Screening, cloning and expression patterns of phosphorus-related genes of Burkholderia multivorans WS-FJ9 at different phosphorus levels

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Abstract
As important plant growth promoting rhizobacteria, phosphate-solubilizing bacteria (PSB) fix nitrogen, dissolve potassium, promote growth, improve the soil microenvironment, and enhance soil fertility. A high-efficiency PSB strain from the pine tree rhizosphere, Burkholderia multivorans WS-FJ9, was screened in our laboratory. In this study, we using a Bio Screener fully automatic microbial growth curve meter to determine the growth of the WS-FJ9 strain in phosphate-removing medium, the growth and mineral phosphate solubilization of WS-FJ9 were obtained by Mo-Sb colorimetry and organophosphate-degradation plate assays. Second-generation sequencing technology was used to obtain genomic information and analyze possible phosphorus decomposition genes. The quantitative expression of these genes under different phosphorus levels was determined by real-time PCR. The results showed that WS-FJ9 had strong adaptability and capacity for mineral phosphate solubilization at low phosphorus levels, which is characterized by its low phosphorus induction and high phosphorus inhibition. The amount of solubilized mineral phosphate could exceed 140 mg/L. The total length of WS-FJ9 was 7,497,552 bp after splicing, and the GC content was 67.37%. Eight phosphate-related genes were selected for further study of their expression patterns at different phosphorus levels. Among them, AP-2, GspE and GspF were only related to organic phosphorus, HlyB was only related to inorganic phosphorus, and PhoR, PhoA, AP-1 and AP-3 were related to both. The strain utilizes multiple pathways for mineral phosphate solubilization, and the degradation processes of different phosphorus sources are interrelated and independent, indicating that WS-FJ9 can adapt to different phosphorus source environments and has good application potential.

Introduction
Phosphorus (P) is an essential nutrient element for plant growth and development. P participates in most plant metabolic processes and is one of the factors that limits crop yield. It has been reported that 74% of the cultivated land in China lacks phosphorus; 95% of the phosphorus in the soil is insoluble, and the phosphorus that can be absorbed and utilized by plants is insufficient to meet plant demands (Chen et al. 2017; Blume et al. 2010). To solve this problem, a large amount of phosphate fertilizer is often used to alleviate the problems caused by phosphorus deficiency in agricultural and
forestry production. However, long-term application of phosphate fertilizer not only causes soil hardening, acidification and water pollution but also may harm human health (Chaney 2012). In addition, the raw materials of phosphate fertilizer mainly come from nonrenewable phosphate rock. Therefore, improving the utilization rate of soil insoluble phosphorus has become the primary limitation in agricultural and forestry development.

Phosphate-solubilizing bacteria (PSB) in the rhizosphere have attracted increasing attention because of their advantages, such as environmental protection, low cost, and high efficiency (Khan et al. 2007; Owen et al. 2015). Over the years, many studies have been carried out on the characteristics and mechanisms of PSB (Lin et al. 2015). It is generally believed that PSB can dissolve insoluble inorganic phosphates by secretion of small molecule organic acids, proton exchange, and complexation and degrade organic phosphorus by secretion of degradation enzymes such as phosphatases and proteases (Qin et al. 2019).

At present, research on the phosphorus solubilization genes of PSB is mainly focused on genes related to the degradation of insoluble inorganic phosphorus. For example, Kim and others transferred a phosphorus solubilization gene (PQQ) to Escherichia coli transgenically, which significantly improved the phosphorus solubilization efficiency of the E. coli (Kim et al. 2003). Song et al. cloned the microbial phosphate solubilization gene GabY from the red soil of Guangxi Province and induced its expression in E. coli, and the recombinant E. coli degraded insoluble inorganic phosphorus (Song et al. 2019). Research on organic phosphorus degradation genes is focused on phosphatase genes, mainly including acid phosphatase, alkaline phosphatase and inositol hexaphosphatase genes. Fraga et al. cloned the acid phosphatase gene napA and transferred it to Burkholderia cepacia IS-16. It was found that the activity of acid phosphatase and the phosphorus dissolving activity of this strain were significantly increased in vitro (Fraga et al. 2001). Due to the wide variety of PSB, there are still relatively few studies on phosphate solubilization pathways and expression patterns of phosphate solubilization genes, which need to be further studied.

