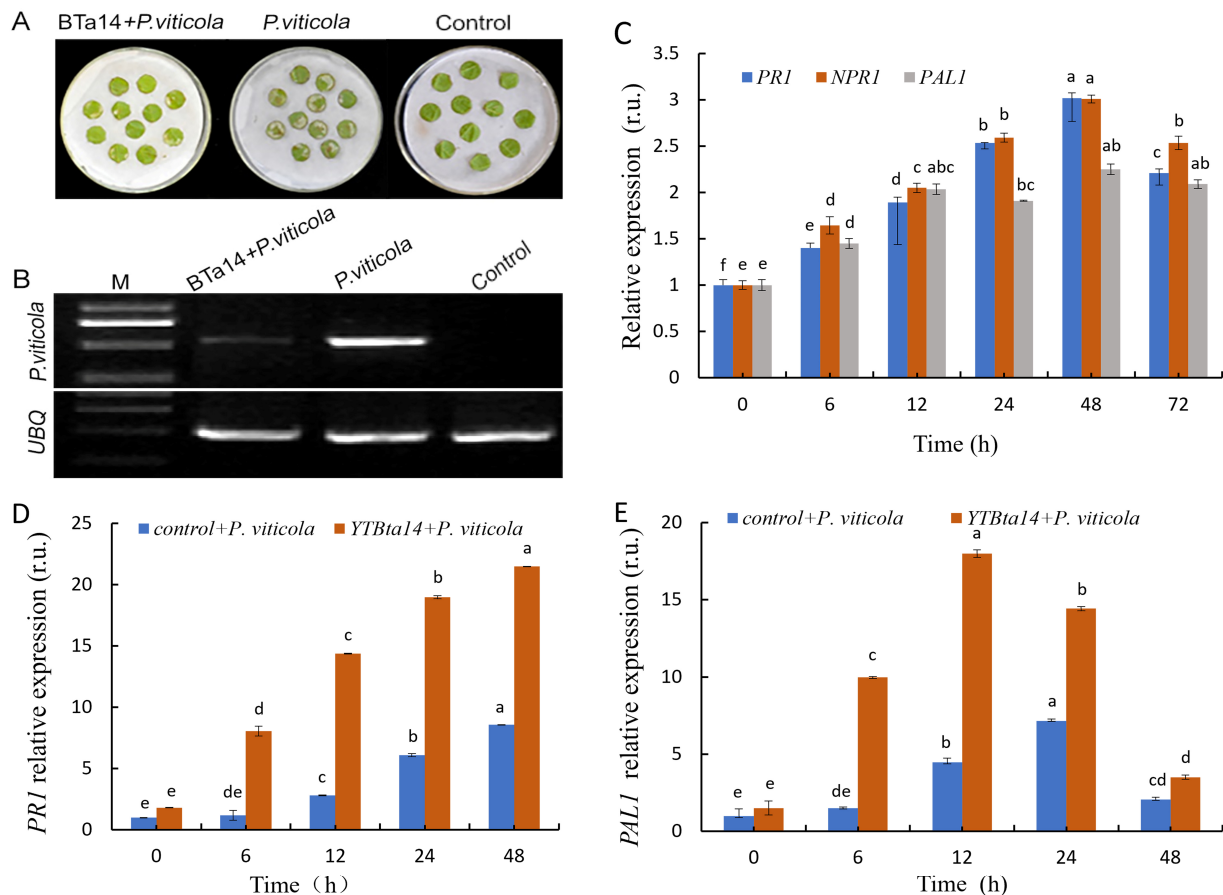


Figure 5. YTBta14 induces defense-related gene expression in grapevine against downy mildew. (A) Disease symptoms of grape leaves at 7 d with *P. viticola* under different treatments. (B) ITS-based quantitative analysis of pathogenic microbe proliferation in *Vitis vinifera* leaves. (C) Expression patterns of hypersensitive response (HR)-related marker genes in YTBta14-treated grapevines by real-time PCR. (D) Relative expression levels of *PR1* gene measured by real-time PCR. (E) Relative expression levels of *PAL1* gene measured by real-time PCR. Notes: X-axis indicates treatment time (hpi). Y-axis represents relative gene expression levels (fold change). Error bars represent standard deviation ($n = 3$). Lowercase letters indicate statistically significant differences ($p < 0.05$) among treatments.

