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Exiguobacterium acetylicum Strain SI17: A Potential Biocontrol Agent against *Peronophythora litchii* Causing Post-Harvest Litchi Downy Blight

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Abstract: Litchi downy blight (LDB) caused by *Peronophythora litchii* destroys 20–30% of litchi fruit every year and causes significant economic losses. Some *Exiguobacterium* strains exhibit considerable promise in both agricultural and industrial sectors. *E. acetylicum* SI17, isolated from the litchi fruit carposphere, demonstrated significant biocontrol activity against LDB through pre-harvest treatment. To elucidate its underlying regulatory mechanisms, the genome of SI17 was sequenced and analyzed, revealing a circular chromosome spanning 3,157,929 bp and containing 3541 protein-coding genes and 101 RNA genes. Notably, 94 genes were implicated in the production of secondary metabolites. Among the 29 *Exiguobacterium* strains so far sequenced, SI17 possessed the largest genome. In the phylogenomic analysis encompassing the entire genome, SI17 was clustered into Group I. Treating litchi fruit with SI17 before harvesting resulted in a decrease in H_2O_2 content in the fruit peel and an increase in superoxide dismutase activity, thus enhancing resistance to LDB. Interestingly, SI17 did not display plate antagonism against *Peronophythora litchii* SC18. It can be inferred that SI17 generates secondary metabolites, which enhance litchi's resistance to LDB. This study represents the first documentation of an *Exiguobacterium* strain exhibiting a role in litchi plant disease and showcasing significant potential for the biological control of LDB.

Keywords: *Exiguobacterium acetylicum* SI17; litchi downy blight; genome sequencing; defense activation; biocontrol efficacy

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1. Introduction

The litchi fruit (*Litchi chinensis*) belongs to the Sapindaceae family and is typically found in subtropical areas of Asia, South Africa, Australia, the United States (especially Hawaii), and Israel [1]. Originating from South China, it is predominantly cultivated in the southern provinces of Fujian, Guangdong, and Hainan. Unfortunately, harvested litchi fruit are highly susceptible to spoilage due to fungal diseases caused by pathogens such as *Peronophythora litchii*, *Geotrichum candidum*, and *Colletotrichum gloeosporiodes*, resulting in a 20–30% loss. Among these, litchi downy blight (LDB), caused by *P. litchii*, is a significant issue [2]. *P. litchii* infects litchi tender leaves, flowers, and mature fruits in the field and post-harvest storage. This hemibiotrophic, homothallic, and polycyclic oomycete produces oospores spherical in shape with smooth walls and a light-yellow color. Oospores can survive in the soil or plant debris for several years and germinate directly or indirectly under favorable conditions. Lemon-shaped deciduous sporangia with papillae at the apex are also produced. Sporangium, wind or water dispersal, germinates directly, producing germ tubes

and penetration hyphae able to penetrate the receptive organs. Alternatively, sporangia can form secondary sporangia or release zoospores. Zoospores, kidney shaped, have two lateral flagella, and rapidly germinate in cool, wet conditions. *P. litchii* causes withering and watery brown spots on the infection sites of tender leaves or fruit and produces downy white sporangiophores, which trigger pre- and post-harvest fruit decay [3]. LDB also affects the growth and development of young leaves, although it is most detrimental during the period from bloom to fruit ripening, particularly in extremely humid and rainy conditions [4]. At present, the prevailing methods for managing LDB largely rely on post-harvest applications of chemical fungicides (like dimethomorph and propamocarb). Regrettably, these residues may persist in fruits, posing carcinogenic hazards to consumers and contributing to environmental pollution [5]. Hence, it is crucial and urgent to explore alternative techniques like biological control to minimize post-harvest decay of litchi fruit. This method provides safe, environmentally friendly, and sustainable results, in line with the goals of global strategic initiatives for eco-conscious and/or organic farming practices [6].

In recent years, there has been rapid progress in utilizing biological control agents (BCAs) to manage post-harvest diseases [7]. These applications have exhibited effectiveness in suppressing *P. litchii* and preventing decay in litchi fruit. Among the BCAs employed, there are various antagonistic bacteria like *Bacillus subtilis* [1,8,9], endophytic strains such as *B. amyloliquefaciens* TB2 and LY-1 [10,11], and *Lactobacillus plantarum* LAB [12]. Additionally, certain metabolites have demonstrated antagonistic effects against *P. litchii*, including hypothemycin from *Paecilomyces* sp. SC0924 [2], zeamines from *Dickeya zeae* [13], and 4-ethylphenol from *Streptomyces fimicarius* BWL-H1 [14]. The primary mechanisms through which BCAs inhibit diseases include the synthesis of antibiotics, release of extracellular enzymes such as protease, chitinase, cellulase, and β-1,3-glucanase, production of siderophores, and generation of indole acetic acid (IAA). They also occupy ecological niches, enhance microbial diversity in the phyllosphere and carposphere [15], and prompt plant defense responses such as systemic acquired resistance, induced systemic resistance, and/or priming [16].