The Burkholderia cepacia complex (Bcc) is widely distributed in the soil and is an important component of plant growth promoting rhizobacteria (PGPR) that can promote the growth of wheat,
rice, poplar and other plants (Nishiyama et al. 2010; V. Trân Van et al. 2000; Li et al. 2014). PSB can not only promote plant growth by fixing nitrogen, dissolving phosphorus and secreting plant hormones (Min et al. 2019) but also produce a variety of antibacterial substances (Chen et al. 2019; Zhang et al. 2018), inhibit soil-borne diseases, and antagonize a variety of plant pathogens (Ren et al. 2006). At the same time, Bcc, as a bioremediation agent, can decompose herbicides and pesticides that are difficult to degrade (Li et al. 2013). To date, there are 17 genotypes of Bcc, and *Burkholderia multivorans* belongs to Bcc genotype II. Some of its strains are human pathogens (Varga et al. 2012), while others have antagonistic effects against some plant pathogens (Sijam et al. 2005). At present, the research on this bacterium is mostly focused on its antagonistic substances, but there have been few reports on the molecular mechanisms for phosphate solubilization of this bacterium.

A high-efficiency PSB from the rhizosphere of pine trees, *B. multivorans* WS-FJ9, was screened in our laboratory. Previous studies have shown that WS-FJ9 has a good ability to promote plant growth, dissolve phosphorus and antagonize a variety of plant pathogenic bacteria (Hou et al. 2012), and preliminary studies have explored its degradation mechanism of inorganic phosphorus by transcriptome analysis (Zeng et al. 2017). However, the ability of this strain to degrade organic phosphorus and the expression levels of phosphorus solubilization genes under different phosphorus levels are not clear. In this study, the growth and mineral phosphate solubilization ability of WS-FJ9 under different phosphorus levels were determined to explore its phosphorus solubilization characteristics when presented with different phosphorus sources. To precisely locate the phosphate solubilization genes and systematically understand the phosphate solubilization pathway of this strain, the second-generation genome of this strain was sequenced to mine genes related to phosphorus solubilization. Furthermore, the expression patterns of these genes under different phosphorus levels were further analyzed to reveal the mechanism of phosphorus solubilization and plant growth promotion of this strain at the molecular level.

**Materials And Methods**

**Strain and culture conditions**

The phosphate-solubilizing bacterium *B. multivorans* WS-FJ9 was isolated from the rhizosphere soil of
a 28-year-old slash pine (*Pinus elliottii*) in Guangzhuang Forestry Center, Fujian, China (Hou et al. 2012) and deposited in the Chinese Center for Type Culture Collection (Accession No. CCTCCM2011435). The Genomic data uploaded to NCBI (Accession No. JAAGNW000000000). After WS-FJ9 was activated, a single colony was removed and transferred into LB medium and cultured at 28 °C for 10 hours at 200 rpm. Then, 1% of the WS-FJ9 strain seed solution was transferred to a phosphate solubilizing medium with different exogenous phosphorus levels. Samples were transferred from bottles into a 100 µL 96-well plate with a liquid pipette, and each sample was repeated 3 times. The 96-well plate with the bacterial solutions was placed in a Bio Screener automatic microbial growth curve instrument for determination of their OD values.  

**Phosphate solubilization measurement**

Five concentrations of exogenous soluble phosphate (0, 1, 5, 10, and 20 mM) were added to Monkina medium (Yang et al. 2014), the National Botanical Research Institute's phosphate growth medium (NBRIP) (Han et al. 2019), respectively. The bacterial suspensions 10 µL were pipetted to the center of plates, with each soluble phosphate level repeated in triplicate. The growth and phosphate solubilization of the bacterium were observed after 5 days of incubation at 30°C. The phosphate solubilization activity was determined by the ratio between the clear zone diameter and the colony diameter. One milliliter of the bacterial suspension was inoculated into the Monkina medium and NBRIP broth medium at each of the soluble phosphate levels in triplicate. Medium without bacterial inoculation served as the control. The supernatants of each of the soluble phosphate treatment and control groups were filtered through 0.22-µm-pore-sized medical millexGP filters (Millipore, USA). The concentrations of soluble phosphate in the filtrates were measured using the ascorbate method (Zhang et al. 2008).  

