Phosphorus (P) is an important element for plant and bacteria growth. The response of bacteria to P transformation into adsorbed forms and their interaction with plants remain unclear. In this study, the mechanisms involved in P adsorption in plants and bacteria were analyzed by a RNA-seq technology. A phosphate-solubilizing bacterium (PSB) *Acinetobacter* sp. strain M01 was used in the transcriptome profiling of expressed genes and during interaction with melon (*Cucumis melo* L.). Results showed that the majority of genes related to cellular component and molecular function were up-regulated. The genes in the melon plant treated with M01 were down-regulated compared with those in the control. Genes for phenylalanine metabolism, amino acid, glycerolipid metabolism were identified in M01 DEGs related to transport and catabolism, carbohydrate metabolisms, and amino acid transport were marked in melon. This study provides an understanding of the gene interaction in PSB to fixed P and melon.

**ABSTRACT**

Phosphorus (P) is an important element for plant and bacteria growth. The response of bacteria to P transformation into adsorbed forms and their interaction with plants remain unclear. In this study, the mechanisms involved in P adsorption in plants and bacteria were analyzed by a RNA-seq technology. A phosphate-solubilizing bacterium (PSB) *Acinetobacter* sp. strain M01 was used in the transcriptome profiling of expressed genes and during interaction with melon (*Cucumis melo* L.). Results showed that the majority of genes related to cellular component and molecular function were up-regulated. The genes in the melon plant treated with M01 were down-regulated compared with those in the control. Genes for phenylalanine metabolism, amino acid, glycerolipid metabolism were identified in M01 DEGs related to transport and catabolism, carbohydrate metabolisms, and amino acid transport were marked in melon. This study provides an understanding of the gene interaction in PSB to fixed P and melon.

**1. Introduction**

In terrestrial ecosystems, nutrients are essential to plant growth and development (Adesemoye et al. 2009; Richardson et al. 2009). Phosphorus (P) is one of the major growth-limiting nutrients and is biologically available from atmospheric source in contrast to nitrogen (Ezawa et al. 2002; Abell et al. 2010). P limitation is important in primary production and other ecosystem processes for various terrestrial ecosystems (Vitousek et al. 2010). P in its fixed form is derived primarily from rock weathering (Adesemoye et al. 2009; Vitousek et al. 2010). Large amounts of inorganic and organic P compounds are present in agricultural greenhouse soils but are immobilized and mostly unavailable. Hence, the availability of P to plants is limited because of P deficiency in soils (Stockdale et al. 2002; Braun et al. 2019).

P is essential for plant development and is involved in various biochemical processes including metabolism of lipids and biosynthesis of nucleic acids and cell membranes (Ha and Tran 2014). Using organic or inorganic P fertilizers is essential to achieve maximum yield but is a high-cost and time-consuming process (Nziguheba et al. 1998). A large amount of P from fertilizers enters immobile pools through precipitation with highly reactive AlOH and FeOH in acidic soils and CaCO3 in calcareous or normal soils (Gyaneshwar et al. 2002; Adesemoye and Kloepfer 2009). Soil P dynamics is characterized by physicochemical (sorption-desorption) and biological (immobilization-mineralization) processes. The reported efficiency of P fertilizers is approximately 10%–25%, and the recovery of applied P is 10%–15% (Sundara et al. 2002). Moreover, the concentration of bioavailable P in soil is extremely low (only 1.0 mg kg–1) (Sucunza et al. 2018). Soil P exists predominantly in inorganic fractions and is adsorbed to soil mineral surfaces (Gyaneshwar et al. 2002; Vitousek et al. 2010). The rates of P diffusion in soil solutions represent the major limitation of adequate P supply for plant growth (Schachtman et al. 1998; Richardson and Simpson 2011).

Microorganisms are integral to the soil P cycle because they mediate the availability of P for plants (Park et al. 2011; Richardson and Simpson 2011; Sharma et al. 2013). The contribution of microbes to the enhancement of P availability in different soils has attracted considerable interest over the years (Estrada-Bonilla et al. 2017; Yang et al. 2017; Wei et al. 2018). Soil microorganisms have a substantial contribution to the total soil P and this biological process is generally helpful for plant uptake (Zhu et al. 2018). Microorganisms enhance the capacity of plants to acquire P from the soil through several mechanisms, such as promoted root growth, increased P into the soil solution, and induced metabolic processes by directly solubilizing P from soil inorganic or organic P (Hayat et al. 2010). Thus, understanding the contribution of soil microorganisms to the improvement of soil-to-plant P uptake will provide an opportunity for increasing the efficiency of P usage (Richardson and Simpson 2011).

Phosphate-solubilizing bacteria (PSB) play a vital function in P cycle and can promote plant growth (Chen et al. 2006). PSB increase phosphate solutions by increasing soil
acidity or phosphatase enzymes, solubilize the inorganic forms of P, dissolve phosphate-complexed minerals (Richardson et al. 2009), and release P directly to surrounding soil systems (Hameeda et al. 2008; Bakhshandeh et al. 2017). Therefore, the use of PSB offsets the high cost of phosphate fertilizers and mobilizes insoluble fertilizers in the affected soils (Vessey 2003; Estrada-Bonilla et al. 2017). The use of phosphate biofertilizers decreases the detrimental effects of phosphate fertilizers on crop and soil health (Wei et al. 2017). PSB in plant rhizospheres play a vital role in releasing P that is readily usable by plants (Park et al. 2011).

