Article

Unraveling the Metabolite Signature of Endophytic *Bacillus velezensis* Strain Showing Defense Response towards *Fusarium oxysporum*

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Abstract: Seedling blight, caused by the fungus *Fusarium oxysporum*, significantly lowers rice production globally. Earlier reports have opined that endophytic bacteria strains could be possible biocontrol agents, but the mechanistic actions involved are still unclear. Therefore, this study aimed to isolate the endophytic bacteria with high inhibitory activity and elucidate its possible mechanisms for inducing resistance by metabolomics. The results showed that mdj-36 had the strongest in vitro pathogen inhibition of *F. oxysporum*, while mdj-34 displayed the lowest inhibitory activity identified as *Bacillus velezensis* strains. Metabolomic analyses demonstrated that *B. velezensis* mdj-36 growth medium could produce higher organic acids, terpenes, and diterpene than *B. velezensis* mdj-34. Further investigation revealed that ‘secondary bile acid biosynthesis’ and ‘glycerophospholipid metabolism’ pathways played essential roles in defense response towards *F. oxysporum*. This study’s findings provide a credible theoretical basis for the possible use of the *B. velezensis* strain against rice seedling blight.

Keywords: rice; seedling blight; metabolome; organic acid; terpenes; diterpene

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1. Introduction

As an essential global staple crop, rice (*Oryza sativa* L.) provides a stable food supply for over four billion people, with the Asian region regarding it as a vital energy source and diet composition [1]. As the world’s population soars, so has demand for rice, but several economic diseases have constrained its increased and sustained production. The rice seedling blight is one of the most destructive fungal diseases prevalent at the seedlings-bed level. As a biotic factor, seedling blight infection can lower agricultural productivity [2]. Rice seedling blight management is complex because of the plant’s high susceptibility levels to the disease. Conventionally, its control uses fungicides such as imazalil, tolclofos-methyl, fenaminosulf, liturium, and hymexazol [3,4], but concerns have been raised over their negative environmental impacts and their effects on humans. These have informed research efforts towards developing alternative techniques to control the disease effectively [5].

The use of biological control agents as an effective alternative to fungicides is gaining attention in modern agricultural research. Endophytic bacteria have been identified as promising biocontrol agents as they can grow in plants with minimal adverse effects [3] and produce natural substances, which can ameliorate disease severity in plants [6,7]. Compared with rhizosphere and soil microorganisms, endophytic bacteria can grow much more favorably in plants. They do not have survival competition with soil bacteria and are less affected by field operation and variations in climate parameters [8]. Some recent research efforts have been concerted towards screening potential endophytic *Bacillus* strains that possess commendable disease-suppressing properties (minimal growth needs, rapid root colonization, and defense protein induction) in view of their application as bioagents [9].

Bacteria strains belonging to the *Bacillus* genera are the most predominant endophytic microorganisms found in plants [10,11], and this is suggestive of their possible role as bioagents against economic pathogens. *B. subtilis* was found to be antagonistic towards *Xanthomonas oryza* pv. *oryzae* inciting bacterial leaf blight disease in rice [12]. It has also been reported that *B. subtilis* can also control sheath blight of rice caused by *Rhizoctonia solani* Kuhn [13]. Microbes associated with the onset and spread of this economic disease have been reported to belong to the *Fusarium* genus, *Rhizoctonia* genus, and *Cochliobolus* genus [14]. The *Fusarium* genus has been implicated as the primary causative agent of seedling blight in China, where rice consumption is daily [2]. Thus, the *F. oxysporum* pathogen is traditionally used to screen endophytic bacteria with antagonism. Based on these antecedents, the present study sets out to isolate, screen, and evaluate potentially effective endophytic bacterial strains to manage seedling blight and unravel the underlying defense response mechanisms towards *F. oxysporum* through metabolomics. These findings could support the theory and application for the use of endophytic bacteria as biocontrol agents.