3.6. YTBta14 Primes Cellular Defense Responses in Grapevine Against Downy Mildew

The oxidative burst can function during the hypersensitive cell death response, acting as a defense activator and a component of cellular defense responses induced following pathogen infection. Analysis of intracellular defense responses induced by *P. viticola* infection after YTBta14 pretreatment revealed that, as shown in Figure 6, YTBta14 treatment alone did not induce an oxidative burst in grapevine cells. Infection with *P. viticola* alone resulted in minimal reactive oxygen species (ROS) accumulation, whereas pretreatment with YTBta14 followed by pathogen infection triggered significant ROS accumulation at 6 hpi. Results from Trypan Blue staining were consistent with this finding, demonstrating that only the combination of YTBta14 pretreatment and subsequent *P. viticola* infection induced a rapid oxidative burst and micro-hypersensitive response (micro-HR) in the leaves.

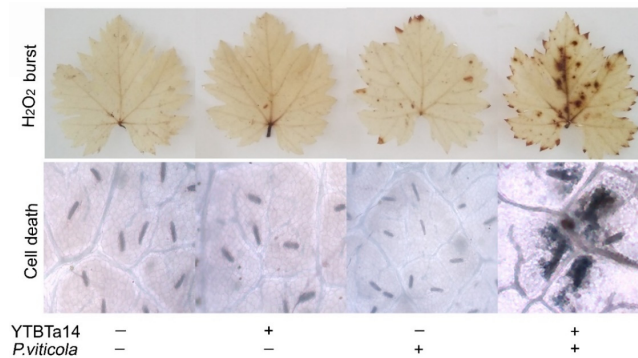


Figure 6. Oxidative burst and hypersensitive response in grapevine induced by YTBTa14 pretreatment and *P. viticola* infection. ROS levels were determined by DAB staining, and cell death was assessed using Trypan Blue staining. ‘+’ treatment with either YTBTa14 or *P. viticola*.

3.7. YTBTa14 Regulates Antioxidant Enzyme Activities in Grape

POD, SOD, and CAT are crucial components of the plant defense system. The grapevines treated with sterile YTBTa14 supernatant showed significantly higher POD activity than the control group throughout the experimental period, reaching peak levels on day 5 and maintaining elevated concentrations from 5 to 11 d (Figure 7A). This indicates that YTBTa14 effectively activates the POD biosynthetic pathway in plants. Similarly, the treated group showed 2.8-fold higher peak SOD activity (day 7) than controls ($p < 0.01$), demonstrating YTBTa14’s defense-enhancing capacity (Figure 7B). Although CAT activity in the treated group peaked on day 1 post-treatment and subsequently declined, it maintained significantly higher levels throughout the experimental period, remaining more than 5-fold greater than control values by day 5. Moreover, the treated group consistently exhibited superior CAT activity at all measured time points compared to controls (Figure 7C). In summary, YTBTa14 significantly elevated the overall activity levels of all three enzymes. This phenomenon suggests that YTBTa14 may enhance the oxidative stress defense capacity of grapevine plant through the systematic upregulation of the antioxidant enzyme system, providing a physiological basis for further elucidating its disease resistance mechanisms.

