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Sphingobium sp. V4, a bacterium degrading multiple allelochemical phenolic acids

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Abstract

Background Continuous cropping challenges constrain the development of agriculture. Three main obstacles limit continuous cropping: autotoxicity of plant allelochemicals, deterioration of physicochemical characteristics of soil, and microflora imbalance. Plant-derived phenolic acids can cause autotoxicity, which is considered the main factor mediating continuous cropping obstacles. Reducing the phenolic acids in continuous cropping soils can decrease the autotoxicity of phenolic acids and ameliorate continuous cropping obstacles. Therefore, it is important to study the microbial resources that degrade allelochemical phenolic acids. Thus, the bacterial strain V4 that can degrade phenolic acids was isolated, identified, and genomically analyzed.

Results Strain V4 isolated from strawberry soil using vanillic acid-mineral agar was identified as a Gram-negative short rod bacterium. Subsequent 16S rRNA phylogenetic analysis revealed that V4 clustered with members of the genus *Sphingobium*. The most closely related species were *Sphingobium lactosutens* DS20^T (99% similarity) and *Sphingobium abikonense* NBRC 16140^T (97.5% similarity). V4 also shared > 95% sequence similarity with other members of *Sphingobium*, so *Sphingobium* sp. V4 was named accordingly. Biochemical tests revealed that the biochemical characteristics of *Sphingobium* sp. V4 were similar to its most similar strains except for some properties. *Sphingobium* sp. V4 effectively degraded vanillic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and syringic acid. V4 grew best at the conditions of 30 °C, pH 6.0–7.0, and 0–0.05% NaCl. 500 mg/L vanillic acid was completely degraded by V4 within 24 h under the optimal conditions. Whole genome analysis showed that *Sphingobium* sp. V4 contained one chromosome and three plasmids. Two genes involved in vanillic acid degradation were found in the V4 genome: the gene encoding vanillate *O*-demethylase oxidoreductase VanB on the chromosome and the gene encoding vanillate monooxygenase on a large plasmid. The organization of vanillate catabolic genes differed from the adjacent organization of the genes, encoding vanillate o-demethylase VanA and VanB subunits, in *Pseudomonas* and *Acinetobacter*.

Conclusions The isolated bacterium *Sphingobium* sp. V4 degraded multiple phenolic acids. Its properties and genome were further analyzed. The study provides support for further investigation and application of this phenolic acid-degrading microorganism to alleviate continuous cropping obstacles in agriculture.

Keywords Continuous cropping obstacles, Allelochemicals, Phenolic acid, Vanillic acid, *Sphingobium* sp. V4, Biodegradation, Genome

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Background

Continuous cropping obstacles present a difficult problem in agricultural production worldwide. Many crops are prone to such continuous cropping obstacles, including peanut, soybean, tomato, cucumber, potato, strawberry, tobacco, and *Panax notoginseng* (Yu et al. 2000; Zhou et al. 2012; Li et al. 2016; Tan et al. 2017). The causes of continuous cropping obstacles are complex and generally related to the autotoxicity of plant allelochemicals, the deterioration of soil physical and chemical properties, and imbalances in soil microflora. Phenolic acids in plant root exudates and stubble decaying substances can cause allelopathy, which is the main mediating factor of continuous cropping difficulties (Chen et al. 2014; Wu et al. 2015; Bai et al. 2019). Levels of phenolic acids, such as p-coumaric acid, vanillic acid, p-hydroxybenzoic acid, and ferulic acid, are significantly higher in continuous cropping soils and have exhibited allelopathy which inhibits the growth of the same or related crops and increases the incidence of soil-borne diseases (Carlsen et al. 2009; Chen et al. 2014; Gulzar and Siddiqui 2015; Bai et al. 2019). Bai et al. reported that coumaric acid, p-hydroxybenzoic acid, vanillic acid, vanillin, and ferulic acid significantly accumulated with increased duration of continuous cropping for tobacco, and the accumulated phenolic acids caused strong allelopathic activity (Bai et al. 2019). Phenolic acids also affect the microbial community structure by acting as carbon and energy sources or as signaling molecules (She et al. 2017; Liu et al. 2017; Xie et al. 2017; Qu and Wang 2008; Chen et al. 2018; Zhou et al. 2018). Peanut root exudates (containing phenolic acids such as p-hydroxybenzoic acid, coumaric acid, etc.) are considered to affect soil-borne pathogens and promote the spore germination, sporulation, and mycelial growth of the soil-borne pathogens, *Fusarium oxysporum* and *Fusarium solani* (Li et al. 2013). *p*-Coumaric acid may increase *F. oxysporum* population densities in soils (Zhou and Wu 2012). Cinnamic acid may promote cucumber *Fusarium* wilt, a disease caused by *F. oxysporum* (Ye et al. 2004). Vanillic acid (0.02 to 0.05 μ mol/g soil) can increase *Fusarium* spp. abundance in cucumber rhizosphere soil (Chen et al. 2018).

Decreasing the phenolic acid allelochemicals in continuous cropping soils helps crops by reducing the autotoxicity of phenolic acids and the occurrence of soil-borne diseases and, thus, alleviates continuous cropping obstacles. The published reports of bacteria that degrade allelochemical phenolic acids are increasing in number, not only for *Pseudomonas*, but also for *Paraburkholderia* and other genera (Mohan and Phale 2017; Wilhelm et al. 2020; Araki et al. 2020; Liu et al. 2023).

In this study, a *Sphingobium* sp. V4 was isolated, identified, and observed to degrade phenolic acids with high efficiency. Its confirmed substrates included vanillic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and syringic acid. Its whole genome and vanillic acid demethylase gene were also analyzed. This work provides a potential bacterial strain for the development of bacterial agents to prevent and control continuous cropping obstacles.

Methods

Strain isolation

Soil samples were collected from a strawberry field located in Zibo, Shandong Province, China, in 2018. The geographic coordinates of the strawberry field were 36° 53′ N and 118° 04′ E. Vanillic acid-mineral (VM) agar medium was used to isolate the vanillic acid-degrading bacterium. The composition of the VM liquid medium was as follows: 0.5 g of vanillic acid, 0.2 g of KNO_3, 5 g of $Na_2HPO_4 \cdot 12H_2O$, 3 g of KH_2PO_4, 0.5 g of NaCl, 0.003 g of $CaCl_2$, 0.003 g of $MgSO_4 \cdot 7H_2O$, and 1000 mL of distilled water. VM agar medium was generated by adding 20 g/L of agar to the VM liquid medium. VM was supplemented with 0.5 g/L (*w/v*) of yeast extract to produce a VM yeast extract (VMY) medium. Sterilization was performed at 121 °C for 20 min in an autoclave. Soil sample suspensions of 10^{-2}, 10^{-3}, and 10^{-4} concentrations were made with sterilized distilled water. Then, 100 μL suspensions of each concentration were spread on the VM agar medium to isolate bacteria. Each concentration was replicated three times. The plates were incubated at 30 °C for 3 to 5 days, and bacterial colonies were observed daily. After the colonies grew well, the strains were isolated and purified on VM agar medium.