Exiguobacterium exhibits significant promise for utilization across various sectors like industry and agriculture, owing to the presence of certain strains that possess diverse functionalities such as enzyme production, bioremediation, degradation of toxic substances, and facilitation of plant growth [17]. For instance, *E.* sp. VSG-1 produces hydrolytic enzymes like cellulase, pectinase, mannanase, xylanase, and tannase, which bolster the conversion of fermented sugar from sugarcane bagasse into bioethanol [18]. Additionally, *E. profundum*, known for its protease production ability, has been employed in the extraction of chitin from shrimp shells [19]. Furthermore, various other hydrolytic enzymes like lipase, amylase, and pullulanase have been isolated from different *Exiguobacterium* strains, further expanding their potential applications [20–23]. *E. alkaliphilum* B-3531D, *E.* sp. AO-11, and *E. mexicanum* M7 exhibit the capacity to metabolize crude oil, suggesting promise for the development of bioremediation solutions for oil-contaminated marine ecosystems [24–26]. Additionally, four *Exiguobacterium* isolates have demonstrated the ability to reduce Cr (VI) levels in polluted environments [27–30]. Moreover, *Exiguobacterium* exhibits significant potential for agricultural usage, with several strains possessing traits that promote plant growth across various crops [31–35]. While disease control is vital in agriculture, there have been no reported applications of *Exiguobacterium* in plant disease management thus far.

The genus *Exiguobacterium* includes species and strains engaged in industry and agriculture, comprising enzyme production, bioremediation, degradation of toxic substances, and plant growth-promoting properties [17]. Cellulase, pectinase, mannanase, xylanase, and tannase produced by *E.* sp. VSG-1 make steam-exploded sugarcane bagasse useful to biofuel fabrication by *Saccharomyces cerevisiae* fermentation [18]. A protease-producing strain of *E. profundum* has been employed in the extraction of chitin from shrimp shells [19]. Lipase, amylase, and pullulanase have been isolated from different *Exiguobacterium* strains, increasing their potential applications [20–23]. *Exiguobacterium* sp. AO-11, *E. alkaliphilum*

B-3531D, and *E. mexicanum* M7 metabolize crude oil, suggesting promise for the bioremediation of oil-contaminated marine ecosystems [24–26]. *Exiguobacterium* strains ZM-2, GS1, PY14, and *E. mexicanum* from chromite mines reduce Cr (VI) levels in polluted environments [27–30]. Moreover, several *Exiguobacterium* strains exhibit significant potential for agricultural applications as plant growth promoting bacteria [31–35]. *Exiguobacterium* has been reported to suppress fungal diseases of cereal crops in Australia [36], and *E. acetylicum* strain 1P MTCC 8707 inhibited the in vitro growth of *Rhizoctonia solani*, *Sclerotium rolfsii*, Pythium sp., and *Fusarium oxysporum* [37]. No applications of *Exiguobacterium* in pre- and post-harvest plant disease management have been reported thus far.

The genomes of several *Exiguobacterium* strains have been sequenced. Based on the concatenated single-copy core genes and 16S rRNA gene sequences, the *Exiguobacterium* strains were clustered into two groups. Group I comprised strains capable of thriving at temperatures of 7 °C or lower, while Group II encompassed the remaining strains [38,39]. The proliferation of transporters, crucial for conveying vital substrates and conferring resilience against environmental stresses, was a key factor propelling the genome expansion in Group I strains, thus broadening their ecological niche [39]. Additionally, genome sequencing enables us to elucidate the agricultural utility of these strains.

E. acetylicum SI17, sourced from the carposphere of litchi fruit, has demonstrated efficacy in combating LDB through biocontrol measures [40]. The volatile organic compounds (VOCs) emitted by SI17, particularly those containing α-Farnesene (AF), exhibited the ability to hinder the growth of *P. litchii*, thus alleviating the severity of LDB [40]. The purpose of the study was to gain deeper insights into the attributes of SI17. We carried out genome sequencing and comparative analysis of its genome.

2. Materials and Methods

2.1. E. acetylicum SI17 Bioinformatics Analysis

2.1.1. Bacterial Culture and DNA Extraction

E. acetylicum SI17 was isolated from the interior of litchi fruit sarcocarp. The cells of *E. acetylicum* SI17 were cultured in a 500 mL flask containing 100 mL of Luria–Bertani (LB) medium (tryptone 10 g/L, yeast extract 5 g/L, and NaCl 10 g/L) at 28 °C with shaking at 200 rpm for 24 h. Subsequently, the cells were harvested at 4 °C through centrifugation at 10,000× *g* for 5 min. Genomic DNA extraction was carried out immediately using the method outlined by Hoffman and Winston [41]. The purity and integrity of the DNA were evaluated by agarose gel electrophoresis, and its concentration was measured using a Qubit™ fluorometer (Invitrogen, Carlsbad, CA, USA).

2.1.2. Genome Sequencing, Assembly, Annotation, and Phylogenomic Analyses

The genome of *E. acetylicum* SI17 was sequenced, assembled, and annotated following the methodology outlined by Zheng et al. [42]. The genome was sequenced on the Nanopore PromethION. Further, twenty-eight complete amino acid sequences of various *Exiguobacterium* strains were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/genome/ (accessed on 24 May 2021)). A phylogenomic tree, based on whole genomes, was then constructed using CVTree3 [43], employing *Bacillus subtilis* as the out-group.

2.2. Biocontrol (Antagonistic) Activity on Litchi Fruit

2.2.1. Inoculation Experiments on Litchi Fruit

The pathogen *P. litchii* SC18 and the biocontrol agent *E. acetylicum* SI17 were cultured using the method described by Zheng et al. [40]. The pathogen was cultured on carrot agar (CA) medium for 7 days. "Feizixiao" litchi fruits, approximately 60% ripe (about 65 days after flowering), were treated by spraying with *E. acetylicum* SI17 suspension at a concentration of 5×10^7 CFU/mL or a 1/10 dilution of LB broth (as control) until completely covered. After seven days, the harvested fruits were moved to a greenhouse maintained at a steady temperature of 25 °C, 95% humidity, with 12 h day and night cycles. Twenty-four hours later, the treatments were sprayed with 30 mL of *P. litchii* SC18 at a

concentration of 5×10^4 sporangia/mL. Disease severity was measured every 12 h from 60 to 96 hpi. Each treatment contained 3 replicates, with 30 fruits per replicate. The disease index was calculated following the procedure outlined by Zheng et al. [40].