To date, knowledge on how PSB interacts with fixed P and plants is limited. Whether bacteria can target specific interactions with fixed P and plants must be determined. RNA sequencing that uses deep-sequencing technologies provides opportunities for directly identifying the abundance of specific genes or quantifying the expression of target genes in the bacteria. Furthermore, this technique has become a regular and important method to analyze plants’ and microbes’ gene expression due to its accuracy in common or rare transcripts (Di Bella et al. 2013). For instance, the identified gene expression of Burkholderia can provide valuable information in understanding the molecular mechanisms of PSB (Zeng, Wu, Wang, et al. 2017). However, the responses of bacteria to low P conditions and interaction with the plant are still poorly analyzed. This study investigated the transcriptomes of a highly efficient PSB Acinetobacter sp. strain M01 through high-throughput sequencing to explore the genes involved in P solubilization and the interaction between PSB and melon seedling. This work has laid a foundation for understanding the potential function of PSB genes in P stress and the improvement of melon plant growth.

2. Materials and methods

2.1. Strain and culture condition

Acinetobacter sp. strain M01 (GenBank accession number: KT964802), a highly efficient PSB (Zhang et al. 2017), was employed for the precise identification of phosphate-solubilizing-related genes (Supplemental Figure S1A). The M01 strain showed traits of promoting plant growth, such as tomatoes. M01 was cultivated on agar plates (5.0 g L\(^{-1}\) of yeast extract, 10.0 g L\(^{-1}\) of peptone, and 15.0 g of agar at pH 6.9–7.1) at 25 ± 2 °C for 5 days. Bacterial cells were stored in 40% glycerol at −80 °C.

2.2. Induction of phosphate-solubilizing genes and melon seedling raising

The phosphate-solubilizing activity of M01 was determined in an activated bacterium culture and a solid medium using the following three culture methods: treatment T1, nutrient agar; treatment T2, National Botanical Research Institute’s phosphate growth medium (NBRIP) containing (grams per liter) 10.0 glucose, 5.0 Ca\(_2\)(PO\(_4\))\(_2\), 5.0 MgCl\(_2\)·6H\(_2\)O, 0.25 MgSO\(_4\)·7H\(_2\)O, 0.2 KCl, and 0.1 (NH\(_4\))\(_2\)SO\(_4\) including 0.5% Ca\(_2\)(PO\(_4\))\(_2\) as an insoluble P source; and treatment T3, NBRIP medium supplemented with soluble P (NaH\(_2\)PO\(_4\)) at 3 g L\(^{-1}\). Bacterial cell concentration was adjusted to 10\(^6\) CFU mL\(^{-1}\). Bacterial cells were grown in the three types of media (T1–T3) at 25 °C for 3 days, from which bacterial growth was detected. The cultured bacteria were transferred to test tubes, frozen in liquid nitrogen, and stored at −80 °C until further analysis. Melon seeds were sown in a 50-hole tray with the grown substrate. When the first true leaves grew, the melon plants were inoculated with PSB M01 (10\(^8\) CFU mL\(^{-1}\)), and some plants were given water as a control. At least 10 plants were collected for each treatment after 15 days. The total melon seedlings were collected and frozen in liquid nitrogen for further RNA analysis. All frozen plant samples were stored at −80°C.

2.3. RNA extraction, library construction, and sequencing

Total RNA was extracted with TRIzol\(^\text{1}\) Reagent (Invitrogen) in accordance with the instructions (Supplemental Figure S1B and C). RNA quality was then determined by using a NanoDrop 2000 spectrophotometer (Thermo Fisher, USA). cDNA was synthesized using 1 μg of total RNA as a template. The reverse transcription reaction conditions were set as 25 °C for 10 min, 42 °C for 15 min, and 70 °C for 15 min. After the reaction, the PCR solution was placed in ice bath for 5 min. All the reaction solutions were prepared on ice. The final quality of cDNA was evaluated by gradient cDNA electrophoresis. Libraries were prepared using the DNA sample from TIANGEN kit (Beijing, China). The paired-end libraries were sequenced using Illumina HiSeq by Sangong Biotech Co., Ltd. (Shanghai, China). All treatments were set in triplicate, including experimental and control per group.

2.4. Quality control and read mapping

All raw reads were subjected to quality control with default parameters (http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic). Rockhopper (http://cs.wellesley.edu/~btjaden/Rockhopper/) was then used to identify clean reads to the reference genome in orientation mode with default parameters. This system uses RNA sequencing reads generated by high-throughput sequencing as inputs (Tjaden 2015).

2.5. De novo assembly and differential gene expression analysis

De novo assembly for all samples was performed to generate transcripts using the Trinity method (Grabherr et al. 2011). Theunigenes were used for BLAST searches in National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/), Swissprot (http://www.expasy.ch/sprot/), and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) databases. Gene ontology (GO) annotation was also conducted with the Blast GO program for the classification (Levin et al. 2010). The unigenes were also uploaded to the KEGG database for pathway assignment (Altermann and Klaenhammer 2005; Pinard et al. 2019). Differentially expressed genes (DEGs) were identified according to the gene expression level (https://biocductor.org/packages/release/bioconductor/edgeR.html), and their functions were determined by GO and KEGG pathway analysis with Goatools (https://github.com/tanghaibao/Goatools). The DEGs were considered significantly enriched in metabolic pathways in accordance with reported calculations (Song et al. 2018). The gene circle of M01 was mapped with Circos v0.64 (http://circos.ca/).
2.6. qRT-PCR analysis

The primers were designed using software Primer 5.0 (Supplemental Table S1). The total RNA was isolated using TIANGEN (Biotech CO., LTD., Beijing) reagent in accordance with the manufacturer’s protocol. Each sample with three replicates was obtained in qRT-PCR (Real-time PCR System, Applied Biosystems, USA) runs with 35 cycles of 95 °C for 40 s, 55 °C for 32 s, and 72 °C for 60 s. Acinetobacter sp. 16S rDNA was used as the control gene.