**Sample preparation for genome sequencing**

The WS-FJ9 strain was washed 3-4 times with 1*PBS until the supernatant was clear. The samples were quickly frozen in liquid nitrogen and stored at - 80 °C. Three tubes of samples were prepared, each of which was approximately 0.5 g. The samples were sent to a sequencing company (Pasano, Shanghai), and high-quality samples of *B. multivorans* WS-FJ9 total DNA were extracted and
RNA extraction and reverse transcription

A bacterial total RNA extraction kit and reverse transcription kit were used according to the manufacturer’s instructions (Vazyme, Nanjing).

Quantitative real-time PCR

To understand the phosphate-solubilization mechanism of the WS-FJ9 strain from multiple angles, the phosphate solubilization genes from different phosphate solubilization pathways were selected to detect their relative expressions by qRT-PCR, including the PhoR gene responsible for sensing the two-component system of external phosphorus sources, which can sense the concentration of soluble phosphorus in the outside world; phosphatase genes AP-1, AP-2, AP-3, which encode the acid phosphatase gene and are important enzymes regulating phosphorus metabolism; organic acid genes, such as PhoA, which encode alkaline phosphatase, which can mineralize the activity of organic acids; HlyB, GspE, and GspF, are related to the secretion system responsible for secreting organic acids and enzymes into the environment. Primer 5.0 software was used to design specific primers for quantitative real-time PCR. The specific primers were designed as follows:
Table 1. Specific primers for qRT-PCR of *Burkholderia multivorans* WS-FJ9 phosphorus-solubilizing genes

| Gene Name | Primer 5’-3’                  |
|-----------|-------------------------------|
| PhoA      | F:ATGTCGACTATCAAGCGCAT         |
|           | R:CTCACCCCACCTGTAGATGC        |
| PhoR      | F:ATCCCGATTTTGTCCGCTACCT      |
|           | R:CGTTCGAGTTCCGATGATGCCTTG    |
| AP-1      | F:GAAGAAAACCTGGATCCGCG         |
|           | R:GGAAGGCCGCGTACAGGTT         |
| AP-2      | F:GGTGGCGAACATCGTGGTG         |
|           | R:CCAGACCTTCGGACGGGTG         |
| AP-3      | F:CGCCTCTGTCTGGATCTC          |
|           | R:GAAGGCGATCTTGGTCAGC         |
| HlyB      | F:ATGTATTTCGGACGACGCT         |
|           | R:AGGAACGAGGTGGTGAGGTT        |
| GspE      | F:AACAGGCCTCGGACATCCA         |
|           | R:GTCGAGTTGCACGTCATTT         |
| GspF      | F:ATCGTGCTGCGTTACCTAT         |
|           | R:ACCAGTGCCGACGAATTG          |

The ChamQ™ SYBR® qPCR Master Mix (Low ROX Premixed) kit and 7500 real-time instrument (Applied Biosystems, Foster City, CA, USA) were used for qRT-PCR. The kit instructions were followed, and the reaction mixture was prepared on ice.

**Statistical analyses**

Statistical analyses were carried out using Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and SPSS software (ver. 23.0 IBM Corp., Armonk, NY, USA). Comparisons among treatments were analyzed for significance using Duncan’s new multiple range test.

**Results**

**Growth of Burkholderia multivorans WS-FJ9 in phosphorus-solubilizing medium with different exogenous phosphorus concentrations**

The growth of *B. multivorans* WS-FJ9 in phosphorus-solubilizing medium with different concentrations of exogenous phosphorus was examined to better understand the phosphate solubilizing ability of WS-FJ9 and determine its phosphate solubilization characteristics. The WS-FJ9 reached the logarithmic
phase preferentially under low phosphorus conditions, and the sequence was as follows: 0 mmol/L ≈ 1 mmol/L > 5 mmol/L > 10 mmol/L > 20 mmol/L (Fig. 1). It is possible that high phosphorus concentrations may hinder the early growth of WS-FJ9. However, whether or not the phosphorus source is sufficient restricts the total number of viable bacteria in the later stage. The number of colonies in the later stage of logarithmic growth was directly proportional to the phosphorus content, that is, 20 mmol/L > 10 mmol/L > 5 mmol/L > 1 mmol/L > 0 mmol/L.