2.2.2. Relative Quantification of *P. litchii* SC18 in Pericarp

Total RNA was extracted from the litchi pericarp by using the RNAprep Pure Plant Kit (Tiangen, Beijing, China). The purity and integrity of the RNA were assessed using agarose gel electrophoresis and the Agilent 2100 system (Agilent Technologies, Santa Clara, CA, USA), while the RNA concentration was measured with the Nanodrop (Thermo Scientific, Waltham, CA, USA) and Qubit 2.0 (Life Technologies, Carlsbad, CA, USA) systems. For cDNA synthesis, one microgram of RNA was used with the PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, Kusatsu, Japan). The qRT-PCR assay was carried out using TB Green™ Premix Ex Taq™ II (Takara, Kusatsu, Japan), and the data were obtained using the Thermal Cycler™ Dice Real Time System III (Takara, Kusatsu, Japan). The relative expression levels were determined by calculating the fold change based on the $2^{-\triangle\triangle Ct}$ method. The primers were designed with Primer 3.0 (ABI, Carlsbad, CA, USA), and the sequences were as follows: *P. litchii* Actin-F: ACATTGCCCTGGACTTCG, *P. litchii* Actin-R: AGCTCCTTGGTCATACGC, litchi Actin-F: CGGGAAATTGTCCGTGAC, litchi Actin-R: GAGGACTTCTGGGCAACG. *P. litchii Actin* was quantified using litchi *Actin* as a reference gene, and the results indicated the relative amount of *P. litchii* SC18 in the litchi peel.

2.2.3. Enzyme Activity in the Pericarp

In 2021, samples were collected from Litchi cv. "Feizixiao" fruits. Nine fruits were taken for each treatment at each time point (0, 48, 84, 96, 108, and 120 h post-inoculation (hpi)). The pericarp was then collected, promptly enveloped in tin foil, flash-frozen in liquid nitrogen, and stored at −80 °C until required for experimental analyses. The levels of H_2O_2 and the activities of the enzymes catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), and peroxidase (POD, EC 1.11.1.7) were meticulously measured. The detection kits for these analyses were obtained from Nanjing Jiancheng Biological Engineering Institute, Nanjing, China (http://www.njjcbio.com/ (accessed on 18 November 2021)).

2.3. Data Analysis

All statistical analyses were performed using SPSS 25.0 (IBM, Armonk, NY, USA). Significant differences between more than two groups were determined using one-way ANOVA and Duncan's multiple comparison test. Statistical significance was set at $p < 0.05$. Histograms were constructed using Microsoft Office Excel 2016 (Microsoft, Los Angeles, CA, USA).

3. Results

3.1. Genome and Phylogenomic Analysis of E. acetylicum SI17

3.1.1. Genome Sequencing, Assembly, and Functional Annotation

The data gathered from the Nanopore platform were used to construct a circular between chromosomes, with a length of 3,157,929 base pairs (bp) and a GC content of 47.28% (Figure 1, Table 1). Additionally, four plasmids were detected, measuring 119,838 bp, 43790 bp, 6694 bp, and 68,355 bp in length, with GC contents of 40.05%, 43.16%, 37.50%, and 37.92%, respectively (Figure 1, Table 1). The genome comprises 3541 predicted protein-coding sequences (CDS), accounting for 88.98% of the total genomic content. These sequences possess an average length of 846 bp, as shown in Table 1. Analysis of the genome components revealed the presence of 154 interspersed repeats, 105 tandem repeats (TR), 70 tRNA genes, 27 rRNA genes, 1 sRNA gene, 3 other RNA genes, 6 genomic islands (GIs), and 5 prophages. Notably, no clustered regularly interspaced short palindromic repeats (CRISPR) sequences were identified, as specified in Table 2.