2.7. Statistical analysis

Microsoft Office Excel was used for data collection. SPSS package (version 19.0) was employed to analyze the data by one-way ANOVA (Fisher’s LSD test). Significant differences were considered at $P < 0.05$ level.

3. Results

3.1. Data processing and analysis

The total genome size of Acinetobacter sp. strain M01 was sequenced as size as 4.4 M (Figure 1 and supplemental Figure S1D), and the raw reads obtained in three treatments (T1, T2, and T3) ranged from 1,913,877,300 bp to 5,988,424,200 bp. T1 had the highest number of raw reads, followed by T3. The Q30 values of the raw reads ranged from 92.7% to 96.60%, and the value of GC was between 43.5% and 47.44%. After filtering, the clean data ranged from 1,843,746,923 bp to 5,708,714,967 bp (Table 1). The highest clean data was obtained in the T1-2 sample. However, the amount of clean data obtained in the T2 samples was lower than that from the T1 and T3 treatments. The Q30 value of the clean data ranged from 92.9% to 96.8%, and the GC content was within the normal range (between 42.98% and 47.20%).

3.2. Comparing genome statistics

The number of total reads in T2 treatment was lower than that in T1 or T3 (Table 2). The percentage of unmapped reads was lower than 10% in all treatments and was even less than 2% in T2. By contrast, the percentages of unique mapped reads were higher than 80% in all samples. The percentage of multiple mapped reads was less than 10.4% and was the highest in T1 combination. The mapping ratio ranged from 90.50% to 98.26%. Although the number of total reads in T2 combination was low, its mapping ratio value was the highest at above 98% (Table 2). On the basis of the gene coverage analysis, the coverage of most sequencing sequences was between 64.88% and 76.92%. T2 had the highest coverage rate, followed by T1. In the nine samples, the coverage rates of genes constituting 60% to 80% of whole genome reached 11.22% and 15.73%, respectively. Other areas covering less than 40% were in small proportion from only 1.65% and 6.25% (Table 3).

![Genome-wide assessment. Circular plot of reads mapped to strain M01 genome.](image)

![Table 1. Base information of three treatments in RNA sequencing.](data)

| Sample | Raw data (bp) | Before Filter | After Filter |
|--------|---------------|---------------|--------------|
| T1-1   | 5,010,774,000 | 96.32         | 96.54        |
| T1-2   | 5,988,424,200 | 96.43         | 96.66        |
| T1-3   | 4,342,693,500 | 96.13         | 96.40        |
| T2-1   | 3,020,536,800 | 93.13         | 93.41        |
| T2-2   | 2,707,779,300 | 93.51         | 93.76        |
| T2-3   | 1,913,877,300 | 92.70         | 92.93        |
| T3-1   | 4,898,119,200 | 96.60         | 96.76        |
| T3-2   | 5,164,125,900 | 96.22         | 96.42        |
| T3-3   | 5,305,719,600 | 95.91         | 96.22        |

Note: Three treatments concluded T1, nutrient condition; T2, scarce P; and T3, scarce P supplemented with soluble phosphate.

![Table 2. Comparative genome statistics of three treatments.](data)

| Sample | Total Reads | Unmapped Reads | Unique Mapped Reads | Multiple Mapped reads | Mapping Ratio (%) |
|--------|-------------|----------------|--------------------|-----------------------|-------------------|
| T1-1   | 31,970,054  | 3,037,155 (9.50%) | 26,324,142 (82.34%) | 2,609,073 (8.16%)     | 90.50             |
| T1-2   | 37,636,654  | 3,481,686 (9.25%) | 30,276,934 (80.45%) | 3,878,034 (10.30%)   | 90.75             |
| T1-3   | 27,652,286  | 2,293,818 (8.30%) | 23,000,912 (83.18%) | 2,357,556 (8.53%)    | 91.70             |
| T2-1   | 19,510,830  | 2,259,960 (11.74%) | 17,250,870 (90.27%) | 2,319,970 (12.19%)   | 98.26             |
| T2-2   | 17,405,670  | 2,026,758 (11.76%) | 15,378,912 (88.24%) | 2,457,622 (13.21%)   | 98.24             |
| T2-3   | 12,230,832  | 1,176,755 (11.77%) | 11,054,077 (86.96%) | 1,552,497 (13.27%)   | 98.23             |
| T3-1   | 31,632,974  | 1,036,450 (3.28%) | 30,200,524 (96.72%) | 396,139 (1.25%)      | 96.72             |
| T3-2   | 33,073,438  | 736,695 (2.22%)  | 31,828,834 (96.24%) | 509,909 (1.54%)      | 97.78             |
| T3-3   | 34,068,740  | 1,257,301 (3.69%) | 32,173,296 (94.44%) | 638,143 (1.87%)      | 96.31             |
3.3. Number of DEGs in PSB and melon seedling