2. Materials and Methods

2.1. Pathogens and Plant Samples

The highly-pathogenic *F. oxysporum* FO2016038 (Institute of Rice, Northeast Agricultural University, China) was the standard pathogen used in this study. It was maintained on potato dextrose agar (PDA) (potato 200 g, glucose 20 g, agar 20 g, sterile water to 1000 mL, pH neutral) medium at 4 °C. Plant samples of rice were collected from an open field (Mudanjiang, Heilongjiang Province, China, 44°44' N, 129°50' E).

2.2. Isolation of Bacteria from Rice

The isolation of endophytic bacteria in rice was performed as previously reported [15]. Samples were first cleaned with sterile water, and tissue surfaces were disinfected with 75% ethanol and 2.5% sodium hypochlorite for 1 and 4 min, respectively. After soaking in 70% ethanol for 30 s and washing with sterile water five times, a measured 0.1 mL aliquot from the final buffer wash was removed and transferred to 9.9 mL Luria-Bertani (LB) broth to serve as a sterile control. The tissues were drained off with a sterile filter paper, then cut into cubes using sterile scissors and ground in a sterile mortar with 10 mL of sterile distilled water for 10 min. The obtained solution was then serially diluted and plated on tryptic soy agar (TSA). Finally, colonies were transferred to fresh TSA plates to obtain pure isolates.

2.3. Screening for Antagonistic Bacterial Strains In Vitro

As previously described, the bacterial isolates' inhibitory effects against *F. oxysporum* were evaluated by placing a 5-mm diameter mycelial disc of the test pathogen in the center, and bacterial isolates were spot inoculated 3-cm distance from the disc on a PDA [16]. As a control (CK), only the plate was inoculated with *F. oxysporum*. After five days of incubation at 28 °C, the inhibition rates were calculated as follows:

$$\text{Inhibition rate (\%)} = 1 - \frac{\text{the diameter of the treated zone of inhibition}}{\text{the diameter of the control zone of inhibition}} \times 100\%$$

2.4. Molecular Identification of Antagonistic Bacterial Strains

Selected antagonistic bacterial strains were identified by 16S rRNA gene analysis. The total DNA was extracted from the culture medium (1 mL) of different isolated strains, and the quality of the genomic DNA was measured using gel monitoring apparatus. The genomic DNA was amplified by using 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1495 R (5'-CTACGGCTACCTTGTTCACGA-3') [17]. The results were sequenced by BGI Company (Beijing, China) and screened using the BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi> accessed on 2 April 2021). A phylogenetic tree was then constructed via the neighbor-joining method using the software of Mega 6.0.

2.5. Application Effect of Endophytic Bacteria on Rice

2.5.1. Preparation of Conidial Suspension of *Fusarium Oxysporum*

Fusarium oxysporum was prepared on a PDA plate medium and incubated at 25 °C for seven days. Then, 5 mL of sterile water was added to the PDA plate medium, and the colony surface was coated lightly with a sterile coating device to obtain a conidia suspension. Subsequently, 2–4 layers of sterile mirror cleaning paper were used to filter the conidia suspension and remove the mycelia or fungus blocks, and a conidia suspension with a concentration of 1×10^5 /mL was then prepared after microscopic examination of its concentration.

2.5.2. Preparation of Fermentation Broth of Endophytic Strain mdj-36

The mdj-36 strain stored at -80 °C was inoculated on LB medium plates to activate the culture. Bacterial colonies of the mdj-36 strain were grown in LB broth at 28 °C and incubated with 180 rpm, and then they were subjected to oscillatory culture to obtain the strain seed solution. Finally, this was added to the fermentation medium at an inoculation amount of 5%, and the strain was incubated at 28 °C for 20 h.