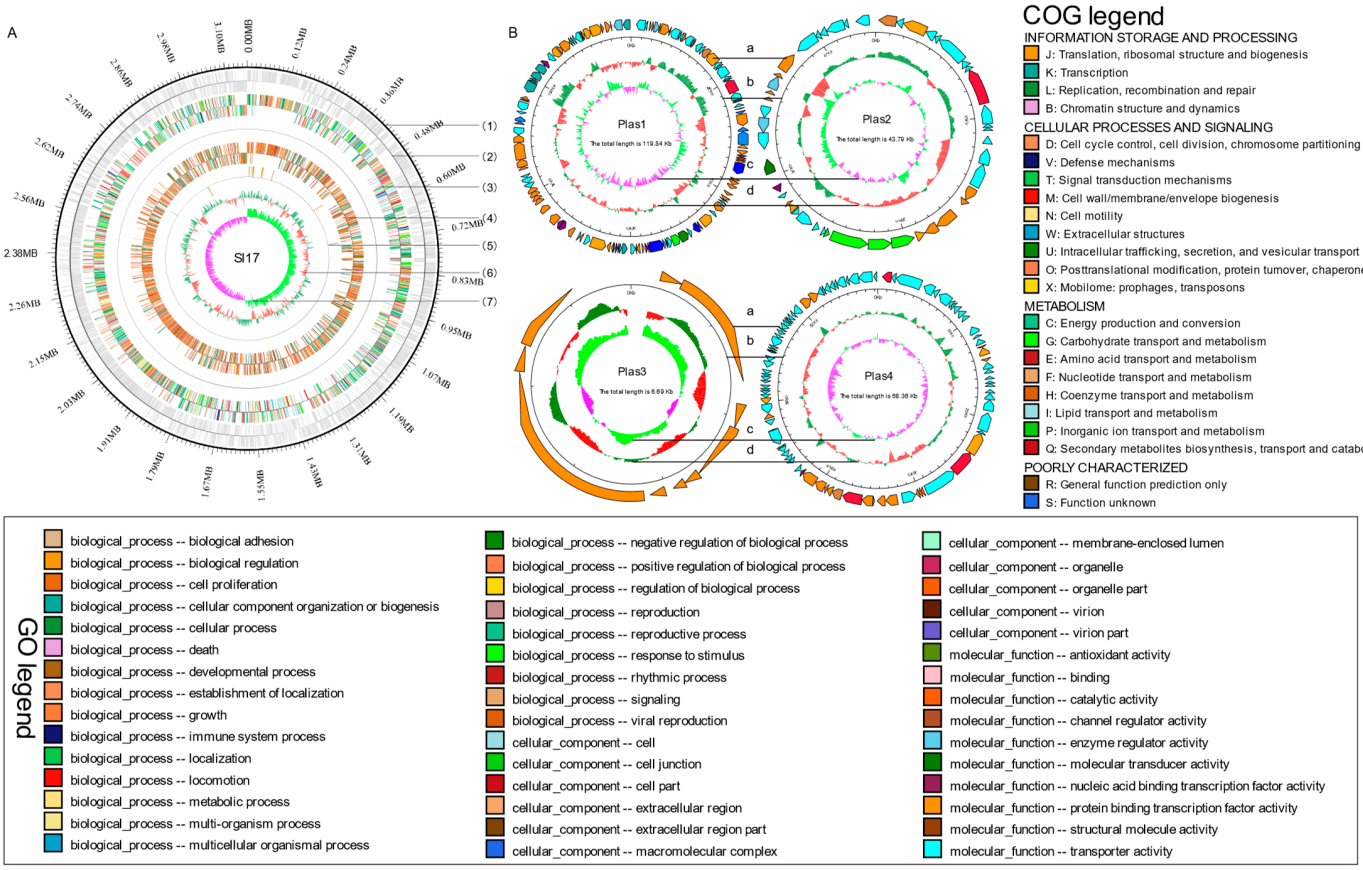

Figure 1. Map of chromosome and four plasmids of *Exiguobacterium acetylicum* SI17. (**A**) chromosome, from outside to inside, the map shows the (1) position of the genome, (2) coding genes on the + and –, strands, (3) COG annotation, (4) GO annotation, (5) non-coding RNA, and (6) GC content. The outer green indicates that the GC content in this region is higher than the genome-wide average, while the inner red indicates the opposite. The height of the peak represents the magnitude of the difference from the average GC content. Higher peaks indicate greater differences from the average GC content. (7) GC skew value (G–C/G + C). When the value is positive, there is a high possibility that CDS is transcribed from the positive chain; Otherwise, there is a high possibility that CDS is transcribed from the negative chain. (**B**) plasmids, from outside to inside, the map shows the (a) COG annotation, (b) position of the genome, (c) GC content, (d) GC skew value.

Table 1. Genomic features of strain SI17.

Features	**Value**				
Number of reads	716,365				
Number of bases (bp)	8,189,664,288				
Mean read length (bp)	11,432.3				
N50 read length (bp)	14,606				
Mean read quality	10.1				
Gene number	3541				
Gene total length (bp)	3,022,368				
Gene average length (bp)	846				
Gene/genome (%)	88.98				
Features	Chromosome	Plasmid 1	Plasmid 2	Plasmid 3	Plasmid 4
Genome size (bp)	3,157,929	119,838	43,790	6694	68,355
G + C content (%)	47.28	40.05	43.16	37.50	37.92

Table 2. Genome component analyses of the strain SI17.

Non-coding RNA (ncRNA)				
Type	Number	Average_Length	Total_Length	In Genome (%)
tRNA	70	77	5394	0.1588
5s rRNA	9	115	1035	
16s rRNA	9	1550	13,950	1.2128
23s rRNA	9	2912	26,208	
sRNA	1	86	86	0.0025
Genomics Islands (GIs)				
GIs_ID	Start	End	Length	GC%
GIs001 (chr)	1,353,417	1,359,120	5704	41.09
GIs002 (chr)	1,511,852	1,532,374	20,523	39.63
GIs003 (chr)	1,710,372	1,718,135	7764	42.17
GIs004 (plas 1)	57,004	61,841	4838	36.59
GIs005 (plas 1)	96,328	103,839	7512	50.12
GIs006 (plas 2)	12,956	20,897	7942	36.15
Prophage				
Prophage_ID	Start	End	Length	GC%
Prophage_1 (Chr1)	804,985	828,528	23,544	47.52
Prophage_2 (Chr1)	1,237,842	1,295,459	57,618	47.55
Prophage_3 (Chr1)	1,333,287	1,341,796	8,510	47.56
Prophage_4 (Chr1)	1,600,271	1,629,792	29,522	49.76
Prophage_5 (Chr1)	1,845,601	1,882,853	37,253	45.65
Interspersed Repeat				
Type	Number	Total Length (bp)	Average length (bp)	In Genome (%)
LTR	89	7967	90	0.2346
DNA	14	1005	72	0.0296
LINE	35	3408	103	0.1003
SINE	14	1110	79	0.0327
RC	2	101	50	0.003
Total	154	13,043	0.384	90
Tandem Repeat				
Type	Number	Repeat Size (bp)	Total Length (bp)	In Genome (%)
Tandem repeat (TR)	55	8~201	5332	0.157
Minisatellite DNA	50	11~51	4348	0.128
Microsatellite DNA	0	0~0	0	0