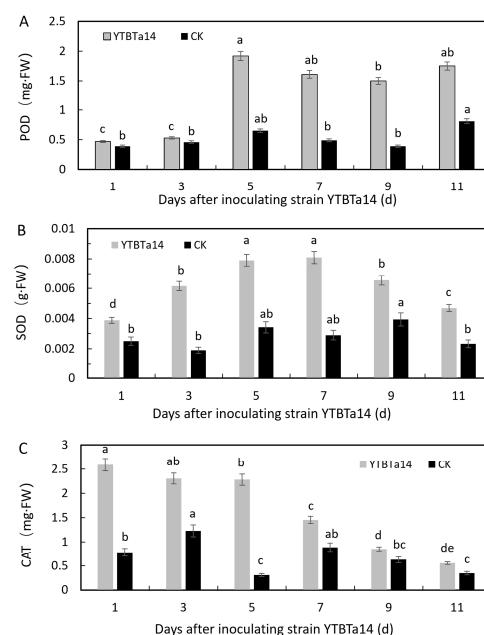


Figure 7. Peroxidase (A), superoxide dismutase (B), and catalase (C) enzyme activities in the leaves. Error bars represent the means $\pm$ standard deviations of three replicate experiments. Different letters above the bars indicate a significant difference at $p < 0.05$ according to Duncan’s multi-range test.

4. Discussion

In recent years, the escalating issues of pesticide resistance and environmental pollution caused by the overuse of chemical pesticides have become increasingly prominent. Consequently, the development of microbial biocontrol agents has emerged as a research hotspot in the field of plant disease control [34,35]. In this study, the strain *Pseudomonas chlororaphis* YTBTa14, which was isolated and identified, exhibited significant broad-spectrum antifungal activity as well as plant growth-promoting effects. Its mechanisms of action involve multiple pathways, including direct antagonism, systemic induced resistance, and phyto-regulation, positioning it as a promising candidate strain for developing dual-functional products that integrate biopesticide and biofertilizer functionalities.

4.1. Broad-Spectrum Antifungal Activity and Biocontrol Potential of YTBTa14

This study demonstrates that strain YTBTa14 exhibits significant inhibitory activity against multiple phytopathogens, showing pronounced efficacy against *C. vitis*, *C. gloeosporioides*, and *P. viticola*. Moreover, substantial inhibitory effects were observed against *B. dothidea*, *A. mali*, *P. nicotianae*, and *R. cerealis*. These findings corroborate the well-documented biocontrol traits of *P. chlororaphis*, including broad-spectrum disease suppression, plant growth promotion [36], enhanced stress tolerance [37,38], and environmental safety [39].

Further evidencing the genus' potential, *in vitro* assays revealed that *P. chlororaphis* G05 suppresses mycelial growth of *F. graminearum*, *C. gloeosporioides*, and *B. cinerea* [40]. Complementarily, foliar applications of *P. chlororaphis* O6 formulations controlled tomato leaf blight and gray mold, while exhibiting dual functionality—concurrently targeting root-knot nematodes (*Meloidogyne* spp.) and aphids via bioactive metabolites [41]. Similarly, rhizosphere-adapted *P. chlororaphis* PCL1606 demonstrated efficacy against soil-borne pathogens through rhizocompetence [17,18], and strain PCL1391 showed race-specific activity against *Colletotrichum lindemuthianum* [42].

Antagonistic stability analysis indicated promising biocontrol potential for strain YTBTa14. The strain maintained activity under 10% NaCl concentration and exhibited optimal antifungal efficacy at neutral pH. Its stability surpassed that of *P. chlororaphis* strain Zong1 [43] and was comparable to the optimal pH range (6.0–7.5) reported for *P. chlororaphis* UFB2 by Setlhare et al. [44].

YTBTa14 also demonstrated notable thermostability. Following treatment of its fermentation filtrate at six temperature gradients (40 °C to 90 °C) for 30 min, the inhibition rate against *P. viticola* (grape downy mildew) decreased with increasing temperature. Nevertheless, the inhibition rate remained above 30% at 60 °C, indicating good thermal stability. This finding aligns with the thermostability observed in the fermentation filtrate of *P. chlororaphis* WS-04, a strain identified by Liu et al. as effective against walnut canker [45].