**Detection of phosphate-solubilizing capacity of Burkholderia multivorans WS-FJ9**

The phosphate degradation by strain WS-FJ9 on a phosphate solubilizing plate was shown in Fig. 2: with the increase in the soluble phosphate concentration, the diameter of WS-FJ9 colonies also increased, while the diameter of the transparent area decreased. At the same time, the growth of strain WS-FJ9 was consistent with the growth in Fig. 1, which indicated that the growth rate of strain WS-FJ9 (that is, the speed at which the logarithmic growth phase was reached) was not faster with a higher soluble phosphorus content in the medium. However, with the increase in soluble phosphorus content in the medium, the longer the time was required to reach the logarithmic growth phase. The final total number of bacteria in each medium depended on the content of soluble phosphorus, that is, the higher the content of soluble phosphorus, the higher the total number of bacteria in the final medium.

According to the ability of solubilizing phosphorus of strain WS-FJ9 under different phosphorus levels (Fig. 3), the strain showed low phosphorus induction and high phosphorus inhibition. Under each treatment, the content of soluble phosphorus was the highest when the bacteria reached the stable stage and decreased slightly in the later stage. Under the condition of low phosphorus, the bacteria reached the stable period the soonest and had the strongest ability to solubilize phosphorus. The reason for the slight decrease in the later period may be that the available phosphorus content in the fermentation broth exceeded a certain threshold value, which inhibited the phosphorus solubilization activity of the WS-FJ9.

**Genome assembly and annotation of Burkholderia multivorans WS-FJ9**

To explore its ability to solubilize phosphate, the genome of strain WS-FJ9 was detected. The original
reads of the WS-FJ9 strain obtained by sequencing were subjected to quality control, quality
evaluation and assembly. The assembled genome characteristics and genome structural prediction
are shown in Table 2 and Table 3, respectively. The total length of the assembled genome was
7,497,552 bp, and the GC content was 67.37%. A total of 1519 genomic short fragments (contigs) and
479 long fragments (scaffolds) were obtained. The maximum scaffold sequence length was 52,157
bp, the minimum scaffold sequence length was 230 bp, the N50 size was 29,847 bp, and the N90 size
was 7,350. It was predicted that the genome of strain WS-FJ9 encoded 7,720 genes covering
5,815,848 bp, accounting for 77.57% of the genome, and the average length of the coding genes was
753.35 bp. In addition, a total of 52 tRNA structures, 85 ncRNA structures, 3 rRNA structures, and 627
CRISPR structures were predicted.

**Table 2. Statistics of genomic characteristics of *Burkholderia multivorans* WS-FJ9**

| Name                | Numerical value | Name                | Numerical value |
|---------------------|-----------------|---------------------|-----------------|
| Genomic size/bp     | 7,497,552       | Max Scaffold Size/bp| 175,577         |
| GC content/         | 67.37           | Min Scaffold Size/bp| 1,006           |
| Scaffold Quantity   | 479             | Total Scaffold Size/bp| 7,497,552      |
| N50                 | 29,847          | N90                 | 7,350           |

**Table 3. Prediction of genome structure of *Burkholderia multivorans* WS-FJ9**

| Name   | Numerical value | Total length(bp) |
|--------|-----------------|------------------|
| CDS    | 1,519           | 7,497,552        |
| tRNA   | 52              | 4,082            |
| rRNA   | 3               | 4,514            |
| CRISPR | 627             | 2,129            |
| ncRNA  | 85              | 11,392           |

**Functional annotation of protein-encoding genes of *Burkholderia multivorans* WS-FJ9**

The functional annotation of protein-encoding genes is at the core content of whole genome analysis
of microorganisms, and it can reveal the biological activities of a species at the molecular level.
According to the functional annotation results of the genomic protein coding genes of WS-FJ9 (Table
4), 6271 protein-encoding genes were compared in the NR database, and 106 protein-encoding genes
were compared in the KEGG database. The differences were mainly related to the volume and focus
of the databases.
**Phosphate-related genes and metabolic pathways of Burkholderia multivorans WS-FJ9**

The genomic data of strain WS-FJ9 revealed many types of phosphate-related genes, which mainly include genes involved in organic acid synthesis and secretion, phosphatase synthesis and secretion and the sensing of external phosphorus sources, related to the regulatory system (Table 5).