Functional annotation was conducted by aligning the predicted gene protein sequences using the diamond alignment tool against several frequently used databases. In the context of Clusters of Orthologous Groups (COG) analysis, a total of 2574 proteins were sorted into 23 distinct functional groups. Apart from the general function prediction category, both translation, ribosomal structure, and biogenesis, as well as the transcription category, exhibited a higher percentage among the 22 categories, encompassing 237 and 230 genes, respectively (Figure S1). In the Gene Ontology (GO) analysis, the 2409 proteins were categorized into three primary groups: biological processes, molecular functions, and cellular components. Interestingly, categories of metabolic processes and cellular processes, catalytic activity and binding, as well as cell part and cell, demonstrated higher gene abundance (Figure S2). Moreover, in the context of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, the metabolism pathway emerged as particularly notable (Figure S3). The Carbohydrate-Active enZYmes (CAZy) database, dedicated to carbohydrate enzymes, covers a broad spectrum of enzyme families pivotal in catalyzing the degradation, alteration, and biosynthesis of carbohydrates. It features five main groups: glycoside hydrolase

(GH), glycosyl transferase (GT), polysaccharide lyase (PL), carbohydrate esterase (CE), and auxiliary activity (AA). Notably, there were 45, 36, 1, 5, 0, and 27 genes categorized under GH, GT, PL, CE, AA, and CBM (carbohydrate-binding module), respectively (Figure S4). Additionally, the Transporter Classification Database (TCDB) analysis (Figure S5) showed that genes classified as porters, including uniporters, symporters, and antiporters (94 genes), along with P-P-bond-hydrolysis-driven transporters (129 genes), collectively represented 74.33% of all predicted transporters (300 genes).

3.1.2. Phylogenomic and Comparative Genomic Analysis

The 29 *Exiguobacterium* genomes were divided into two groups. Notably, *E. acetylicum* SI17 was clustered in the first group, showing greater homology to *E. indicum* and *E. enclensis* (Figure 2). Despite differences in GC content, there were no significant variations in genome size, gene count, proteins, rRNAs, or tRNAs between the two groups (Table S1). To the best of our knowledge, this is the first documented instance of using *Exiguobacterium* strains for controlling plant diseases. This suggests that the Group I strains may have potential applications in agriculture.

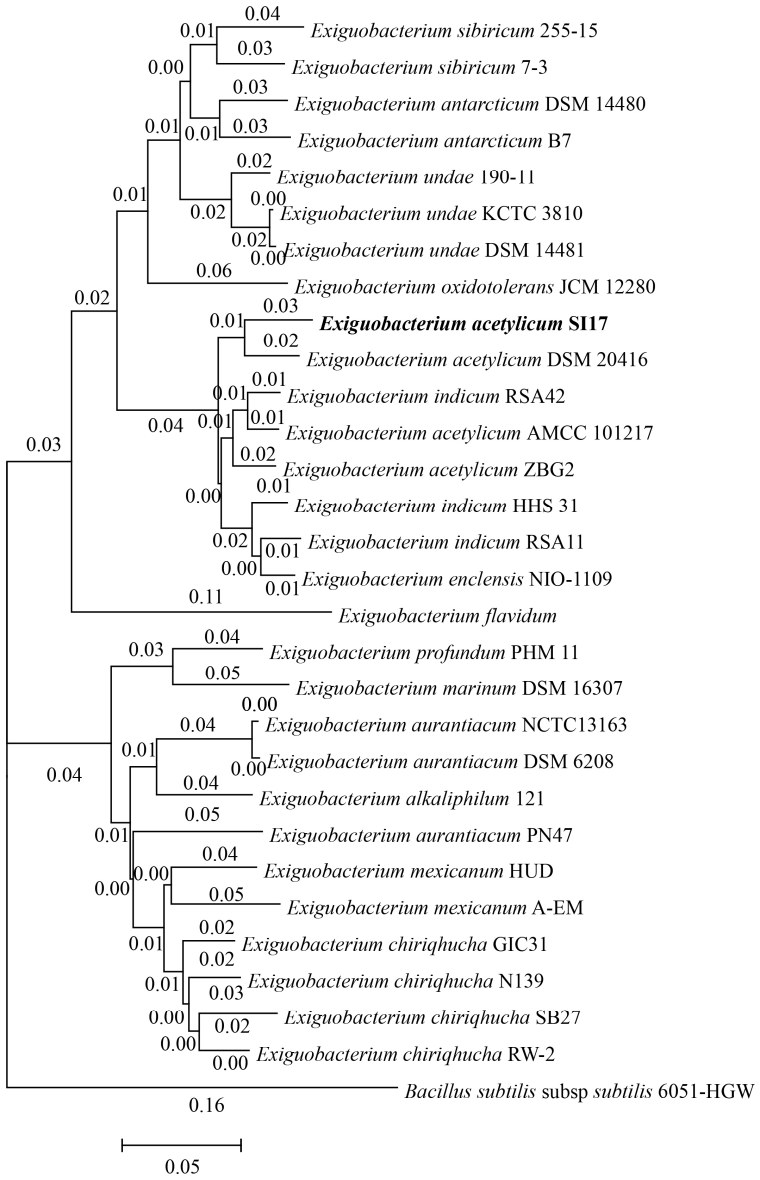

Figure 2. Phylogenomic tree based on whole-genome sequences of 29 *Exiguobacterium* strains. *Bacillus subtilis* was used as an out-group. Strain *E. acetylicum* SI17 is highlighted in bold.