As shown in Figure 2(A), the fragments per kilobase of transcript per million fragments mapped distributions of the nine samples were consistent and showed the same peak distribution. However, in the PCA analysis, the distribution of the nine samples was clustered differently (Figure 2(B)). The density of transcripts per million among the six melon plant samples had similar distribution (Figure 2(C–E)). The T1 samples aggregated, whereas the T2 samples were dispersed. The gene expression varied among the three treatments under different conditions. A total of 531 (T1 vs. T2; Figure 3(A)), 498 (T1 vs. T3; Figure 3(B)), and 111 (T2 vs. T3; Figure 3(C)) up-regulated and 514, 508, and 144 down-regulated genes were obtained. T1 vs. T2 was similar to T1 vs. T3, whereas T2 vs. T3 showed few DEGs. For the DEGs in melon plants, a total of 1095 (M1 vs. C1; Figure 3(D)), 1048 (M2 vs. C2; Figure 3(E)), and 1084 (M3 vs. C3; Figure 3(F)) up-regulated and 1012, 1329, and 267 down-regulated genes were identified. The number of DEGs and dispersed regions significantly differed among the three treatments. The number of up- and down-regulated genes was similar for T1 vs. T2 and T1 vs. T3 but low ($P < 0.05$) in T2 vs. T3 (Figure 4(A)). A total of 18424 DEGs were common in melon inoculated with M01 treatment (Figure 4(B)). Differential gene heatmap analysis showed significant differences in the gene expression levels of T1, T2, and T3. However, the gene distribution regions of T2 and T3 were similar to that of T1 (Figure 5(A)). Compared with that in control, more genes in melon plant (M01) were down-regulated (Figure 5(B)).

3.4. GO analysis of DEGs in PSB and melon seedling

GO analysis showed that in T1 vs. T2, 92 up- and 46 down-regulated genes were mostly involved in metabolic processes, 62 up- and 44 down-regulated genes involved in cellular processes, 61 up- and 12 down-regulated genes in cellular components, 37 up- and 3down-regulated genes in macromolecular complexes, 61 up- and 30 down-regulated
genes in binding function and catalytic activity, and 54 up- and 45 down-regulated genes in molecular function (Table 4).

In T1 vs. T3, 90 up- and 58 down-regulated genes were involved in metabolic and other biological processes, 61 up- and 56 down-regulated genes in cellular processes, 58 up- and 17 down-regulated genes in cell part, 31 up- and 4 down-regulated genes in macromolecular complexes in cellular components, 55 up- and 30 down-regulated genes in binding processes, and 58 up- and 47 down-regulated genes in catalytic activities (Table 4).

The number of biological process genes was higher than the other two comparison groups, and the lowest gene number was found in the molecular function. In T1 vs. T2 (Figure 6), the number of up-regulated genes related to biological processes was the largest, especially for metabolic processes. Genes related to cell part and binding were the highest in cellular component and molecular function, respectively. In T1 vs. T3 (Supplemental Figure S2), the

Figure 3. Volcano plot of three treatments. A, B, C, D, E, and F represent T1 vs. T2, T1 vs. T3, T2 vs. T3, M1 vs. C1, M2 vs. C2, and M3 vs. C3, respectively.

Figure 4. DEG analysis in three bacterial treatments and melon plant samples (A, T1 vs. T2, T1 vs. T3, T2 vs. T3; B, M1, M2, and M3).
genes showed the same distribution as T1 vs. T2. However, the number of genes in catalytic activity was the highest in molecular function. In T2 vs. T3 (Supplemental Figure S3), the total number of genes in the three processes was reduced compared with that in the two comparison groups. The number of up-regulated was similar to that of down-regulated genes. On the basis of the GO enrichment analysis, gene expression (GO:0010467) was involved in the biological process. Genes involved in cellular component include intracellular (GO:0005622), cells (GO:0005623), intracellular ribonucleoprotein complex (GO:0030529); macromolecular complex (GO:0032991); organelle (GO:0043226); non-membrane bound organelle (GO:0043228) (Figure 7); intracellular organelle (GO:0043229); intracellular nonmembrane bound organelle (GO:0043232); intracellular part (GO:0044424); cell part (GO:0044464); and ribonucleoprotein complex (GO:1990904). The genes involved in

---

**Figure 5.** Clustering heat map of DEGs in three treatments (A) and melon plant (B).

**Figure 6.** Summary of GO enrichment analysis (T1 vs. T2). Three categories, namely, biological processes, cellular components, and molecular functions, are shown. Up and down classification of DEGs are marked.
molecular function include RNA binding (GO:0003723) and structural molecule activity (GO:0005198) (Figure 7). In the melon plant, most DEGs were located in response to stimulus (GO:0050896), response to chemical (GO:0042221), and hormone (GO:0009725) (Figure 8(A)).