According to KEGG analysis of this strain, a total of 106 genes of the WS-FJ9 genome were annotated, enriched in 32 metabolic pathways, and can be divided into 9 types. Among them, the pathways with the most genes were mainly signaling and cellular processes (29), genetic information processing (24), metabolism (21), carbohydrate metabolism (18), and metabolism of cofactors and vitamins (13). There were more than ten pathways related to phosphorus degradation: the two-component system (Fig. 4), bacterial secretion system (Fig. 5), phosphonate and phosphinate metabolism, inositol phosphate metabolism, pentose phosphate pathway, glycerol phospholipid metabolism, oxidative
phosphorylation, ABC transport system, phosphotransferase system (PTS), phosphatidylinositol signal system, phospholipase D signal pathway, etc. Therefore, the WS-FJ9 strain has the same traditional phosphorus degradation pathway as most of the PSB and has additional phosphorus solubilization pathways.

**Quantitative real-time PCR of phosphorus-related genes in Burkholderia multivorans WS-FJ9 strains under different exogenous phosphorus conditions**

The eight phosphate solubilization genes from different phosphate solubilization pathways were detected their relative expressions by qRT-PCR. The result showed that the expression patterns of the eight genes can be roughly divided into 4 categories:

(1) *PhoR* was sensitive to the phosphorus concentration and had the same expression pattern in both organic and inorganic phosphorus media (Fig. 6B). The expression level of the gene was high under phosphorus-free conditions (approximately 1.0 times), but it was low after the addition of exogenous phosphorus, and there was no significant difference in expression (approximately 0.2 mol / 0.4 times).

(2) *PhoA* (Fig. 6A), *AP-1* (Fig. 6C), and *AP-3* (Fig. 6E) were sensitive to the phosphorus concentration but had different expression patterns in both organic and inorganic phosphorus media. In the organic phosphorus medium with a low exogenous phosphorus concentration (0-1 mmol / L), the three genes were upregulated 2.2 times, 1.1 times, and 1.5 times, respectively. When the concentration of exogenous phosphorus was high, these genes were downregulated 0.4 times, 0.5 times, and 0.5 times, respectively. In the inorganic phosphorus medium, the expression level of *PhoA* was slightly higher than in the absence of phosphorus and under high phosphorus and slightly lower at other levels; the difference, at approximately 0.5 times, was not significant. With the increase in exogenous phosphorus concentration, the expression of *AP-1* and *AP-3* decreased, but the differences were not significant. The above three genes had slightly larger responses to the exogenous phosphorus content in the organic phosphorus medium.

(3) *AP-2* (Fig. 6D), *GspE* (Fig. 6G), and *GspF* (Fig. 6H) were sensitive to the phosphorus concentration only in organic phosphorus medium. There was no difference in the expression of these genes in the inorganic phosphorus medium (all approximately 1.0 times). In the organic phosphorus medium, *AP-2*
had a low expression (1-1.3 times) when the exogenous phosphorus content was low (0-5 mmol / L) and a high expression (2.7 times) when the exogenous phosphorus content was high. The expression patterns of GspE and GspF were as follows: when the exogenous phosphorus concentration was low (0-1 mmol / L), and the level of gene expression was high, up to 1.7 times and 2.4 times, respectively; when the exogenous phosphorus concentration was high, and the one of gene expression was low, approximately 0.8 and 0.5 times, respectively.