The purified colony was inoculated into VM liquid medium, and the grown cell culture was added to fresh VM liquid medium with a 10% (*v/v*) inoculation ratio to test its ability to degrade vanillic acid. Cultivation conditions were maintained at 30°C and 150 r/min for 5 days. Samples were collected at 0 and 5 days. The content of vanillic acid was determined by high-performance liquid chromatography (HPLC) (Shimadzu, Japan) with an XDB-C18 reversed-phase column (4.6 mm × 250 mm). For HPLC, 0.16% acetic acid and methanol (40:60) were used as the mobile phase at a flow rate of 1.0 mL/min. The column temperature was 30 °C, and the detector wavelength was 280 nm. The substrate degradation rate was calculated as [(initial substrate content − final substrate content)/initial substrate content] × 100%. A control was established in which 10% (v/v) sterilized distilled water was added instead of V4 culture. The degradation test was repeated three times.

Phylogeny of 16S rRNA gene sequences

The genomic DNA of the vanillic acid-degrading bacterium V4 was extracted using the Bacterial Genomic DNA Rapid Extraction Kit (Sangon) according to the manufacturer's instructions and used as the template. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) with the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1541R (5′-AAGGAGGTGATCCAGCCGCA-3′) (Weisburg et al. 1991). Each 25 μL PCR reaction system consisted of the following reagents: 2×Taq PCR Mix (Biomiga) 12.5 μL; 10 μM 27F primer 1 μL; 10 μM 1541R primer 1μL; template 1μL; ddH_2O 9.5μL. The thermal cycling program was as follows: initial denaturation for 5 min at 95 °C; 30 cycles of denaturation for 30 s at 94 °C; annealing for 60 s at 55 °C; and elongation for 90 s at 72°C, a final extension for 10 min at 72 °C. The PCR product was purified using a UNIQ-10 Column Micro DNA Gel Extraction Kit (Sangon) according to the manufacturer's instructions. The purified PCR product was sequenced by Biosune Biotechnology Co., Ltd. (Shanghai, China). The obtained gene sequence was compared with sequences in GenBank using BLAST on the National Center for Biotechnology Information (NCBI) website. The related bacterial 16S rRNA gene sequences were downloaded. The sequence similarities between the 16S rRNA genes were calculated using DNAstar software (Burland 2000). ClustalX 2.1 was used for sequence alignment, and the resulting alignment was used for phylogenetic analysis. The average evolutionary divergence over all sequence pairs was computed using maximum composite likelihood model in MEGA 11. The average divergence value for related 16S rRNA genes was 0.05 ± 0.00, confirming that the gene dataset was suitable for generating a neighbor-joining tree. Accordingly, the phylogenetic tree of 16S rRNA sequences was constructed using the neighbor-joining method in MEGA 11 (Tamura et al. 2021).

Morphological and biochemical characterization

Colonies grown on VM agar and Nutrient Agar (NA) (1% peptone, 0.3% Beef extract, 0.5% NaCl, 1.5% agar, pH 7.2 ± 0.2) were observed. Colonies grown on NA were used for Gram staining and observations of bacterial morphology with an optical microscope. The type of Gram staining reaction was also identified by the KOH string method (Powers 1995). Bacterial cells grown on nutrient broth (NB) (1% peptone, 0.3% Beef extract, 0.5% NaCl, pH 7.2 ± 0.2) were used for morphological observation with a scanning electronic microscope (FEI quanta FEG250, USA). A catalase activity test was performed as described previously (Lin et al. 2014). Biochemical characteristics, including oxidase, nitrate reduction, L-tryptophan fermentation, glucose fermentation, hydrolysis of L-arginine, urea, aesculin, gelatin, and 4-nitrophenyl-β-D-galactopyranoside, and assimilation of carbon compounds, were tested using the API 20NE kit (bioMérieux). Other enzyme activities were tested using the API ZYM kit (bioMérieux) according to the manufacturer's instructions.

Phenolic acid degradation profile

Five phenolic acid media containing, respectively, 0.5 g/L of vanillic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, or syringic acid as substrate (except for the phenolic acid substrate, the other components were the same as those in the VMY medium) were prepared to detect the phenolic acid substrate profile of strain V4. The V4 culture grown in VMY medium was centrifuged (8000×*g* for 10 min at 4 °C), the supernatant was discarded, and the precipitate was suspended in sterilized distilled water to prepare the inoculum (OD_{600} = 0.6). Then, 10% inoculum (*v/v*) was added into a sterile phenolic acid medium with vanillic acid, ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, or syringic acid as substrate, respectively. The cultures were incubated at 30 °C and 150 r/min for 5 days, and samples were collected at the beginning and the end of the culture period. After filtration, the substrate content was detected by HPLC (using the same HPLC conditions described above). The substrate degradation rate was calculated as [(initial substrate content − final substrate content)/initial substrate content] × 100% to determine the substrate degradation spectrum of the phenolic acid-degrading bacteria. Each phenolic acid substrate experiment had a control in which 10% (*v/v*) sterilized distilled water was added instead of strain V4 inoculum. The cultivation condition and substrate detection method of the control were the same as those used for the experiment groups. Each of the experiments was repeated three times.

Influence of environmental conditions on growth of V4

To evaluate the influence of temperature on phenolic acid degradation, 10% (*v/v*) V4 cultures in VMY medium for each of the different groups were cultured in shaker-incubators at 150 r/min at 20 °C, 30 °C, 37 °C, and 45 °C. Cultures were sampled at 0, 1, and 7 d for measurements of the OD_{600}. To evaluate the influence of pH on phenolic acid degradation, VMY media were prepared, and the pH was adjusted with 5 M NaOH or 5 M HCl to pH values of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, respectively. 10% (*v/v*) V4 culture in each medium was incubated at 30°C and 150 r/min, and cultures were sampled at 0, 1, and 7 days for measurement of the OD_{600}. To determine the influence of salinity, different concentrations of NaCl were added to VMY media (as done for the temperature assay, though