3.5. KEGG and KOG analysis of DEGs

KEGG analysis revealed several important pathways, such as alanine, aspartate, and glutamate metabolism (ko00250), valine, leucine, and isoleucine degradation (ko00280), and phenylalanine metabolism in amino acid metabolism (ko00360). Glycolysis/gluconeogenesis (Ko00010) and pentose phosphate (ko00030) pathways were involved in the carbohydrate metabolic pathway. The metabolic pathways involving the cellular community—prokaryotes were identified as biofilm formation – Pseudomonas aeruginosa (ko02025). The paths involving lipid metabolism included fatty acid biosynthesis (ko00061), glycerolipid (ko00561), and ether lipid (ko00565) metabolism. Pathways as related to ribosome were located in comparisons of three treatments in strain M01 (Figure 9(A–C)). In melon, plant hormone signal transduction (ko04075), and phenylpropanoid biosynthesis (ko00940), and flavonoid biosynthesis (ko00941) were identified (Figure 8(B)). KOG function classification analysis (Figure 8(C)) showed that most of the DEGs related to the functional groups in melon plant were involved in secondary metabolite biosynthesis, transport and catabolism, carbohydrate transport and metabolism, lipid transport and metabolism, and amino acid transport and metabolism. Moreover, the bacterial metabolic pathway of cofactors and vitamins was regulated by the biotin (ko00780) metabolic process. Terpenoids and polyketides were metabolized through geraniol degradation (Ko00281). A two-component system (ko02020) was identified in the signal transduction pathway, and ribosome (Ko03010) was detected in the translation pathway (Figure 10).

3.6. qRT-PCR validation

The data were validated through qRT-PCR analysis, and four genes were selected. The data from qRT-PCR were consistent with those obtained from RNA-seq (Supplemental Figure S4).}

4. Discussion

PSB is a kind of beneficial bacteria that interact with plant and can positively affect plant development. The role of
PSB and soil P on plant growth has been widely studied (Ghosh et al. 2015; Zhang et al. 2016; Vezzani et al. 2018). This work focused on the role of scarce P in regulating bacterial cell growth and the interaction of PSB with melon plant, especially during survival in extremely P deficient conditions. Inoculation with different PSB improves P plant acquisition and growth (Richardson et al. 2009; Rasul et al. 2019). In this study, the least amount of clean data was obtained from the T2 treatment, suggesting that strain M01 expresses a small number of genes under scarce P conditions. Although the number of total reads in T2 combination was low, its mapping ratio value was the highest at above 98% (Table 2). This value suggested that unnecessary genes are blocked or helpful genes are regulated in the T2 treatment (under P deprivation). This phenomenon may be attributed to restrained cell growth due to limited nutrients. Under nutritional stress, bacteria exhibit low specific growth rates, for instance, *Bacillus subtilis* keeps long periods of non-dividing situation (Cummings and Macfarlane 1997; Castro-Cerritos et al. 2018) that may lead to reduced gene expression. Moreover, many genes were upregulated in melon plant under PSB M01 inoculation (Figure 2), indicating that M01 can stimulate the gene expression to improve plant growth.

However, the addition of soluble P in T3 treatment increased the number of clean data, indicating that P deficit regulates the expression of genes related to bacterial growth. The T3 treatment nearly had the same amount of...
clean data as the T1 treatment (Table 1), suggesting that bacteria respond to soluble P and increase the number of genes they express to promote their growth. Recent works showed that arbuscular mycorrhizal fungi respond to phosphate (Ezawa and Saito 2018) and confirmed that phytoate overexpression in Arabidopsis improves plant growth under phosphate deficiency (Belgaroui et al. 2018). Previous studies on P effects on bacteria growth focused on the ability of bacteria to solubilize phosphate (Mehta and Nautiyal 2001; Chen et al. 2006; Zeng, Wu, Wen 2017). In the present research, strain M01 showed varied gene expression levels under different P conditions. When added with soluble phosphate, Burkholderia multivorans strain WS-FJ9 exhibits different phosphate solubilization rates, increased P concentration, and decreased halo diameter in each colony (Qingwei et al. 2017). Serratia sp. can increase the expression of pqq genes under P limitation, and mutant (pqqE-) shows substantial decrease in P soluble amount and gluconic acid production (Ludueña, Anzuay, Angelini, et al. 2017; Ludueña, Anzuay, Magallanes-Noguera, et al. 2017). In the present study, Acinetobacter sp. strain M01 regulated the expression of various genes under different P conditions (T1, nutrient; T2, scarce P; and T3, scarce P supplemented with soluble phosphate). These findings indicate that bacterial growth is greatly affected by P, and bacteria regulate different genes in response to P conditions.

Although many studies have focused on the characterization of PSB isolated from different soils (Liu et al. 2015; Otieno et al. 2015; Zhang et al. 2017), the role of P in bacterial cell growth and the bacterial response to P requires further research. Current observations showed that the gene expression levels significantly varied among the three treatments (Figure 6). Nutrients can limit bacterial growth in water system and calcareous or acid soils (Elser et al. 1995; Farjalla et al. 2002; Demoling et al. 2007; Göransson et al. 2011). The number of up- and down-regulated genes was the highest in T1 vs. T2 but the lowest in in T2 vs. T3 (Figure 3). Therefore, M01 regulated only a few genes to grow under P deprivation, and these genes might be strongly related to P adsorption. GO analysis showed that in T1 vs. T2, most of the up-regulated genes were involved in metabolic and cellular processes, and most of the down-regulated genes were associated with cellular components and molecular functions (Table 4). Only a few related genes were involved in metabolic and cellular processes in all treatments and molecular functions in T2 vs. T3. However, the up-regulated genes related to biological processes were the most abundant (Figure 6), suggesting that a considerable number of these genes regulate P transformation.