3.2. Biocontrol Activity of *E. acetylicum* SI17 on LDB

3.2.1. Pre-Harvest *E. acetylicum* SI17 Treatment Suppressed LDB

Pre-harvest inoculation experiments on litchi fruit indicated that *E. acetylicum* SI17-treated fruits had a significantly lower disease index at 60, 72, and 84 h, but this difference lessened over time (Figure 3A,B).

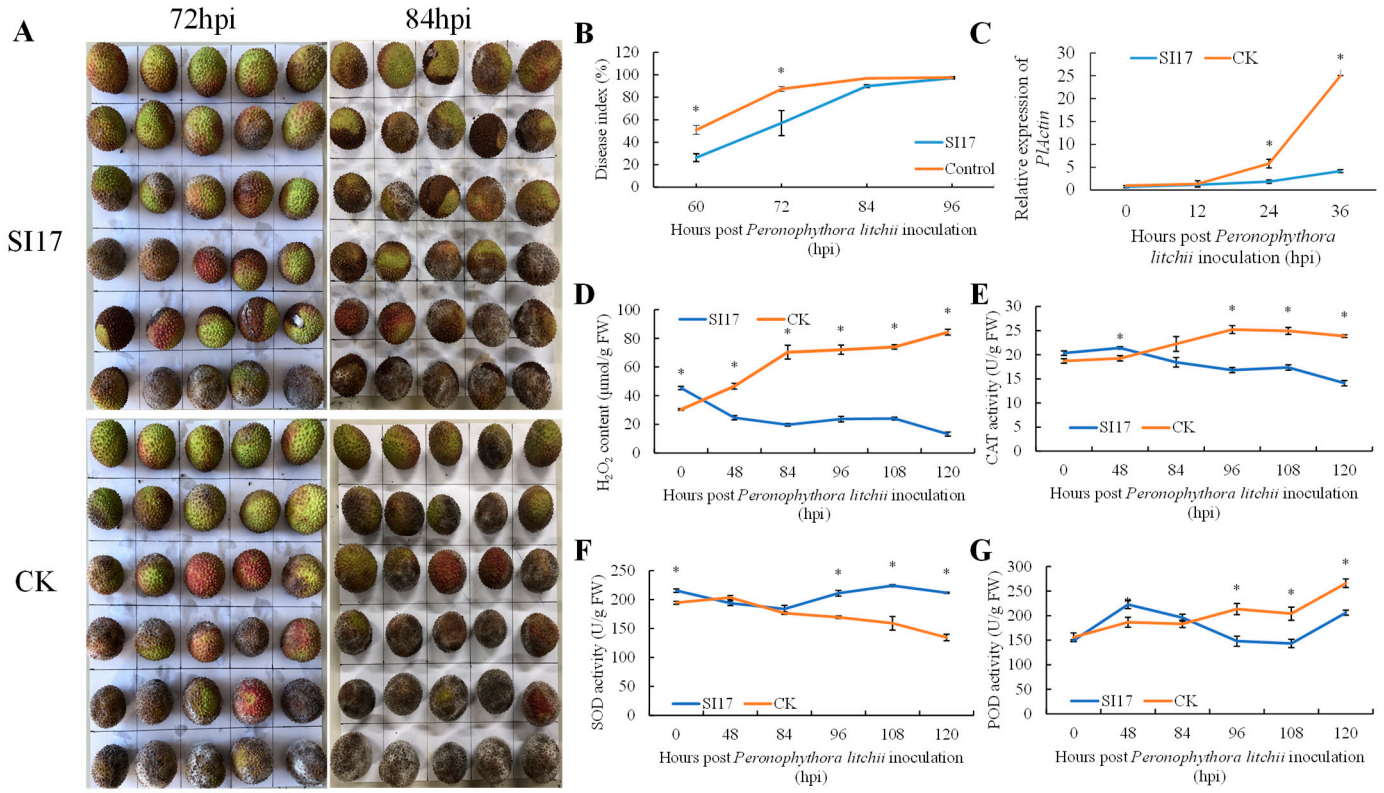

Figure 3. Interaction between *Exiguobacterium acetylicum* strain SI17 pre-harvest treatment and *Peronophythora litchi* on litchi cv. "Feizixiao" fruits: (**A**) litchi downy blight symptoms and (**B**) disease index (B) development, (**C**) *PlActin* expression, (**D**) H_2O_2 content, (**E**) catalase (CAT), (**F**) superoxide dismutase (SOD), (**G**) peroxidase (POD) activities. CK = control. The * above bars indicate significant differences at $p < 0.05$ in Duncan's multiple range test.

3.2.2. Relative Quantification of *P. litchii* SC18 in Pericarp after *E. acetylicum* SI17 Treatment

Following the treatment with *P. litchii* SC18, the relative quantity of pathogens was measured using qRT-PCR. In both the *E. acetylicum* SI17 treatment and control groups, relative pathogen levels were low before 12 hpi (Figure 3C). By 24 hpi, pathogen levels in the *E. acetylicum* SI17 treatment group were three times lower than those in the control group, and by 36 hpi, they were six times lower (Figure 3C). As time progressed, the pathogen growth rate in the control group was much higher than that in the *E. acetylicum* SI17 treatment group.