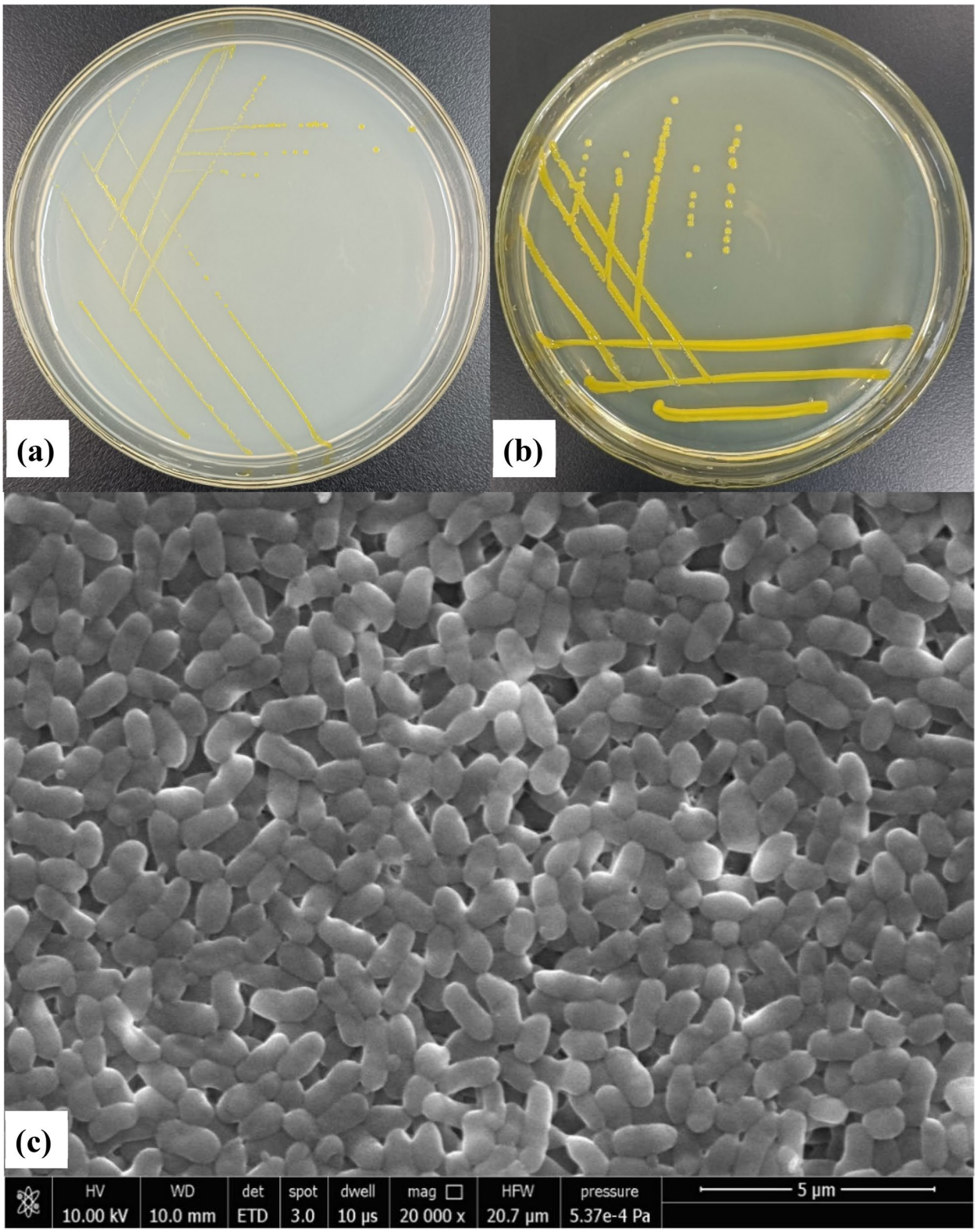

Fig. 1 Colony morphology and Cell morphology of V4. **a** Colony morphology on VM agar. **b** Colony morphology on NA. **c** Cell morphology under scanning electronic microscope

it had no addition of NaCl). The final concentrations of NaCl were 0%, 0.05%, 1.0%, 2.0%, 3.0%, 4.0%, and 5.0%, respectively. After inoculation with 10% (*v/v*) V4 culture, each group was incubated at 30 °C and 150 r/min, and cultures were sampled at 0, 1, and 7 days for measurement of the OD_{600}. For evaluating the influence of environmental conditions on the growth of V4, each experiment was conducted with three replicates, and the significant increase ($P < 0.05$) of OD_{600} indicated growth of the V4 strain.

Vanillic acid degradation curve and growth curve

For vanillic acid degradation curve determination, 10% (*v/v*) V4 culture grown in VMY medium was inoculated into a fresh VMY medium. Flasks were cultivated at 30 °C and 150 r/min. Cultures were sampled at 0, 4, 8, 12, 24, 36, and 48 h. Then, the vanillic acid degradation rate was assayed by determining the content with the HPLC technique described above. OD_{600} at each time was also measured. A control was set up in which 10% (*v/v*) sterilized distilled water was added in the VMY medium. Experiments were repeated three times.

Whole genome sequencing and analysis

The whole genomic DNA of strain $V4^T$ was sequenced by Beijing Novogene Bioinformatics Technology Co., Ltd. The SMRTbell™ Template Kit (version 1.0) (PacBio, USA) was used to construct a 10-kb SMRTbell library. The NEBNext®Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, USA) was used to construct a 350-bp library. The library was quantified using a Qubit 4.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), and insert size was estimated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Sequencing was performed using the PacBio Sequel platform and Illumina PE150 platform, according to the respective manufacturer's instructions. The genome was assembled using SMRT Link 5.0.1 (Ardui et al. 2018; Reiner et al. 2018) (https://www.pacb.com/support/software-downloads/). The GeneMarkS program was used to retrieve the protein-coding genes (Besemer et al. 2001). Transfer RNA (tRNA) genes were predicted by the tRNAscan-SE (Lowe and Eddy 1997). Ribosome RNA (rRNA) genes were analyzed by the rRNAmmer (Lagesen et al. 2007). Genes were annotated using BLAST and the NCBI Non-Redundant Protein Database (NR) (Li et al. 2002). Gene function was also annotated using the Gene Ontology (GO) database, while pathways were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al. 2004, 2006). Proteins were phylogenetically classified using the Clusters of Orthologous Groups (COG) database (Galperin et al. 2015).

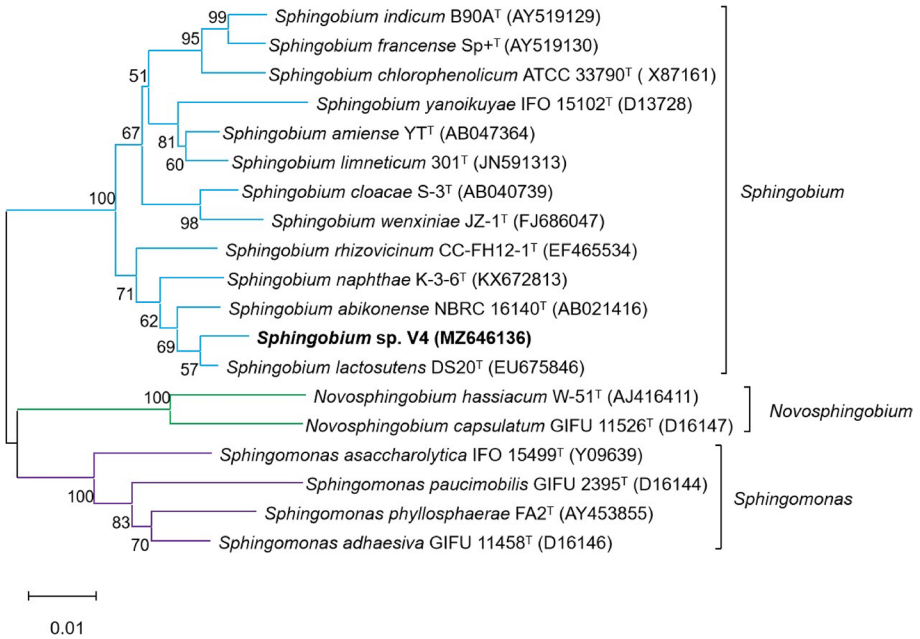

Fig. 2 Phylogenetic tree of V4 and related strains based on 16S rRNA gene sequences. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 19 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1499 positions in the final dataset. Evolutionary analyses were conducted in MEGA11

Table 1 Differential characteristics between strain V4 and related species belonging to the genus *Sphingobium*

Characteristic	1	2	3
pH range	5.0–9.0	6.0–10.0	6.0–10.0
Highest salt tolerance (%, w/v)	4.0	5.0	5.0
Catalase/oxidase	+/+	−/+	−/+
Nitrate reduction	+	w	−
L-tryptophan fermentation	+	−	−
Enzyme activity (API ZYM)			
Esterase lipase (C8)	+	−	−
Lipase (C14)	+	+	−
Trypsin	+	−	w
Acid phosphatase	−	+	+
α-Chymotrypsin	+	+	−
α-Galactosidase	+	−	+
β-Galactosidase	w	−	−
β-Glucuronidase	+	+	−
Assimilation of (API 20NE)			
D-Mannose	−	+	+
N-Acetylglucosamine	−	+	+
Potassium gluconate	+	−	−
Capric acid	−	+	+

Strains: 1, V4; 2, *S. lactosutens* DS20^T; 3, *S. abikonense* NBRC 16140^T. Data of 1 was from this study, and data of 2 and 3 were taken from the literature (Kumari et al. 2009; Chaudhary et al. 2017). +, positive; −, negative; *w*, weakly positive

Statistical analysis

Statistical analyses were conducted using the SPSS 16.0 software package (SPSS Inc., USA). The degradation rates or OD_{600} of different groups are presented as means (SD) in text and chart content. Independent-samples *t*-tests were used to compare the differences between groups in phenolic acid degradation tests. One-way ANOVA was used to analyze the effect of environmental factors on bacterial growth.