The majority of the genes were related to cellular components, including intracellular and intracellular ribonucleoprotein complexes, macromolecular complexes, nonmembrane-bound organelles, and intracellular organelles. The genes were also involved in molecular functions, including RNA binding and structural molecule activity (Table 5). These genes are important to bacterial survival under long-term stationary phase in Escherichia coli (Arunasi et al. 2014). KEGG analysis identified alanine, aspartate, and glutamate metabolism; valine, leucine, and isoleucine degradation; phenylalanine metabolism; and
amino acid metabolism (Figure 9(A,B)). KOG function classification analysis in melon detected secondary metabolite biosynthesis, transport and catabolism, carbohydrate transport and metabolism, and amino acid transport and metabolism (Figure 8(C)). These findings provide evidence that strain M01 benefits P uptake by regulating cell development pathways, cellular component, and molecular function, including RNA binding and structural molecule activity. Phosphatidic acid is a key intermediate in lipid metabolism (Athenstaedt and Daum 1999) and may be involved in P solubilization. In this work, only some pathways such as lipid metabolism (fatty acid biosynthesis), glycerolipid metabolism, and ether lipid metabolism pathways were related to P transformation.

Understanding the interaction of PSB with the plant is critical for developing suitable strategies to improve plant growth. In this study, M01 used different pathways to acquire P. PSB can transport P by affecting the pathway of glucose metabolism (Qingwei et al. 2017). Glucose-metabolizing enzymes or glucose transport process may also be related to phosphate regulation (Basu and Phale 2006; Nikel et al. 2015). Genes in bacteria involved in ABC-type transporter are overexpressed under phosphate deficiency (Bardin et al. 1996; Qingwei et al. 2017). In the current work, biotin, terpenoids, and polyketides were metabolized through geraniol degradation, which is related to phosphate solubilization. Signal transduction occurred through a two-component system, and the translation pathway included regulating ribosome (Figure 9(A–C)). The two-component transcription factor regulates the expression of several genes that function in the mineralization of phosphate in B. subtilis (Hulett et al. 1994). The genes that encode the two-component system, phoR and phoP, play an important role in phosphate response (Barreales et al. 2018; Guo et al. 2018).

This work found that PSB could potentially interact with melon seedling. The gene expression significantly varied among T1, T2, and T3 (Figure 10), suggesting that M01 exhibits different gene patterns related to P levels in the three treatments. More genes in melon plant were down-regulated compared with those in the control (Figure 5(B)). However, the growth and gene expression rates of M01 were relatively

### Table 5. Differentially expressed genes analysis in three treatments.

| Type                          | Term                                                                 | T1 vs. T2 | T1 vs. T3 | T2 vs. T3 |
|-------------------------------|----------------------------------------------------------------------|-----------|-----------|-----------|
| **Biological Process**        |                                                                      |           |           |           |
| Cellular homeostasis          | GO:0019725                                                          | 0.333     | 0.667     | 0.111     |
| Macromolecule biosynthetic process | GO:0009059                                                      | 0.349     | 0.365     | 0.159     |
| Cellular macromolecule biosynthetic process | GO:0034645                     | 0.349     | 0.365     | 0.159     |
| Biocatalytic regulation       | GO:0009059                                                          | 0.349     | 0.365     | 0.159     |
| Regulation of biological process | GO:0009059                                                    | 0.407     | 0.462     | 0.143     |
| Regulation of metabolic process | GO:0009059                                                   | 0.407     | 0.432     | 0.136     |
| Regulation of macromolecule biological process | GO:0009059                     | 0.444     | 0.458     | 0.139     |
| Homeostatic process           | GO:0042592                                                          | 0.444     | 0.458     | 0.139     |
| Regulation of metabolic process | GO:0009059                                                  | 0.444     | 0.458     | 0.139     |
| Regulation of macromolecule metabolic process | GO:0009059                     | 0.449     | 0.435     | 0.130     |
| **Cellular Component**        |                                                                      |           |           |           |
| Intracellular                 |                                                                      |           |           |           |
| cell                          | GO:0005623                                                          | 0.430     | 0.437     | 0.093     |
| Outer membrane                | GO:0019867                                                          | 0.400     | 1.000     | 0.400     |
| Intracellular part            | GO:0004424                                                          | 0.430     | 0.437     | 0.093     |
| Cell wall                     | GO:0004424                                                          | 0.430     | 0.437     | 0.093     |
| Macromolecular complex        | GO:0034291                                                          | 0.541     | 0.473     | 0.095     |
| **Molecular Function**        |                                                                      |           |           |           |
| Transferase activity, transferring acyl groups | GO:0016746                     | 0.154     | 0.308     | 0.308     |
| Transferase activity, transferring acyl groups other than amino-acyl groups | GO:0016746                     | 0.222     | 0.444     | 0.444     |
| N-acyltransferase activity    | GO:0016410                                                          | 0.233     | 0.333     | 0.667     |
| Acetyltransferase activity    | GO:0016407                                                          | 0.333     | 0.333     | 0.667     |
| Heterocyclic compound binding | GO:001363                                                           | 0.354     | 0.339     | 0.069     |
| Organic cyclic compound binding | GO:001363                                      | 0.359     | 0.333     | 0.068     |
| Nucleic acid binding transcription factor activity | GO:001071               | 0.364     | 0.485     | 0.182     |
| Nucleic acid binding          | GO:0003676                                                          | 0.435     | 0.380     | 0.076     |
high in T1, suggesting that this strain does not regulate specific genes to solubilize phosphate when the nutrient growing agars satisfied its growth requirements. Within the shortage of soluble phosphate, M01 regulated different genes to acquire P. Bacteria respond differently to phosphate limitation (Devine 2018; Ghosh et al. 2019). Hence, strain M01 can improve P uptake to meet its cell development despite being under the insoluble phosphate condition. These findings highlight that Acinetobacter sp. strain M01 can improve P uptake from scarce P sources and regulate gene expression in melon seedling.