However, it should be noted that laboratory-based antagonism and inoculation assays differ significantly from field conditions. Successful biocontrol critically depends on the colonization capacity of the antagonistic strain, which largely determines its efficacy in practical settings [46]. Therefore, further investigation is required to assess YTBTa14's stable performance under field conditions, including its colonization dynamics in the grape rhizosphere and on grapevines.

P. chlororaphis is capable of synthesizing a diverse array of antimicrobial substances, encompassing phenazines, cyclic lipopeptides, and volatile organic compounds (VOCs). These compounds have been shown to exert inhibitory effects on fungi, bacteria, and oomycetes [47]. For example, it produces metabolites such as phenazine (which can both directly antagonize pathogen growth and activate plant defense) [48,49], pyrrolnitrin [40], and resorcinol. These metabolites demonstrate potential for promoting plant growth and facilitating the colonization of biocontrol bacteria in diverse plant hosts [41]. No-

tably, YTBta14 exhibits efficacy against pathogens of many crops, suggesting the possible presence of multiple antimicrobial compounds. Further characterization of its specific metabolite profile via mass spectrometry (e.g., LC-MS) is warranted.

4.2. Growth-Promoting Effects of *Pseudomonas chlororaphis* YTBta14

Research demonstrates that *P. chlororaphis* exhibits significant growth-promoting effects across multiple crops. Previous studies confirm its efficacy in enhancing the growth and development of agricultural plants such as tomato, maize, and wheat [50,51]. In this study, isolated *P. chlororaphis* strain YTBta14 demonstrated marked growth-promoting properties in both wheat and grape systems: their fermentation filtrates significantly increased wheat seed germination rate, primary root length, and coleoptile elongation, while concurrently enhancing grapevine plant height. Quantitative analyses revealed a 91.07% increase in grape root length and a substantial 103.39% increase in plant height compared to control groups.

This growth-promoting phenomenon demonstrates broad applicability across plant-microbe interaction systems. Qin et al. [52] reported that *P. fluorescens* strain XG32 significantly improved growth parameters (plant height, root length, biomass) in pepper seedlings. Similarly, *P. saponiphila*, an endophyte isolated from *Dendrobium officinale* by Wu et al. [53], enhanced pepper seedling growth. These findings align closely with our observations.

Regarding the underlying mechanism, existing evidence suggests modulation of endogenous phytohormone levels. Strain YTBta14 treatment significantly elevated concentrations of growth hormones—including IAA, GA and CTK—in plants. This hormonal shift presumably enhances mineral nutrient and water uptake efficiency, ultimately driving growth promotion. This mechanism is consistent with the established literature: approximately 82% of plant growth-promoting rhizobacteria (PGPRs) synthesize phytohormones (e.g., IAA, CTK, GA) via tryptophan or metabolic intermediates, thereby stimulating excessive lateral root and root hair development to facilitate growth [54–56].

4.3. Molecular and Physiological Evidence for Induced Systemic Resistance

Research indicates *P. fluorescens* enhances host resistance by upregulating defense-related genes and promoting antimicrobial protein synthesis [12]. For example, strain WCS417r triggers broad-spectrum ISR in *Arabidopsis thaliana* [57], while *P. putida* BTP1 activates jasmonic acid (JA) signaling and elevates PAL activity to induce systemic resistance in tomato [58]. Similarly, *Pseudomonas* sp. Sn48 and *Pantoea* sp. Sa14 significantly upregulate *PR1*, *PR2*, and *PR4* expression in grapevines challenged by *Agrobacterium tumefaciens* [59].

Our study integrates physiological and molecular analyses to dissect *P. viticola* defense mechanisms in grapevines primed by strain YTBta14. Key findings include the following:

(1) Early Defense Marker Induction: YTBta14 rapidly upregulates expression of *PR1*, *NPR1* (SA pathway master regulator), and *PAL1* (phenylpropanoid key enzyme) in leaves, suggesting coordinated SA and JA-mediated resistance activation.