Results

Strain isolation and cell morphology

A strain of vanillic acid-degrading bacterium, V4, was isolated on a VM agar plate. V4 degraded 500 mg/L vanillic acid in VM medium with a degradation rate of 100% ± 0% in 5 days, while the degradation rate of the control was −8.8% ± 2.02%. The difference between the V4 test group and the control group was extremely significant ($P < 0.01$). V4 formed small, round, smooth, and opaque colonies on the VM agar plate after incubation at 30 °C for 3 days, and the color of colonies changed gradually from milky white to yellow (Fig. 1a). V4 formed small, round, smooth, opaque, and yellow-colored colonies on the NA plate (Fig. 1b) after incubation at 30 °C for 1–2 days. Gram-staining and KOH string tests revealed that V4 was Gram-negative. Scanning electronic microscope observation revealed that V4 was short rod-shaped (0.5–0.6 μm × 0.7–1.2 μm), and the cell morphology is shown in Fig. 1c.

Phylogeny of 16S rRNA gene sequences

The 16S rRNA gene of strain V4 was sequenced, and the obtained sequence was used as the query for a BLAST search using NCBI. The 16S rRNA gene sequences of representative strains with high similarity to V4 were aligned using ClustalX 2.1. The phylogenetic tree of 16S rRNA genes of V4 and related strains was constructed using MEGA 11 (Fig. 2). The topology of the phylogenetic tree revealed that the V4 strain formed a clade with members of the genus *Sphingobium*. V4 was most closely related to *Sphingobium lactosutens* DS20^T (=CCM 7540^T = MTCC 9471^T) (Kumari et al. 2009) (99% sequence similarity). V4 also shared 97.5% sequence similarity with *Sphingobium abikonense* NBRC 16140^T (IAM 12404^T = KCTC 2864^T) (Kumari et al. 2009). Other species were relatively far from V4 but their sequence similarities with V4 were still over 95%, which indicated that V4 was a member of

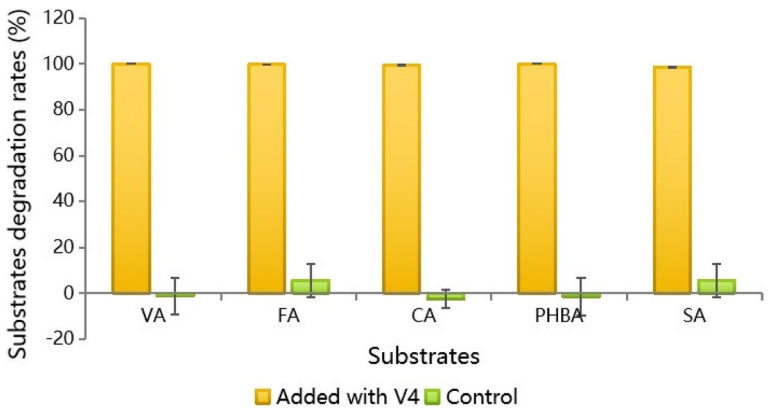

Fig. 3 Phenolic acid degradation rates. VA, vanillic acid; FA, ferulic acid; CA, *p*-coumaric acid; PHBA, *p*-hydroxy benzoic acid; SA, syringic acid

the genus *Sphingobium*. Based on 16S rRNA sequence similarities and the phylogenetic analysis, *S. lactosutens* DS20^T and *S. abikonense* NBRC 16140^T were selected as reference strains for comparisons of physiological and biochemical characteristics, and their differential characteristics are summarized in Table 1.

Biochemical characterization

In the catalase test, V4 tested positive. In the API 20NE tests, V4 was positive for nitrate reduction, L-tryptophan fermentation, aesculin hydrolysis, and 4-nitrophenyl-β-D-galactopyranoside hydrolysis, while negative for glucose fermentation, hydrolysis of L-arginine, urea, and