3.2.3. Pre-Harvest *E. acetylicum* SI17 Treatment Enhanced the Activity of Defense-Related Enzymes

After inoculation with *P. litchii*, H_2O_2 levels in the *E. acetylicum* SI17-treated litchi pericarp steadily declined, unlike the control group, which showed a rapid H_2O_2 buildup before 84 h, followed by a gradual increase. Overall, H_2O_2 levels in the *E. acetylicum* SI17-treated litchi pericarp were lower compared to the control (Figure 3D). In the treatment group, the activity of SOD initially decreased before 84 h and then peaked at 108 h. Conversely, in the control group, SOD activity continued to decline over time (Figure 3F). CAT activity displayed an upward trend in the control group, whereas in the *E. acetylicum*

SI17-treated litchi pericarp, it showed a downward trend (Figure 3E). Prior to 84 h, CAT activity in the *E. acetylicum* SI17 treatment group surpassed that of the control group but subsequently declined (Figure 3E). POD activity in the control group increased steadily over time, whereas in the *E. acetylicum* SI17 treatment group, it initially rose, then declined before rising again, peaking at 48 h and reaching its lowest point at 108 h (Figure 3F).

4. Discussion

E. acetylicum has been observed to enhance wheat seedling growth [32] and break down shrimp shell waste [44]. Yet, its impact on plant diseases remains unexplored until now. Our research reveals that *E. acetylicum* SI17 demonstrates efficacy in managing LDB. By sequencing the whole genome of *E. acetylicum* SI17, we identified the genetic basis for this functional trait. Compared to the other 28 *Exiguobacterium* genomes, *E. acetylicum* SI17 stands out with the largest genome size and the highest counts of genes, proteins, and tRNAs (Table S1). *Exiguobacterium* is clustered into two groups based on its ability to withstand temperatures as low as 7 °C, yet there was no significant difference in functionality between these groups. *E. oxidotolerans* (Group I) and *E. profundum* PHM11 (Group II) demonstrated the capacity to enhance the growth and yield of *Bacopa monnieri* and rice, respectively, particularly under conditions of salt stress [33,45]. Additionally, *E. acetylicum* (Group I) and *E. marinum* a-1 (Group II) exhibited capabilities in degrading harmful substances, such as cyanide-containing effluents and polypropylene, respectively [46,47]. This suggests that not only species within Group I but also those within Group II possess the ability to manage plant diseases.

Existing research has shown that using post-harvest *E. acetylicum* SI17 treatment is highly effective in controlling LDB. Additionally, exposing litchi fruits to SI17 VOCs before harvest significantly decreases the severity of LDB [40]. In this study, we examined the effectiveness of pre-harvest *E. acetylicum* SI17 treatment in controlling LDB to explore its potential for field application. Our findings indicate promising results (Figure 3A,B). H_2O_2, as a representative of reactive oxygen species (ROS), can be excessively generated in response to various biotic and abiotic stresses, resulting in its heightened reactivity and toxic effects on plants [48]. The accumulation of ROS is a widespread defense mechanism for higher plants to resist pathogen attacks. However, excessive ROS can cause plant damage and facilitate pathogen infection [49]. SOD, CAT, and POD are essential enzymes that act as antioxidants, responsible for neutralizing ROS [50]. Many studies have shown that biocontrol agents can effectively clear excess ROS that accumulate due to pathogen induction. For example, *Bacillus velezensis* could enhance the activity of ROS-scavenging enzymes, thereby enhancing the eggplant fruits' disease resistance [51]. A similar situation occurred in *Bacillus amyloliquefaciens* GJ1 against citrus Huanglongbing [52] and *Streptomyces griseorubiginosus* LJS06 against cucumber anthracnose [53]. Litchi pre-treated with SI17 demonstrated an increased ability to scavenge H_2O_2 (Figure 3D), primarily due to heightened SOD activity rather than CAT and POD activities (Figure 3E–G).

Fermentation (C), suspension (F), and supernatant (Q) of *E. acetylicum* SI17 demonstrated no inhibitory effect on the growth of *P. litchii* SC18 (Figure S6). Therefore, it is speculated that *E. acetylicum* SI17 may control disease by enhancing plant resistance rather than directly suppressing *P. litchii* SC18 growth. This is one way for a biocontrol agent to resist disease. Biocontrol agent *Pseudomonas chlororaphis* PA23 could induce systemic acquired resistance against *Sclerotinia sclerotiorum* of *Brassica napus* [54]. Furthermore, various strains of *Exiguobacterium* have been found to produce diverse hydrolytic enzymes [17]. Genome analysis provided additional insights into the key features of *E. acetylicum* SI17 in its ability to combat LDB. Within this strain, four gene clusters, comprising a total of 94 genes, were identified to be linked with the biosynthesis of secondary metabolites (Table S2). These clusters comprised one non-ribosomal peptide synthetase (NRPS), one siderophore, and two terpenes (Table S2). Various metabolites produced by biocontrol agents showed disease resistance in plants. Phenazine-1-carboxylic acid produced by *Pseudomonas chlororaphis* YL-1 showed effectiveness against *Acidovorax citrulli* [55]. Nep1-like proteins generated by

Pythium oligandrum enhanced plant resistance against *Phytophthora* pathogens and *Sclerotinia sclerotiorum* [56]. Hence, it can be deduced that *E. acetylicum* SI17 could enhance plant resistance through the production of diverse secondary metabolites, although the specific functionalities of these metabolites necessitate further verification.