2.5.3. Rice Seedling Bed Resistance Test

The rice seeds were soaked at 25 °C for two days, and the seeds were seeded in a 7 cm $\times$ 7 cm $\times$ 6 cm hole plate, with 20 seeds in each hole. This study tested four treatments; when the rice was grown to the second leaf, it was sprayed with mdj-36 fermentation broth (1×10^8 CFU/mL), hymexazol (100 mg/L), thiophanate-methyl (100 mg/L), and control (water). After 24 h, the conidial suspension of *F. oxysporum* at a concentration of 1×10^5 CFU/mL was inoculated on the rice leaves. The four treated rice seedlings were grown at room temperature. Three holes were selected randomly from each repeat, and the incidence and control effect was investigated after ten days. The incidence and control efficiencies were calculated as follows:

$$\text{Incidence (\%)} = \frac{\text{number of diseased plants}}{\text{total number of investigated plants}} \times 100\%$$

$$\text{Control efficiency (\%)} = 1 - \frac{\text{treatment incidence}}{\text{control incidence}} \times 100\%$$

2.6. Metabolome Analysis

2.6.1. Sample Preparation and Metabolites Extraction

The mdj-36 and mdj-34 strains were singly cultured in 100 mL of sterile LB broth and fermented at 180 rpm and 28 °C for 48 h, respectively. The cells were then obtained by centrifugation (8000 rpm, 4 °C, 20 min), and 50 mL of each supernatant was filtered using size 0.22- μ m Millipore filters for storage in liquid nitrogen. These samples were then transported to the Tianjin Novogene Metabolomics Platform for metabolomics analyses. After thawing at 4 °C and homogenization, some supernatant (200 μ L) was diluted to the final concentration containing 53% methanol by Liquid Chromatography Mass Spectrometry (LC-MS) grade water. The samples were subsequently transferred to a fresh Eppendorf tube and centrifuged at 15,000 $\times$ g and 4 °C for 10 min. Next, a 0.22- μ m membrane was used to filter the supernatants and injected into the LC-MS/MS system for analysis [18,19].

2.6.2. UHPLC-MS/MS Analysis

Ultra-High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (UHPLC-MS/MS) analyses were performed using a Vanquish UHPLC system (Thermo Fisher, Germany) coupled with an Orbitrap Q ExactiveTM HF mass spectrometer (Thermo Fisher, Germany). Samples were injected onto a Hypesil Gold column (100 $\times$ 2.1 mm, 1.9 μ m) using a 17 min linear gradient at a flow rate of 0.2 mL/min. The eluents for the positive polarity mode were eluent A (0.1% FA in water) and eluent B (methanol). The eluents for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9.0)

and eluent B (methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2–100% B, 12.0 min; 100% B, 14.0 min; 100–2% B, 14.1 min; 2% B, 17 min. Q Exactive™ HF mass spectrometer was operated in positive/negative polarity mode with a spray voltage of 3.2 kV, capillary temperature of 320 °C, sheath gas flow rate of 40 arb, and auxiliary gas flow rate of 10 arb [20].

2.6.3. Data Processing and Metabolite Identification

The raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.1 (CD3.1, Thermo Fisher, Waltham, USA) to perform peak alignment, peak picking, and quantitation for each metabolite. The main parameters were set as follows: retention time tolerance, 0.2 min; actual mass tolerance, 5ppm; signal intensity tolerance, 30%; signal/noise ratio, 3; and minimum intensity, 100,000. After that, peak intensities were normalized to the total spectral intensity. We used the normalized data to predict the molecular formula based on additive ions, molecular ion peaks, and fragment ions. Peaks were matched with the mzCloud, mzVault, and MassList databases to obtain accurate qualitative and relative quantitative results. Statistical analyses were performed using the statistical software R (R version R-3.4.3) and Python (Python 2.7.6 version). When data were not normally distributed, normal transformations were attempted using the area normalization method.

2.6.4. Data Analysis

These metabolites were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.genome.jp/kegg/pathway.html>), HMDB database (<https://hmdb.ca/metabolites>) and LIPID Maps database (<http://www.lipidmaps.org/>). Principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) was performed at metaX (a flexible and comprehensive software for processing metabolomics data). We applied univariate analysis (*t*-test) to calculate the statistical significance (*p*-value). The metabolites with VIP > 1 and *p*-value < 0.05 and fold change ≥ 2 or Fold Change (FC) ≤ 0.5 were considered to be differential metabolites. Volcano plots were used to filter interest-based metabolites on log₂(fold-change) and −log₁₀*p*-value of metabolites.