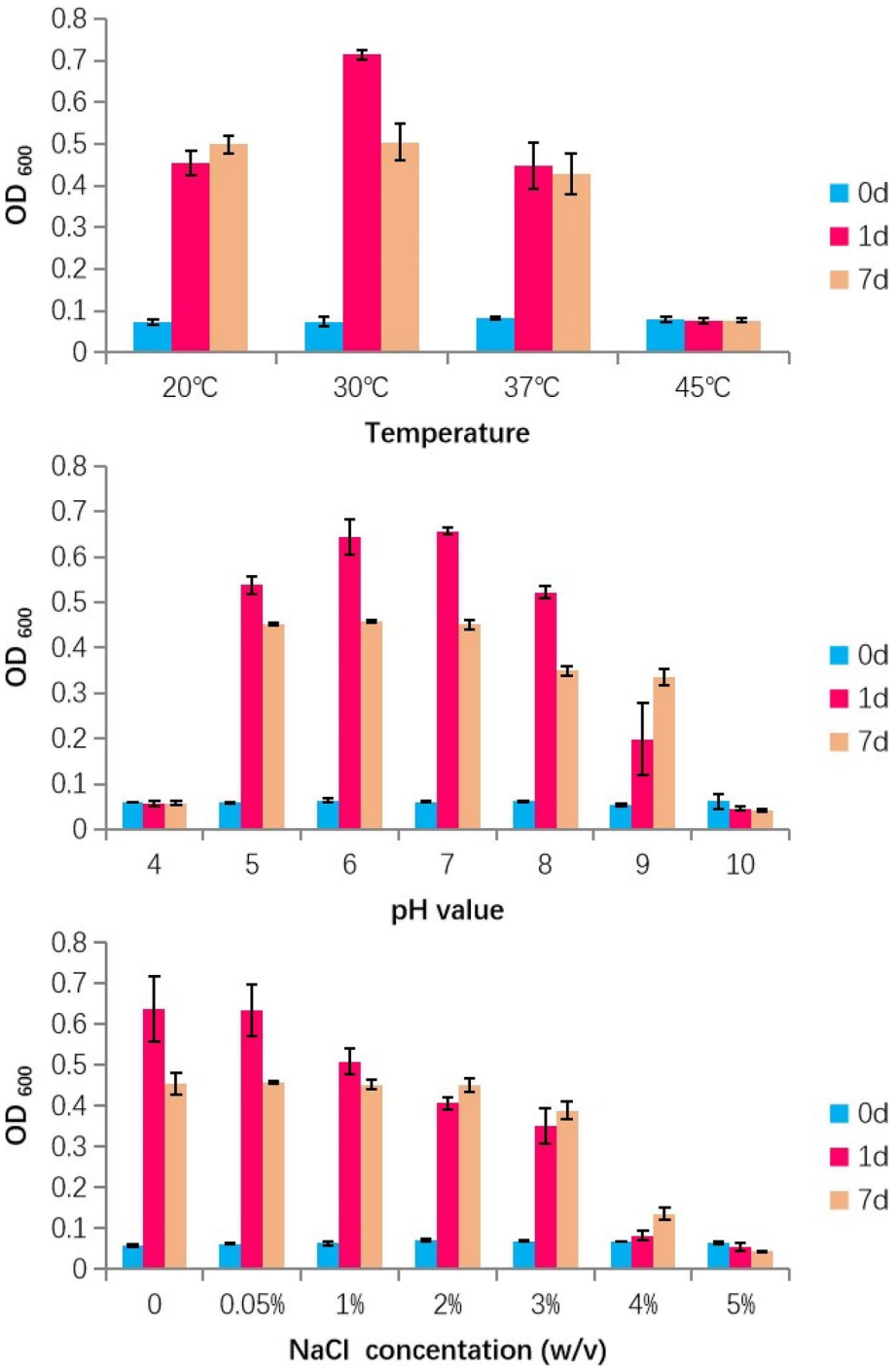

Fig. 4 Influence of environmental conditions on V4 growth

gelatin. Cells assimilated D-glucose, L-arabinose, D-maltose, potassium gluconate, and trisodium citrate, but did not assimilate D-mannose, D-mannitol, N-acetylglucosamine, capric acid, adipic acid, malic acid, or phenylacetic acid. Cytochrome oxidase was positive in V4. In the API ZYM tests, V4 was positive for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase; weakly positive for alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, and β-galactosidase; and negative for leucine arylamidase and acid phosphatase. The detected biochemical characteristics of the V4 strain were similar but different from those of closely related species in *Sphingobium* (Chaudhary et al. 2017; Kumari et al. 2009); the differentiating characteristics between V4 and the closely related species are summarized in Table 1.

Phenolic acid degradation profile

Five different phenolic acids were tested for substrate degradation. Based on the substrate degradation results, it is clear that V4 degraded vanillic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxy benzoic acid, and syringic acid (Fig. 3). After 5 days of culture, degradation rates of the five phenolic acids in the experiment groups containing the V4 strain reached 100 ± 0%, 99.88 ± 0.02%, 99.43 ± 0.19%, 100 ± 0%, and 98.51 ± 0.21%, respectively, while the degradation rates of the control groups were − 1.31 ± 7.82%, 5.53 ± 7.29%, − 2.53 ± 3.93%, − 1.52 ± 8.34%, and 5.52 ± 7.23%, respectively. This indicated that the five phenolic acids tested exhibited no obvious degradation in the control groups, and strain V4 had high degradation ability for the five phenolic acids tested, among which vanillic acid and *p*-hydroxy benzoic acid were completely degraded. Independent-samples *t*-tests revealed that there was an extremely significant difference ($P < 0.01$) between the V4 test group and the control group of each phenolic acid.

At first, we used VM medium to cultivate V4, but we found it grew slowly, besides, V4 cells cultivated in VM medium adhered to the conical flask wall, which brought adverse effect on sampling and subsequent research. So, we added 0.05% yeast extract to improve the growth of V4 in the subsequent study. Accordingly, the subsequent substrate degradation test used VMY medium. Some organic pollutants cannot be used as the only carbon source and energy for microorganisms, and only when there are other organic compounds to provide microbial carbon source or energy can organic pollutants be degraded, the phenomenon was cometabolism. In this study, V4 not only degraded 100% ± 0% of vanillic acid in the VMY medium, but also degraded 100% ± 0% of vanillic acid in the VM medium (without yeast extract). This indicated that the degradation of vanillic acid (a typical phenolic acid) was not the result of cometabolism, but direct degradation.

Influence of environmental conditions on the growth of V4

In the temperature growth test, OD_{600} measurements showed that V4 grew at temperatures from 20 to 37 °C, with an optimal temperature of 30 °C. One-way ANOVA analysis of OD_{600} at 1 day revealed there were extremely significant differences ($P < 0.01$) between 30 °C and other temperature groups. In the pH growth test, OD_{600} measurements showed that V4 grew at pH values from 5.0 to 9.0, with the optimal pH between 6.0 and 7.0. The difference between the pH 6.0 group and the pH 7.0 group was not significant ($P > 0.05$). The differences between the pH 6.0 or 7.0 group and the other pH groups were extremely significant ($P < 0.01$) at 1 day. In the NaCl concentration growth test, OD_{600} measurements indicated that V4 grew at NaCl concentrations of 0 to 4.0%, with an optimal NaCl concentration of 0

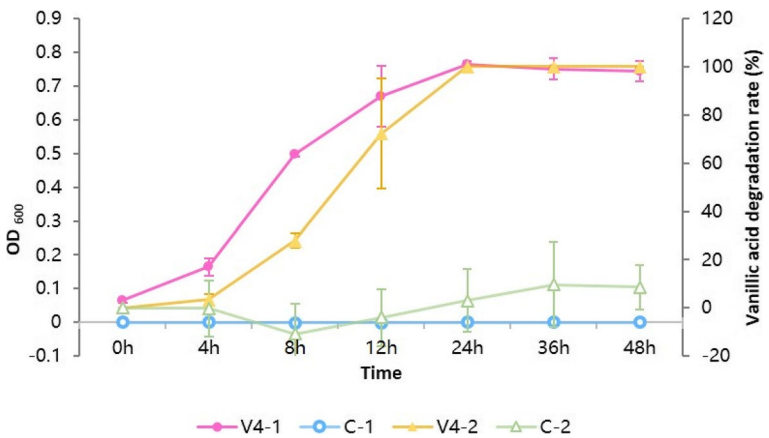

Fig. 5 Vanillic acid degradation curve and V4 growth curve. V4-1 and C-1 represent respectively the OD_{600} of the V4 test group and control group; V4-2 and C-2 represent respectively the degradation rate of the V4 test group and control group

to 0.05% (Fig. 4). The difference between 0% group and 0.05% group was not significant ($P > 0.05$). The differences between the 0% and 0.05% group and the other NaCl groups were extremely significant ($P < 0.01$) at 1 day. These results provided reference values for V4 cultivation and future V4 applications in the agricultural environment.

Vanillic acid degradation curve and V4 growth curve

In the VMY medium, both V4 bacterial growth and vanillic acid degradation were detected. The growth curve and vanillic acid degradation curve are shown in Fig. 5. It can be seen that both OD_{600} and vanillic acid degradation rate increased within 24 h in the V4 test group. At 24 h, the degradation of vanillic acid was 100 ± 0% in the