(2) SA Pathway Initiation: *PR1* expression peaked at 48 h (4-fold increase), following *NPR1* upregulation at 24 h. This sequential pattern mirrors *NPR1*-dependent systemic acquired resistance [12,60], indicating early SA pathway engagement. Concurrent sustained *PAL1* upregulation (2-fold at 48 h) correlates with lignin deposition and phytoalexin synthesis [61], aligning with observed pathogen suppression.

(3) Systemic Antioxidant Enhancement: YTBta14 elevates superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities systemically. This parallels SOD, POD induction during *Pseudomonas syringae* infection [62] and enzyme hyperactivity in *Ralstonia solanacearum* responses [63].

(4) Primed ROS Response: Crucially, YTBa14 pre-treatment triggered a robust H₂O₂ burst (6 hpi) and micro-hypersensitive response (micro-HR) upon pathogen challenge—a defense mechanism restricting pathogen spread [64]. YTBa14 alone did not induce ROS accumulation, confirming its priming role in sensitizing hosts to subsequent attack [65], likely via epigenetic modifications or regulatory protein pre-accumulation [66].

Collectively, YTBa14 upregulates *PR1* (SA marker) and *PAL1*, induces H₂O₂ burst, and enhances SOD, POD activities. This integrated response demonstrates concurrent SA and JA/ethylene pathway activation (via *PAL1*), establishing a multi-pathway defense network that potentiates systemic resistance in grapevines.

5. Conclusions

In summary, *Pseudomonas chlororaphis* YTBa14 demonstrates significant potential as a biocontrol and plant growth-promoting agent. The strain exhibits broad-spectrum antifungal activity against key grapevine pathogens, including *C. gloeosporioides*, *C. vitis*, and *P. viticola*, with inhibition rates exceeding 50%. Phylogenetic analysis confirmed its classification as *P. chlororaphis*, and its environmental adaptability and antagonistic stability under varying pH, temperature, and salinity conditions further support its suitability for agricultural applications. Additionally, YTBa14 enhances crop growth by modulating endogenous hormone levels (IAA, GA, and CTK) and significantly improving germination, root elongation, and biomass in wheat and grapevines. The strain also primes systemic resistance in plants, accelerating defense-related gene expression (*PR1*, *NPR1*, *PAL1*) and inducing oxidative bursts and antioxidant enzyme activity (POD, SOD, CAT) to combat *P. viticola* infection. These findings highlight YTBa14's dual role in pathogen suppression and growth promotion, providing a robust foundation for its development as a sustainable biocontrol agent in agriculture. Further research should explore field efficacy and large-scale application strategies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture15171822/s1>, Supplementary Figure S1. The main flowchart of this study.

Author Contributions: Methodology, B.L. (Baoyan Li) and B.L. (Baoyou Liu); Software, B.L. (Baoyan Li); J.S. and H.Z.; Validation, J.S. and W.Z.; Formal Analysis, B.L. (Baoyan Li); J.S. and P.W.; Investigation, B.L. (Baoyan Li); Y.W. and M.T.; Resources, B.L. (Baoyan Li); B.L. (Baoyou Liu) and J.W.; Data Curation, B.L. (Baoyan Li); Y.D., Q.S. and B.L. (Baoyou Liu); Writing—Original Draft, B.L. (Baoyan Li); Q.S., J.W. and B.L. (Baoyou Liu); Visualization, B.L. (Baoyan Li) and Y.W.; Supervision, Y.D., J.W. and Q.S.; Project Administration, B.L. (Baoyou Liu); Funding Acquisition, B.L. (Baoyan Li); Y.D. and B.L. (Baoyou Liu). All authors have read and agreed to the published version of the manuscript.

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