The Pheatmap package plotted the data of differential metabolites in R language to create a cluster heat map. The functions of these metabolites and metabolic pathways were studied using the KEGG database. The metabolic pathways enrichment of differential metabolites was performed when the ratio was satisfied by $x/n > y/N$, and the metabolic pathway was considered enrichment when the *p*-value of metabolic pathway < 0.05. At this level, the metabolic pathway was considered statistically significant enrichment.

2.7. Statistical Analysis

Data obtained from this research were from at least three independent assays, and the values reported were expressed as mean ± standard deviation (SD). Statistically, we used the one-way ANOVA method to compare datasets, followed by Duncan's multiple range test. This study measured statistical differences at $p < 0.05$.

3. Results

3.1. Antifungal Activity

All endophytic bacteria were isolated from the rice samples. A total of 10 strains were preliminarily identified as *Bacillus* strains using morphological characteristics techniques. They all showed inhibitory activity against *F. oxysporum* FO2016038 in dual culture bioassay (Table 1). The maximum inhibition rate was found in mdj-36, as revealed from more than 50% mycelial inhibition. The inhibition rate in mdj-34 was significantly lower ($p < 0.05$) than other strains. Therefore, these two isolates were selected to ascertain the antagonistic mechanism against *F. oxysporum* further.

Table 1. Inhibition rate of *F. oxysporum* FO2016038 by bacteria isolated from rice samples.

Strain	Inhibition Rate (%)
mdj-1	49.17 ± 2.02 ^b
mdj-3	43.83 ± 1.49 ^c
mdj-5	45.39 ± 2.15 ^{b,c}
mdj-10	36.11 ± 1.97 ^d
mdj-18	30.01 ± 2.03 ^e
mdj-27	41.95 ± 3.53 ^c
mdj-30	46.40 ± 3.50 ^{b,c}
mdj-34	26.46 ± 1.76 ^f
mdj-36	56.67 ± 3.06 ^a
mdj-37	43.80 ± 2.50 ^c
CK	0

All values were expressed as mean ± SD. Varying significant differences were noticed between tested strains ($p < 0.05$).

3.2. Isolation and Identification of Antagonistic Bacteria

To gain further insights into the underlying mechanism of different antagonistic activity, mdj-36 and mdj-34 were identified by 16S rRNA gene analysis. We then constructed a phylogenetic tree based on these results using the neighboring method. The results demonstrated that the two isolates belonged to the *Bacillus velezensis* strain (Figure 1).

Figure 1. The phylogenetic tree based on DNA sequences encoding 16S rRNA gene for the two selected strains. Bootstrap values (%) presented at the branches were calculated from 1000 replications.

3.3. Control Effect of Endophytic Bacteria on Rice Seedling Blight

Seedling bed efficacy assessments showed that the incidence of *B. velezensis* mdj-36 treatment was significantly lower ($p < 0.05$) than that of the hymexazol, thiophanate-methyl, and control groups (Figure 2A). Also, *B. velezensis* mdj-36 treatment had the highest control efficiency of $73.57 \pm 6.11\%$, which were significantly higher than that of hymexazol and thiophanate-methyl (Figure 2B). The above data implied that *B. velezensis* mdj-36 strain could be used as a biocontrol agent for rice seedling blight.

Figure 2. Efficacy evaluation of antagonistic *B. velezensis* mdj-36 against *Fusarium oxysporum*-induced rice seedling blight for ten days. **(A)** incidence, and **(B)** control efficiency. All values were expressed as mean $\pm$ SD. Varying significant differences were noticed between tested strains ($p < 0.05$).