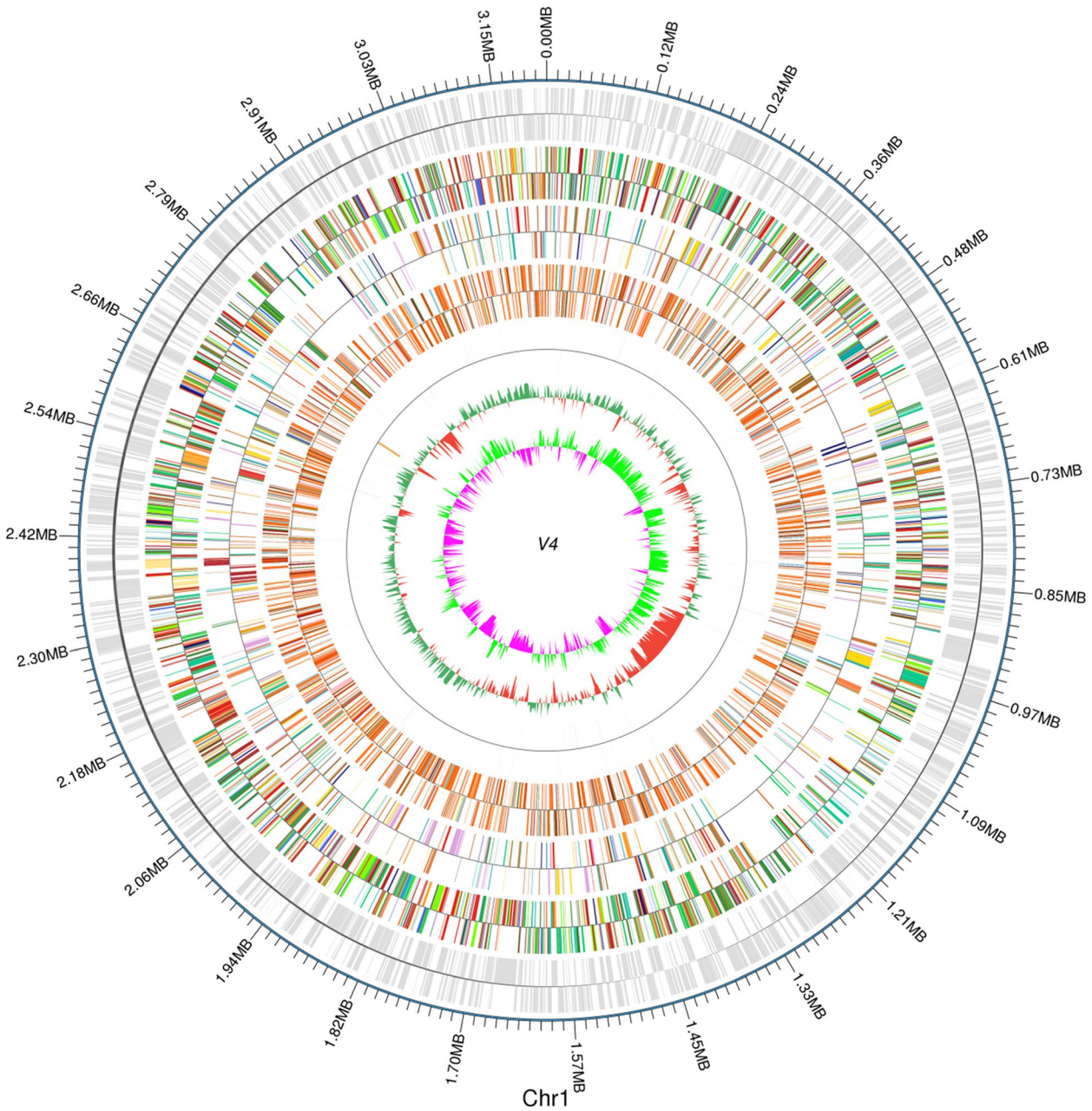

Fig. 6 Chromosome map of *Sphingobium* sp. V4. From outside to inside: the first layer shows the genome location information; the second layer shows genes encoded on positive and negative strands; the third layer shows the COG functional class of genes on positive and negative strands; the fourth layer shows KEGG functional class of genes on positive and negative strands; the fifth layer shows GO functional class of genes on the positive and negative strand; the sixth layer shows ncRNA; the seventh layer shows the genome G + C mol%; the eighth layer shows the genome GC skew value

V4 test group; therefore, it is obvious that a stationary stage is reached, while the degradation rate of the control was 3.03 ± 13.06%; the difference between the V4 test group and the control group was extremely significant ($P < 0.01$). From 8 to 12 h, vanillic acid was degraded most rapidly by the V4 bacterium. During this time, the degradation constant was 11.10%/h; it revealed the degradation efficiency of vanillic acid in V4.

Whole genome sequencing and analysis

To identify candidate genes involved in vanillic acid degradation, we performed whole genome sequencing on *Sphingobium* sp. V4. The genome assembly indicated that the *Sphingobium* V4 genome included a circular chromosome (3,209,747 bp) (Fig. 6) and three circular plasmids (1,247,975; 33,206; and 50,153 bp, respectively) (Figs. 7, 8, and 9). The total length was 4,541,081 bp, and the GC content was 64.5%, which fell within the range of 62–67% observed for other members of *Sphingobium* (Takeuchi et al. 2001).

The V4 genome contained 4395 coding genes, 59 tRNA genes, and three 5S, three 16S, and three 23S RNA genes. NR annotation was performed on 4200 protein-encoding genes (PEGs), including a *VanB* gene encoding vanillate *O*-demethylase oxidoreductase VanB on the chromosome (locus = Chr1:701,288:701,737: -) and a *VanA* gene encoding vanillate monooxygenase (vanillate *O*-demethylase oxygenase subunit VanA) on the 1,247,975-bp plasmid (locus = Plas1:504,261:505,313: +), which appeared to be candidate genes for vanillate degradation in strain V4. The VanA amino acid sequence similarities between V4 and *Pseudomonas* sp. HR199 (Priefert et al. 1997) and between V4 and *Pseudomonas resinovorans* strain CA10 (Shintani et al. 2013) were 30.9% and 54.9%, respectively. The VanB amino acid sequence similarities between V4 and *Pseudomonas* sp. HR199 (Priefert et al. 1997) and between V4 and *P. resinovorans* strain CA10 (Shintani et al. 2013) were 22.8% and 21.5% respectively. COG provided annotations for 3216 genes, and KEGG provided annotations for 4074 genes. GO provided annotations for 2765 entries spanning a total of 44 classifications belonging to molecular function, cellular component, and biological process categories (Fig. 10).

Discussion

Phenolic acids from plant secondary metabolites are key allelopathic compounds that not only affect soil microbial abundance, activity, and community composition, but also induce allelopathy in sensitive plants and thus pose continuous cropping obstacles (Zhalnina et al. 2018; Bai

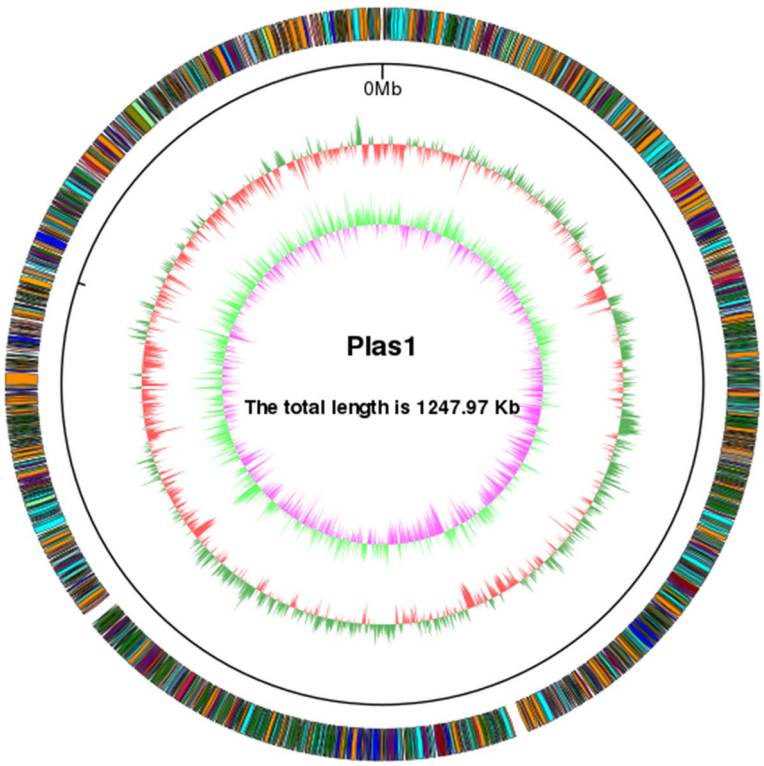

Fig. 7 Map of plasmid 1. From outside to inside: the first layer shows the COG functional class of genes; the second layer shows the genome location information; the third layer shows genome G + C mol%; the fourth layer shows genome GC skew value