5. Conclusions

Acinetobacter sp. strain M01 regulated various genes when subjected to low P conditions and interacted with the melon plant. The findings provide evidence that strain M01 can adsorb P by regulating pathways such as cell development, cellular component, and molecular function, including RNA binding and structural molecular activity. The metabolism of lipid (fatty acid biosynthesis), glycolipid, and ether lipid was related to P transformation. Moreover, the metabolism of biotin, terpenoids, and polyketides via geraniol degradation was identified in strain M01. During the interaction between PSB and melon seedling, the DEGs in melon related to secondary metabolites biosynthesis, transport, and catabolism, carbohydrate and lipids metabolism, and amino acid transport were marked. These findings elucidated the M01 roles of solubilize phosphate genes in the P transformation and improvement of the melon seedling. Understanding the interaction of beneficial PSB with insoluble P and plants would provide important information for their application in plants.

Acknowledgements

The authors thank the following individuals for their assistance: Yu Cao, Jing Chen and Renzhong Zhou, undergraduate students from the School of Horticulture of Anhui Agricultural University, for their assistance in the experiment. The authors also thank anonymous reviewers for their helpful comments and suggestions to improve this manuscript. ZJ: Conceptualization; Methodology; Investigation; Writing – review & editing; WPC: Investigation; Methodology; XQQ: Data curation; Investigation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Science Foundation of China [grant number 31701968], and the Anhui Key Basic Research Project [grant numbers 201904a06020004 and 202004a06020057], and the Second Level Youth Development Fund [grant number 2021Y1.022] from Anhui Academy of Agricultural Sciences.

Data availability statement

All data generated or analyzed during this study are included in this article and its supplementary materials. Sangong Biotech (https://www.sangon.com/) is now the placeholder that will be updated prior to publication. The release time of the data has been delayed but is available from the corresponding author on reasonable request.

Notes on contributors

Jian Zhang is a researcher in the Institute of Horticulture, Anhui Academy of Agricultural Sciences, China. His recent publications regarding research topics are Molecular characterization of beneficial bacteria, Plant-microbes interaction, Soil improvement and Plant-soil nutrients uptake.

Pengcheng Wang is a researcher in the Institute of Horticulture, Anhui Academy of Agricultural Sciences, China.

Qingqing Xiao is a researcher and lecturer in the Department of Biological and Environmental Engineering, Hefei University, China.

ORCID

Jian Zhang https://orcid.org/0000-0003-3775-0078

References

Abell JM, Ozkundalci D, Hamilton DP. 2010. Nitrogen and phosphorus limitation of phytoplankton growth in New Zealand lakes: implications for eutrophication control. Ecosystems. 13(7):966–977.

Adesemoye AO, Kloepper JW. 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol. 85(1):1–12.

Adesemoye AO, Torbert HA, Kloepper JW. 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol. 58(4):921–929.

Altermann E, Klaenhammer TR. 2005. Pathwayvoyager: pathway mapping using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. BMC Genomics. 6(1):60.

Arunarsi K, Adil M, Khan PAA, Shivaji S, Ahmed N. 2014. Global gene expression analysis of long-term stationary phase effects in E. coli K12 MG1655. PLoS One. 9(5):e96701.

Attenstaedt K, Daum G. 1999. Phosphatidic acid, a key intermediate in lipid metabolism. Eur J Biochem. 266(1):1–16.

Bakhshandeh E, Pirdashhti H, Lendeh KS. 2017. Phosphate and potassium-solubilizing bacteria effect on the growth of rice. Ecol Engine. 103:164–169.

Bardin S, Dan S, Osteras M, Finan TM. 1996. A phosphate transport system is required for symbiotic nitrogen fixation by Rhizobium meliloti. J Bacteriol. 178(15):4540–4547.

Barreales EG, Payero TD, de Pedro A, Aparicio JF, Virolle MJ. 2018. The metabolism of lipid (fatty acid biosynthesis), glycolipid, and ether lipid was related to P transformation. Moreover, the metabolism of biotin, terpenoids, and polyketides via geraniol degradation was identified in strain M01. During the interaction between PSB and melon seedling, the DEGs in melon related to secondary metabolites biosynthesis, transport, and catabolism, carbohydrate and lipids metabolism, and amino acid transport were marked. These findings elucidated the M01 roles of solubilize phosphate genes in the P transformation and improvement of the melon seedling. Understanding the interaction of beneficial PSB with insoluble P and plants would provide important information for their application in plants.

Belgaroui N, Lacombe B, Rouached H, Hanin M. 2018. Phytase overexpression in Arabidopsis improves plant growth under osmotic stress and in combination with phosphate deficiency. Sci Rep. 8(1):1137.

Braun S, Warrinner R, Börjesson G, Ulén B, Smolders E, Gustafsson JP. 2019. Assessing the ability of soil tests to estimate labile phosphorus in agricultural soils: evidence from isotopic exchange. Geoderma. 337:350–358.