3.4. Metabolomics Characteristics of All Samples

Principal-component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) of the 12 analyzed samples' metabolomics profiles were conducted to provide information on the differences between *B. velezensis* mdj-36 and mdj-34 growth media. As shown in Figure 3, PCA analysis of metabolites determined through LC-MS (+) and LC-MS (−) found significant differences in mdj-36 and mdj-34 growth media's metabolic compositions. This observation was consistent with the PLS-DA analysis.

Figure 3. Principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) of the metabolism differences between *B. velezensis* mdj-36 and mdj-34 growth media based on Liquid Chromatography Mass Spectrometry (LC-MS). **(A)** PCA analysis in the positive mode; **(B)** PCA analysis in the negative mode; **(C)** PLS-DA analysis in the positive mode; and **(D)** PLS-DA analysis in the negative mode.

3.5. Differentially Expressed Metabolites (DEMs) Analysis

We identified 489 DEMs in the *B. velezensis* mdj_36 and *B. velezensis* mdj_34 growth media. Among these, 166 DEMs had significant variations and so were monitored closely. We identified 103 up-regulated metabolites and 63 down-regulated metabolites between the *B. velezensis* mdj_36 and mdj_34 growth media (Figure 4).

Figure 4. Heatmap analysis of the differentially expressed metabolites (DEMs) in the *B. velezensis* mdj_36 and mdj_34 growth media. The tree indicates the similarity of metabolites, and the color corresponds to the amount of each metabolite. Each column represents one sample.

We used the Z-score method to assess the different metabolites of the *B. velezensis* mdj_36 and mdj_34 growth media based on LC-MS/MS analysis. The top 30 DEMs identified by *p*-value are reported (Figure 5). Substantial changes were observed in the following compounds—amino acids, organic acids, diterpenes (5 ϵ , 9 ϵ , 16 ϵ)-17-Hydroxykauran-19-oic acid), sesquiterpene hydrocarbon (γ -muurolene), ketone (mesterolone and muscone), and glycol (2-Amino-1,3,4-octadecanetriol, 2-Amino-1,3-octadecanediol, 16-Heptadecyne-1,2,4-triol). Notably, three amino acids (trans-4-Hydroxy-L-proline, N-Oleoyl Glycine, and L-Alanyl-L-leucine) were found in significantly high proportions in the *B. velezensis* mdj_36 growth media. In this media, we identified six organic acids with remarkably high levels: γ -aminobutyric acid, α -linolenic acid, α -eleostearic acid, benzoic acid, docosahexaenoic acid, and 9-Oxo-10(E),12(E)-octadecadienoic acid.

Figure 5. A plot comparing significantly different metabolites in the *B. velezensis* mdj_36 and mdj_34 growth media based on LC-MS/MS. The horizontal axis is Z-score, the vertical axis designates significantly-altered metabolites. The figure only shows the Top 30 significantly changed metabolites based on the Z-score value.

3.6. Biological Pathway Enrichment Analysis

DEMs were further studied using the KEGG enrichment analysis protocol. As shown in Figure 6, the 20 most enriched pathways include 'secondary bile acid biosynthesis', 'glycerophospholipid metabolism', 'biosynthesis of unsaturated fatty acids', 'arginine and proline metabolism', 'fatty acid biosynthesis', 'benzoate degradation', 'taurine and hypotaurine metabolism', 'sphingolipid metabolism', 'xylene degradation', 'glyoxylate and dicarboxylate metabolism', 'sulfur metabolism', 'carbon metabolism', 'bacterial chemotaxis', 'purine metabolism', 'glycine, serine and threonine metabolism', 'beta-Alanine metabolism', 'biosynthesis of antibiotics', 'degradation of aromatic compounds', and 'Vancomycin re-

sistance'. Furthermore, the 'secondary bile acid biosynthesis' and 'glycerophospholipid metabolism' were the most significant enrichment pathways.

Figure 6. Bubble plot of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment for DEMs comparing *B. velezensis* mdj_36 and mdj-34 growth media. The rich factor is the ratio of the DEM number to the background number in an individual pathway. The rich factor value and degree of pathway enrichment are directly proportional. The dots' size represents the number of proteins, and the color of the dots represents the range of $-\log_{10}p\text{-value}$, and a lower $-\log_{10}p\text{-value}$ indicates more significant pathway enrichment.