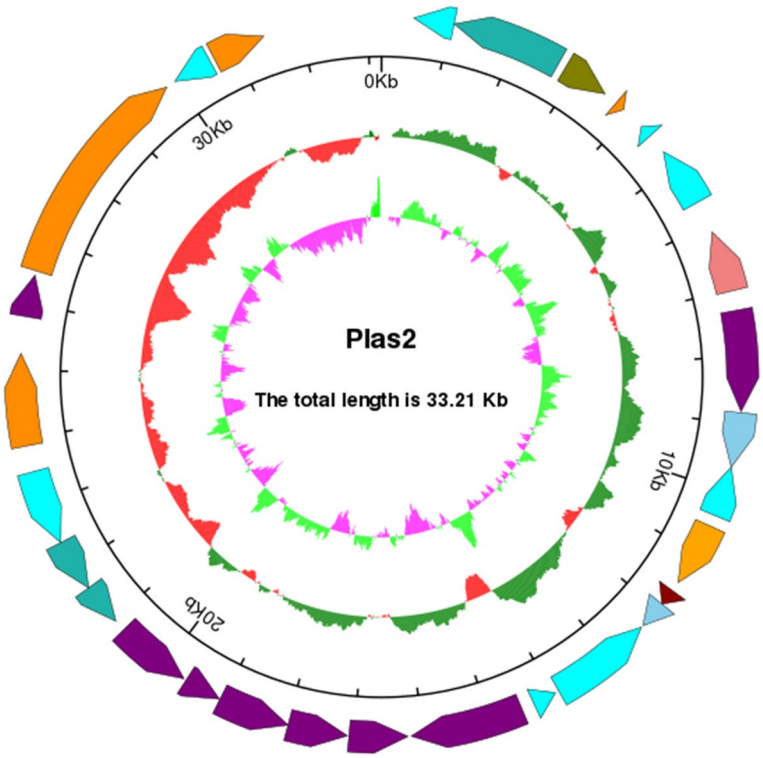

Fig. 8 Map of plasmid 2. From outside to inside: the first layer shows the COG functional class of genes; the second layer shows the genome location information; the third layer shows the genome G + C mol%; the fourth layer shows the genome GC skew value

et al. 2019; Zwetsloot et al. 2020). Accordingly, it is important to find and exploit microorganisms that can degrade phenolic acids in order to improve the soil environment and alleviate such continuous cropping obstacles. In recent years, some aromatic- and phenolic acid-degrading *Paraburkholderia* strains have been used in soil decomposition (Zwetsloot et al. 2020; Wilhelm et al. 2020). *Paraburkholderia madseniana* is a phenolic acid-degrader isolated from forest soil. It can degrade benzoic acid, phthalic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid (Wilhelm et al. 2020); these properties support the role of *P. madseniana* in priming the degradation of soil organic matter. In the present study, *Sphingobium* sp. V4 was revealed to degrade phenolic acids, including vanillic acid, ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, and syringic acid. These capacities suggest *Sphingobium* sp. V4 is a useful bacterium for phenolic acid degradation of agricultural soil. Additionally, the influence of environmental conditions on V4 growth was also investigated, providing details that could critically guide the application of V4 bacteria to degrade phenol acid under suitable conditions, because efficient growth is associated with efficient phenolic acid degradation. Notably, long-term continuous cropping usually leads to the deterioration of soil physicochemical characteristics, among which soil acidification is an important aspect (Shen et al. 2018). *Sphingobium* sp. V4 not only grows best at pH 6–7, but also grows well at pH 5. These properties are advantageous for the application of V4 to degrade phenolic acids in acidic soil under continuous cropping.

Sphingobium is a contributor to the catabolism of diverse organic pollutants (Takeuchi et al. 2001). Among the members of *Sphingobium*, *S. abikonense* NBRC 16140^{T}, isolated from oil-contaminated soil, can metabolize dibenzothiophene and sulfur-containing organic compounds (Kumari et al. 2009; Anzai et al. 2000; Yamada et al. 1968). *Sphingobium naphthae* K-3-6^{T}, isolated from oil-contaminated soil, can degrade aliphatic hydrocarbons (Chaudhary et al. 2017). *Sphingobium wenxiniae* JZ-1^{T}, isolated from activated sludge at a synthetic pyrethroid (SP)-manufacturing wastewater treatment facility, can degrade synthetic pyrethroid pesticides (Wang et al. Wang et al. 2009, Wang et al. 2011). *Sphingobium amiense*, isolated from river sediment, can degrade nonylphenol (Ushiba et al. 2003). Additionally, *Sphingobium* sp. SYK-6, isolated from pulping wastewater, is one of the best-characterized degraders of lignin-derived aromatics. SYK-6 can degrade phenolic acids, such as vanillic acid, ferulic acid, and syringic acid (Abe et al. 2005; Masai et al. 2007). Here, *Sphingobium* sp. V4, isolated from a strawberry field, was determined to degrade a wide variety of phenolic acids

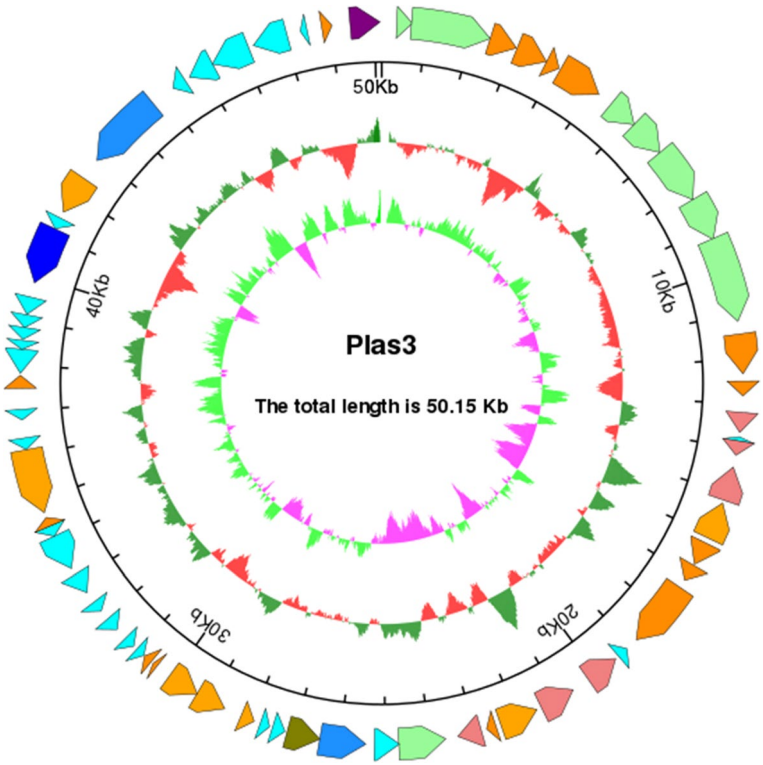

Fig. 9 Map of plasmid 3. From outside to inside: the first layer shows the COG functional class of genes; the second layer shows the genome location information; the third layer shows the genome G + C mol%; the fourth layer shows the genome GC skew value

(i.e., vanillic acid, ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, and syringic acid). This property indicates that *Sphingobium* sp. V4 is a promising bacterium for elimination of phenolic acids and agricultural soil improvement.