(4) HlyB was only sensitive to the phosphorus concentration in inorganic phosphorus medium (Fig. 6F). In the organic phosphorus medium, that gene showed no differences in expression under different phosphorus concentrations (all approximately 1.0). In the inorganic phosphorus medium, when the exogenous phosphorus concentration was low (0-1 mmol / L), the gene expression was upregulated (1-1.3 times). When the concentration of exogenous phosphorus was high, and the gene expression was downregulated (approximately 0.4-0.6 times).

Discussion
PSB, as a type of PGPR, can convert insoluble phosphorus into available phosphorus that can be absorbed and used by crops. At the same time, PSB fix nitrogen, dissolve potassium, promote plant growth and improve the soil micro-environment. These advantages have attracted widespread attention, and PSB have gradually become a sustainable alternative to solve soil phosphorus deficiency worldwide. B. multivorans WS-FJ9 is a high-efficiency PSB obtained from the pine rhizosphere and examined in our laboratory. This strain can degrade both insoluble organic phosphorus and inorganic phosphorus. The amount of phosphate solubilized by the WS-FJ9 strain under different phosphorus levels reached approximately 140 mg / L. At the same time, WS-FJ9 can dissolve inorganic phosphorus at up to 6.2 mM (approximately 1860 mg / L) (Zeng et al. 2017). Guo et al. (2018) screened four strains of PSB from rhizosphere soil of jujube. Among them, Bacillus sp. P7 and Acinetobacter sp.P13 had the strongest ability to decompose organic phosphorus, and the amount of solubilized phosphorus reached 118.84-127.74 mg / L. Jin et al. (2016) screened and isolated an organophosphate-dissolving bacteria Stenotrophomonas maltophilia JYD-4 from Taxus chinensis var. mairei rhizosphere, and the amount of solubilized phosphorus reached 72.38 mg / L.
Teng et al. (2019) isolation and characterization 11 kinds of phosphate solubilizing bacteria from rhizosphere soils of the Yeyahu Wetland, and Pseudomonas sp. J-IP1 had the strongest ability to resolve inorganic phosphorus up to 430.40 mg / L. Ibarra-Galeana et al. (2017) isolated 3 kinds of phosphate solubilizing bacteria from maize rhizospheric soils of northern Sinaloa, of which Sinorhizobium meliloti had the strongest phosphate-dissolving ability of about 592.85 mg / L. The WS-FJ9 strain has the ability to degrade organic phosphorus and inorganic phosphorus, and the comprehensive phosphorus-dissolving ability belongs to the upper and middle levels. Therefore, the strain has a wide application range and has good application potential on phosphorus-deficient soil.

To explore the phosphorus solubilization mechanism of the strain, the whole-genome shotgun strategy was used along with second-generation sequencing technology (Next-Generation Sequencing, NGS). The total length of the assembled genome was 7,497,552 bp, and the GC content was 67.37%, which is relatively high, so the gene density is relatively high, and the ability of this strain to resist high temperatures and an alkaline environment is also strong (Zhou et al. 2014). Through KEGG pathway analysis, multiple pathways related to dephosphorization were found in this strain, including a two-component system, bacterial secretion system, phosphonate and phosphinate metabolism, inositol phosphate metabolism, pentose phosphate pathway, glycerol phospholipid metabolism, oxidative phosphorylation, ABC transport system, phosphotransferase system (PTS), phosphatidylinositol signal system, phospholipase D signal pathway, etc. The existence of a two-component system and bacterial secretion system strongly explained the phosphate solubilizing abilities of the strains that exhibited "low phosphorus induction and high phosphorus inhibition" in previous research, as well as the secretion of organic acids and phosphatase to degrade insoluble inorganic and organic phosphorus.