4. Discussion

Seedling blight is caused by the fungus *F. oxysporum* through infection of healthy seedlings by the systemic spreading of spores or mycelia [21]. As a mitigation strategy, biocontrol techniques have been proposed because they are environment-friendly and contribute towards a more sustainable agricultural system globally. The primary objective of plant disease biocontrol is to utilize one or more living organisms with antagonistic effects against a plant pathogen to achieve plant disease suppression. Microbes are isolated as a first step, and pure cultures' inhibitory effects are assessed [22]. In the present study, out of the ten bacterial strains that were isolated from the rice tissues, mdj-36 showed the strongest in vitro pathogen inhibition of *F. oxysporum*, while mdj-34 displayed the lowest antagonistic activity. Both were identified as *B. velezensis* strains based on the 16S rRNA gene sequences. Several reports have described *Bacillus* strains as promising biocontrol agents for plant diseases [23]. Accumulated evidence has also shown that *B. subtilis*, *B. amyloliquefaciens*, and *B. pumilis* had biocontrol efficiency [21,24–26]. Recently, it has been reported that *B. velezensis* B006 could produce surfactin to control suppressing cucumber and pepper root rot diseases caused by *F. oxysporum* and *Phytophthora capsica* [20]. Cui et al. have isolated an endophytic bacteria *B. velezensis* 8-4, which exhibited biocontrol

activity against *Streptomyces galilaeus* on potato and displayed inhibitory effects against four other potato pathogens, named *F. avenaceum*, *R. solani* Phoma foveat, and *Colletotrichum coccodes* [17]. Abdelkhalek et al. have reported that *B. velezensis* PEA1 could inhibit *F. oxysporum* growth and induces systemic resistance to cucumber mosaic virus [27].

Metabolomics techniques have promising future applications in sustainable agriculture as they provide an effective platform for the chemical screening, comparison, and validation of the metabolites from bacterial fermentation [28]. To mine the metabolite signature of endophytic bacteria showing defense response towards *F. oxysporum*, we used the metabolomics approach based on LC-MS to compare the *B. velezensis* mdj_36 and mdj_34 growth media. The current study identified significantly high levels of six organic acids in *B. velezensis* mdj_36 growth media, including γ -aminobutyric acid. An earlier study demonstrated that γ -aminobutyric acid had strong antifungal effects in harvested fruits [29]. Also, phenolic acids have been effective against major fungal pathogens like *Candida albicans* strains, which are also infectious to humans [30]. The growth inhibition of *F. oxysporum* by benzoic acid has been reported previously [31].

This study also explored the mechanistic molecular roles of fatty acids in seedling blight inhibition. Although fatty acids are known to possess several antimicrobial properties, increased incidences of acquired microbial resistances that reduce the efficacy of conventional antimicrobial therapies have intensified research activities searching for alternative agents. Also, fatty acids are more environment-friendly compared to chemical fungicides [32]. Previous studies have reported the potential fungicidal properties of some fatty acids [33]. In our research, the fatty acid investigated was α -Linolenic acid. Our findings agree with a recent report by Munir et al., who reported that endophyte *B. subtilis* L1-21 in citrus could up-regulate α -Linolenic acid to protect citrus against *Candidatus Liberibacter asiaticus* [34]. Moreover, the concentration of α -eleostearic acid derived from α -linolenic acid was also higher in *B. velezensis* mdj_36 growth media than *B. velezensis* mdj_34 [35]. Surup et al. reported that α -eleostearic acid isolated from cultures of the tropical Ascomycete *Hypoxylon rickii* showed antimicrobial and cytotoxic activities [36].