Vanillic acid is one of the typical phenolic acids. It has strong allelopathy to crops which is involved in continuous cropping obstacles (Wu et al. 2015). So we select vanillic acid as the main compound in this study. The degradation of vanillic acid has been investigated in *Pseudomonas* spp. The first step in vanillic acid degradation is demethylation, which is catalyzed by a two-component vanillate demethylase (VanA and VanB subunits) that converts vanillic acid to protocatechuic acid. Brunel and Davison characterized the *vanA* and *vanB* genes in *Pseudomonas*, which are located adjacently in the genome (Brunel and Davison 1988). *Pseudomonas* sp. HR199 (DSM 7063) (Priefert et al. 1997), *P. fluorescens* BF13 (Civolani et al. 2000), and *Acinetobacter* have a similar organization of their *vanA* and *vanB* genes (Morawski et al. 2000). Nevertheless, *Sphingobium* sp. SYK-6 degrades vanillic acid by the single component enzyme ligM, which is encoded by the *ligM* gene (Abe et al. 2005; Masai et al. 2007). In the present study, V4 genomic sequencing revealed a *vanB* gene on the chromosome and a *vanA* gene on a large plasmid. Many prokaryotes harbor large plasmids, which are involved in the degradation of recalcitrant organic compounds and in metabolic flexibility (Stolz 2014; Rinke 2022). As mobilome components, plasmids are in constant exchange with more stable chromosomes and contribute to horizontal gene transfer (HGT), an important evolution force among prokaryotes (Koonin and Wolf 2008). Thus, the large plasmid containing *vanA* and the chromosome containing *vanB* in the isolated V4 strain are the outcome of prokaryotic evolution, consistent with the genomic diversity of prokaryotes. Other properties of vanillic acid degradation enzymes as well as the genes merit investigation in future research.

Conclusions

The present study isolated the phenolic acid-degrading bacterium V4. Morphological, biochemical, and phylogenetic analysis revealed V4 to be a member of the genus *Sphingobium*, referred to as *Sphingobium* sp. V4. *Sphingobium* sp. V4 effectively degraded vanillic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and syringic acid. V4 degraded 500 mg/L vanillic acid completely within 24 h. *Sphingobium* sp. V4 grew under a wide range of environmental conditions. It not only grew best

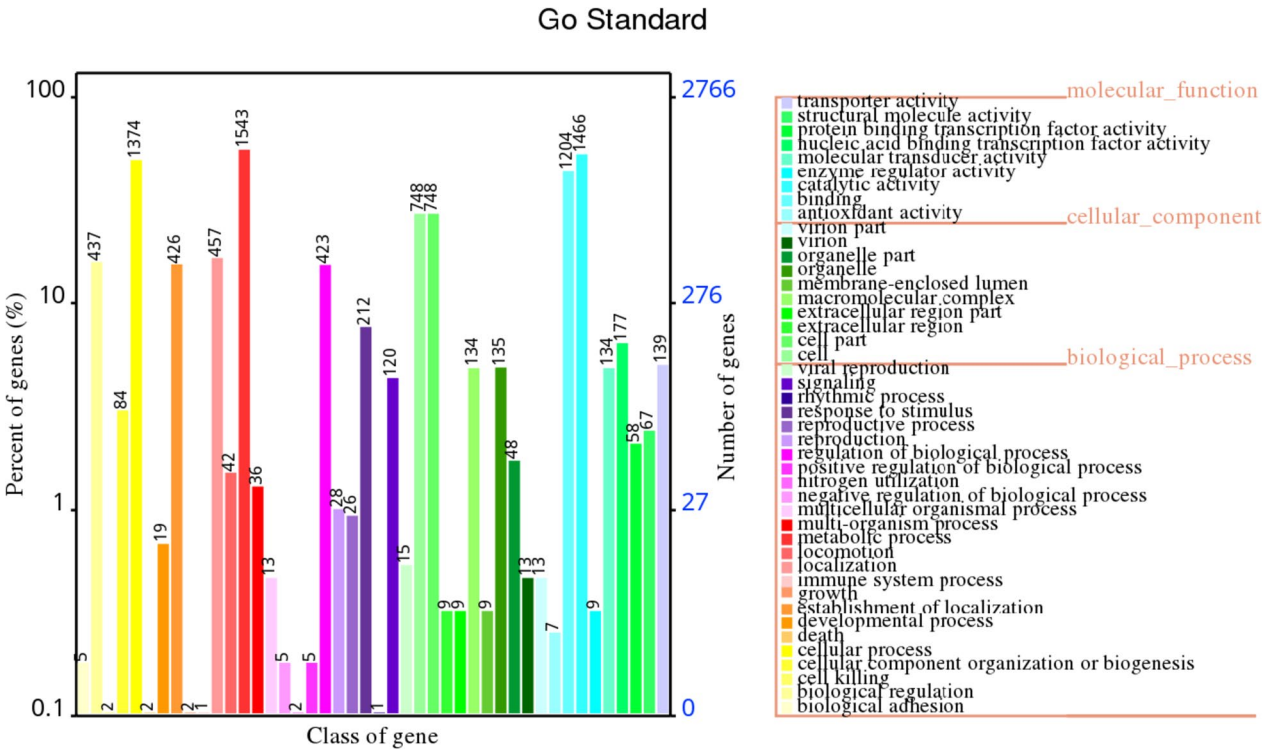

Fig. 10 Histogram showing the distribution of Gene Ontology (GO) terms. Abscissa represents GO terms and ordinate represents the gene number. Different colors are used to distinguish biological processes, cellular components, and molecular functions

at pH 6–7, but also grew well at pH 5. These properties are advantageous for the application of V4 in acidic continuous cropping soil. Whole genome analysis revealed that *Sphingobium* sp. V4 contained one chromosome and three plasmids. Two genes involved in vanillic acid degradation were found in the V4 genome: the gene encoding vanillate *O*-demethylase oxidoreductase VanB was located on the chromosome, while the gene encoding vanillate monooxygenase (vanillate *O*-demethylase oxygenase subunit VanA) was located on a large plasmid, illustrating the genomic diversity of prokaryotes. The present study provides support for further investigation and application of the phenolic acid-degrading microorganism to alleviate continuous cropping obstacles in agriculture.

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Authors' contributions
C. Z. and Q. M. designed the experiment, analyzed the data, and wrote and revised the manuscript. C. Z., S. L., Q. G., D. L., and Z. L. performed the experiment. H. L., Q. Z., and H. L. assisted in the result analysis and revised the manuscript. Z. D. assisted in the experiment technique, and W. G. and Y. G. conducted part of the experiment.

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Availability of data and materials
The data generated and analyzed during this study are available in this article. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole genome sequence of *Sphingobium* sp. V4 are MZ646136 and CP081001-CP081004 (GCA_029590555.1), respectively.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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