5. Conclusions

The *Exiguobacterium acetylicum* SI17 exhibited a biocontrol effect on LDB caused by *P. litchii*. This study marks the initial documentation of an *Exiguobacterium* strain being employed for managing plant diseases. To deepen our comprehension of its regulatory mechanism, we conducted a full genome sequencing of *E. acetylicum* SI17, revealing a larger genome size with high numbers of genes, proteins, and tRNAs. Additionally, the genome was clustered into Group I. Litchi fruit pre-treated with *E. acetylicum* SI17 showed increased SOD activity and improved capacity to scavenge H_2O_2. Pre-harvest treatment with *E. acetylicum* SI17 suppressed LDB, suggesting promising prospects for its practical application. These findings lead to the hypothesis that *E. acetylicum* SI17 might generate a metabolite that enhances litchi's resistance, highlighting the need for more detailed exploration and identification.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10080888/s1, Table S1: Characteristic of *Exiguobacterium* genomes used for phylogenomic analysis; Table S2: Secondary metabolism gene clusters in *E. acetylicum* SI17; Figure S1: Clusters of Orthologous Groups (COG) function classifications. B: Chromatin structure and dynamics; C: Energy production and conversion; D: Cell cycle control, cell division, chromosome partitioning; E: Amino acid transport and metabolism; F: Nucleotide transport and metabolism; G: Carbohydrate transport and metabolism; H: Coenzyme transport and metabolism; I: Lipid transport and metabolism; J: Translation, ribosomal structure and biogenesis; K: Transcription; L: Replication, recombination and repair; M: Cell wall/membrane/envelope biogenesis; N: Cell motility; O: Posttranslational modification, protein turnover, chaperones; P: Inorganic ion transport and metabolism; Q: Secondary metabolites biosynthesis, transport and catabolism; R: General function prediction only; S: Function unknown; T: Signal transduction mechanisms; U: Intracellular trafficking, secretion, and vesicular transport; V: Defense mechanisms; W: Extracellular structures; X: Mobilome, prophages, transposons; Figure S2: Gene Ontology (GO) analysis. Biological process (from left to right): biological adhesion, biological regulation, cell proliferation, cellular component organization or biogenesis, cellular process, death, developmental process, establishment of localization, growth, immune system process, localization, locomotion, metabolic process, multi-organism process, multicellular organismal process; negative regulation of biological process, positive regulation of biological process, regulation of biological process, reproduction, reproductive process, response to stimulus; rhythmic process, signaling; viral reproduction; cellular component (from left to right): cell, cell junction, cell part, extracellular region, extracellular region part, macromolecular complex, membrane-enclosed lumen, organelle, organelle part, virion, virion part; molecular function (from left to right): Antioxidant activity, binding, catalytic activity, channel regulator activity, enzyme regulator activity, molecular transducer activity, nucleic acid binding transcription factor activity, protein binding transcription factor activity, structural molecule activity, transporter activity; Figure S3: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation. Cellular Processes (A): Transport and catabolism (1), Cellular community-prokaryotes (2), cell motility (3), cell growth and death (4); Environmental Information Processing (B): Signal transduction (5), Membrane transport (6); Genetic Information Processing (C): Translation (7), Transcription (8), Replication and repair (9), Folding, sorting and degradation (10); Human Diseases (D): Substance dependence (11), Neurodegenerative diseases (12), Infectious diseases (13), Immune diseases (14), Endocrine and metabolic diseases (15), Drug resistance (16), Cardiovascular diseases (17), Cancers (18); Metabolism (E): Xenobiotics biodegradation and metabolism (19), Nucleotide metabolism (20), Metabolism of terpenoids and polyketides (21), Metabolism of other amino acids (22), Metabolism of cofactors and vitamins (23), Lipid metabolism (24), Glycan biosynthesis and metabolism (25), Energy metabolism (26), Carbohydrate metabolism (27), Biosynthesis of other secondary metabolites (28), Amino acid metabolism (29); Organismal Systems (F): Nervous system (30), Immune system (31), Excretory system (32), Environmental adaptation (33), Endocrine system (34), Digestive system (35),

Aging (36); Figure S4: Carbohydrate-Active enZYmes Database (CAZy) annotation. AA: auxiliary activity; CBM: carbohydrate-binding module; CE: carbohydrate esterase; GH: glycoside hydrolase; GT: glycosyl transferase; PL: polysaccharide lyase; Figure S5: Transporter Classification Database (TCDB) annotation. 1.A: alpha-Type Channels; 1.B: beta-Barrel Porins; 1.C: Pore-Forming Toxins (Proteins and Peptides); 1.E: Holins; 2.A: Porters (uniporters, symporters, antiporters); 3.A: P-P-bond-hydrolysis-driven transporters; 3.D: Oxidoreduction-driven transporters; 3.E: Light absorption-driven transporters; 4.A: Phosphotransfer-driven Group Translocators; 4.C: Acyl CoA ligase-coupled transporters; 5.A: Transmembrane 2-electron transfer carriers; 8.A: Auxiliary transport proteins; 9.A: Recognized transporters of unknown biochemical mechanism; 9.B: Putative transport proteins; Figure S6: Plate antagonism against *Peronophythora litchii* SC18 of fermentation (C), suspension (F), and supernatant (Q) of SI17.

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Data Availability Statement: The Genbank accession number of *Exiguobacterium acetylicum* SI17 genome and four plastid sequences are CP075897.1-CP075901.1. All other datasets for this study are included in the article/Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

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