In the previous studies on the phosphate solubilization pathways of PSB mainly focused on the analysis and discussion of the pathways related to organic acid and phosphatase synthesis (Yin et al. 2011). Studies have shown that the phosphate solubilizing ability of PSB is mostly regulated by the concentration of exogenous phosphorus, and most of the organic acids, enzymes and other substances involved in phosphate solubilization are secreted externally to degrade insoluble
phosphorus (Zeng et al. 2017; Geng et al. 2019). On the basis of the above, this study has increased our understanding of two phosphate solubilization systems that "communicate" between the strain and the outside environment, namely, the two-component system and the bacterial secretion system. Alexander found that there were significant differences in the expression of the PhoA, PhoC, and PhoD genes controlled by phosphate in Streptomyces coelicolor. Under low phosphorus conditions, PhoA and PhoD were upregulated, while PhoC showed the opposite expression patterns (Alexander et al. 2007). In this study, the expression of PhoA in the WS-FJ9 strain was consistent with that in S. coelicolor, and both showed high expression under low phosphorus. However, the expression patterns of genes related to the bacterial secretion system under different exogenous phosphorus conditions have not been reported, so this study selected key genes in the type I and type II secretion systems for analysis. By measuring the expression of phosphate solubilization genes in strain WS-FJ9 at different phosphorus levels, we found that genes from different phosphate solubilization systems and pathways have different expression patterns. AP-2, GspE, and GspF were sensitive to the phosphorus concentration only in organic phosphorus media, and HlyB was sensitive only in inorganic phosphorus medium. PhoR was sensitive in both organic and inorganic phosphorus media. PhoA, AP-1, and AP-3 were sensitive to the phosphorus concentration, but they had different expression patterns in both organic and inorganic phosphorus media. This indicates that these genes are directly or indirectly regulated by soluble phosphate and play an important role in responding to exogenous soluble phosphate.

Because the phosphate solubilization characteristics of WS-FJ9 were induced by low-phosphorus and high-phosphorus inhibition, that is, regulated by the phosphorus concentration, it is speculated that the genes that are sensitive to the phosphorus concentration and show high expression play an important role under this condition. Yang et al. (2016) found that the phosphate solubilization gene GDH of Pseudomonas sp. Wj1 and Enterobacter sp. Wj3 have different expression patterns under different phosphorus levels. It is speculated that the two strains have different phosphorus solubilization mechanisms. Based on this, in view of the multiple expression patterns of the phosphate solubilizing genes of strain WS-FJ9, we speculate that this strain has multiple phosphate
solubilization mechanisms, which are regulated by phosphate solubilization genes from different phosphate solubilization pathways. These genes are not only related to and independent of each other in the degradation of organic phosphorus and inorganic phosphorus but also support each other, which enables strain WS-FJ9 to adapt to different phosphorus source environments. Therefore, the strain has good application prospects. In the future, we can examine phosphate-sensing genes or secretion system genes to gain a more comprehensive and in-depth understanding of the phosphorus solubilization mechanism of this strain. In addition, the genomic data obtained from strain WS-FJ9 can be used to accurately locate relevant genes, which provides a good basis for future research on energy and material metabolism pathways and the related regulatory mechanisms.

Declarations

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Authors’ contributions
YQL performed and studied most of the experiments in manuscripts and analyzed experimental data and drafted linked content of the manuscript. XQW as research supervisor of YQL was involved in planning of research work; analysis and interpretation of data; WLK participated in the grammar and experimental planning of the manuscript; YHW and WHL and XLX were involved in the planning and execution of the research work; analysis and interpretation of the data; All the authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

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Availability of data and materials
All the data and materials have been provided in main manuscript.

**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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Figures
Figure 1
Growth of Burkholderia multivorans WS-FJ9 in phosphate-solubilizing medium with different exogenous phosphorus concentrations

Figure 2
Growth and phosphorus solubilization of WS-FJ9 on phosphorus solubilizing plates with different exogenous phosphorus concentrations A. 0 mmol/L; B. 1 mmol/L; C. 5 mmol/L; D. 10 mmol/L; E. 20 mmol/L
Figure 3

Detection of phosphate-solubilizing ability of Burkholderia multivorans WS-FJ9 under different phosphorus levels

Figure 4

KEGG pathway of gene PhoR and PhoA of Burkholderia multivorans WS-FJ9 [Two-component system]
KEGG pathway of gene HlyB, GspE, GspF of Burkholderia multivorans WS-FJ9 (Bacterial secretion system (T1SS, T2SS))
Expression of phosphorus solubilization genes in Burkholderia multivorans WS-FJ9 at different phosphorus levels A. gene PhoA; B. gene PhoR; C. gene AP-1; D. gene AP-2; E. gene AP-3; F. gene HlyB; G. gene GspE; H. gene GspF