Our metabolomics results reveal that the levels of some polyunsaturated fatty acids (FUFAs) were substantially elevated in the *B. velezensis* mdj_36 growth media. Interestingly, these include docosahexaenoic acid (DHA) and 9-oxo-10(E),12(E)-octadecadienoic acid (9-oxoODA). An earlier study by Bajpal et al. showed that DHA significantly inhibited spore formation in seven economic plant pathogens in an initial screening protocol, and this was validated in an in vivo screening [37]. Similarly, Bilikova et al. demonstrated that 9-oxo-10(E)-12(Z)-octadecadienoic acid inhibited the pathogenic activities of *Paenibacillus larvae* [38]. Also, the results of a dose-dependent study by Cantrell et al. showed that some *Gomphus floccosus*-derived fatty acids suppressed the activities of several fungal plant pathogens, including *F. oxysporum* and *B. cinerea* [39]. Thus, our observations support existing evidence that these bioactive organic compounds can have future applications in raising major crops' resistance against notable economic pathogens.

Some suppressive properties have also been observed with the kaurene diterpene (5 ϵ ,9 ϵ ,16 ϵ)-17-Hydroxykauran-19-oic acid, a diterpene containing a rigid tetracyclic skeleton known as kaurenoic acid [40]. In combination with similar compounds, a significant increase in inhibitory activities has been reported [41]. Sequel to these, Helliwell et al. and Nozaki et al. confirm that kaurene compounds, being intermediates in plant and fungal metabolites' biosynthesis, have a range of bactericidal and fungicidal activities [42,43]. A previous study also found that five diterpenes with the kaurane skeleton (ent-kaur-16-en-19-oic acid, ent-19-methoxy-19-oxokauran-17-oic acid, annoglabasin B, ent-17-hydroxykaur-15-en-19-oic acid, and ent-15b,16b-epoxy-17-hydroxy-kauran-19-oic acid) were isolated from *A. glabra* leaves has also shown antifungal effects [44,45]. Earlier, a GC-MS study by Cheng et al. revealed that the essential oils from Japanese cedar heartwood contained δ -cadinene, isodene, and γ -muurolene, conferring it with many fungicidal properties [46]. Recently, some major and minor terpenes exhibited antifungal properties against several plant pathogens, including *B. cinerea*, *C. gloeosporioides*, and *P. cinnamomic* [47], reiterat-

ing the current study's position that naturally-occurring compounds should be further explored in mitigating the activities of plant fungal pathogens.

Among the pathways explored in our study, 'secondary bile acid biosynthesis' and 'glycerophospholipid metabolism,' which have the lowest $-\log_{10}p$ -value compared with other routes, is the most significant enrichment pathway. Consistent with our results, Guinan et al. recently opined that the notable fungal pathogen's activities, *Candida albicans*, could be inhibited by secondary bile acids [48]. Also, bile acids stimulate a more significant defense pathway than a fungal cerebroside obtained from the rice pathogen, *Magnaporthe grisea* [49]. As an integral cell membrane structure, the glycerophospholipid bilayer performs a dual role in protecting cell components from the external environment and ensuring uninterrupted internal cell biological functions [50]. These findings revealed that *B. velezensis* mdj_36 could play a better role against *F. oxysporum* than *B. velezensis* mdj_34 through enhanced cell structure and biosynthesis of essential proteins.

5. Conclusions

Seedling blight is a significant limitation to global rice production. In this study, an endophytic bacteria *B. velezensis* strain mdj-36 was isolated from rice samples. It exhibited a high inhibitory ability against *F. oxysporum*, known as the causal agent of seedling blight. The metabolomics results revealed that *B. velezensis* mdj-36 could produce higher levels of organic acids, terpenes, and diterpene than that in *B. velezensis* mdj-34, which is sensitive to *F. oxysporum*. Further analysis highlighted the vital role of 'secondary bile acid biosynthesis' and 'glycerophospholipid metabolism' pathways. In all, our study has provided additional information regarding the molecular mechanisms underlying of *B. velezensis* strain as a biocontrol agent against rice seedling blight. It can be a sound theoretical basis for further studies of this microbe as a disease-suppressing agent.

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