Castro-Cerritos KV, Lopez-Torres A, Obregón-Herrera A, Wrobel K, Wrobel K, Pedraza-Reyes M. 2018. LC–MS/MS proteomic analysis of starved Bacillus subtilis cells overexpressing ribonucleotide reductase (aurEFP): implications in stress-associated mutagenesis. Curr Genet. 64(1):215–222.

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium-phosphate solubilizing abilities. Appl Soil Ecol. 34(1):33–41.

Cummings JH, Macfarlane GT. 1997. Role of intestinal bacteria in calcium phosphate solubilizing abilities. Appl Soil Ecol. 34(1):33–41.

Demoling F, Figueroa D, Bååth E. 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biol Biochem. 39(10):2485–2495.

Devine KM. 2018. Activation of the PhoPR-mediated response to phosphate limitation is regulated by wall teichoic acid metabolism in Bacillus subtilis. Front Microbiol. 9:2678.
Di Bella JM, Bao Y, Gloor GB, Burton JP, Reid G. 2013. High throughput-put sequencing methods and analysis for microbiome research. J Microbiol Methods. 95(3):401–414.

Elser JJ, Stabler LB, Hassett RP. 1995. Nutrient limitation of bacterial growth and rates of bacteriivory in lakes and oceans: a comparative study. Aquat Microb Ecol. 9(2):105–110.

Estrada-Bonilla GA, Lopes CM, Durrer A, Alves PRL, Passaglia N. Cardoso EJB. 2017. Effect of phosphate-solubilizing bacteria on phosphorus dynamics and the bacterial community during composting of sugarcane industry waste. Syst Appl Microbiol. 40(5):308–313.

Ezawa T, Saito K. 2018. How do arbuscular mycorrhizal fungi handle phosphate? New insight into fine-tuning of phosphate metabolism. New Phytolet. 220(4):1116–1121.

Farjalla VF, Esteves FA, Bozelli RL, Roland F. 2002. Nutrient limitation of bacterial growth and the bacterial community during composting of sugarcane industry waste. Syst Appl Microbiol. 24(5):413–422.

Ferreira AS, Verma AK, Lachance MA. 2008. Phosphate-solubilizing microbes: sustainable approach for managing phosphorus deficiency by biotechnological approaches. Crit Rev Biotechnol. 28(4):249–274.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Adiconis X, Nusbaum C, Thompson DA, Hulett F, Lee J, Shi L, Sun G, Chesnut R, Sharkova E, Duggan M, Kapp E, Xie Y, Gill BS, Pedersen AM, Georgiou K, Gunter D, Lassmann T, Mikkelsen TS, Kai S, Flatauer C, Arkin AP, Friedberg EC, Bainbridge W, Cross M, Urban AE, Mauceli E, Weinstock GM, Englander W, Vergez L, Bouck J, FitzHugh W, Burt DW, Nusbaum C, Hennigan J, Smith M, Zimin A, Zeng Q., et al.,2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 29(7):644–652.

Guo QG, Dong LH, Wang PP, Li SZ, Zhao WS, Lu XY, Zhang XY, Ping M. 2018. The PhoR/PhoP two-component system regulates fengycin production in Bacillus subtilis NCD-2 under low-phosphate conditions. J Integr Agr. 17(1):149–157.

Gyaneshwar P, Naresh Kumar G, Parekh LJ, Poole PS. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil. 245 (1):83–93.

Ha S, Tran LS. 2014. Understanding plant responses to phosphate starvation for improvement of plant tolerance to phosphorus deficiency by biotechnological approaches. Crit Rev Biotechnol. 34 (1):16–30.

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G. 2008. Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol Res. 163(2):234–240.

Hayat R, Ali S, Amara U, Khalid R, Ahmed I. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. 60(4):579–598.

Hulett F, Lee J, Shi L, Sun G, Chesnut R, Shankova E, Duggan M, Kapp E. 1998. Sequential action of two-component genetic switches regulates the PHO regulon in Bacillus subtilis. J Bacteriol. 176(5):1348–1358.

Levin JZ, Yassour M, Adiconis X, Nusbaum C, Thompson DA, Friedman N, Ginirke A, Regev A. 2010. Comprehensive comparative analysis of strand-specific RNA sequencing methods. Nat Methods. 7:709–715.

Liu Z, Li YC, Zhang S, Fu Y, Fan X, Patel JS, Zhang M. 2015. Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. Appl Soil Ecol. 96:217–224.

Ludueña LM, Anzuay MS, Angelini JG, Barros G, Luna MF, MdP, Fabra A, Taurian T. 2017. Role of bacterial pyrrolidinolone quinone in phosphate-solubilizing ability and in plant growth promotion on strain Serratia sp. S119. Symbiosis. 72(1):31–43.

Ludueña LM, Anzuay MS, Magallanes-Noguera C, Tonelli ML, Ibáñez FJ, Angelini JG, Fabra A, McIntosh M, Taurian T. 2017. Effect of single-stranded DNA and molecules from peanut root nodules on ppgE gene expression and ppg promoter activity in the phosphate-solubilizing strain Serratia sp. S119. Res Microbiol. 168 (8):710–721.

Mehta S, Nautiyal CS. 2001. An efficient method for qualitative screening of phosphate-solubilizing bacteria. Curr Microbiol. 43(1):51–56.

Nikol PI, Chavarria M, Fuhrer T, Sauer U, De Lorenzo V. 2015. Pseudomonas putida KT2440 strain metabolizes glucose through a cycle formed by enzymes of the Entner-Doudoroff, Embden-
mutivorans WS-FJ9 under different levels of soluble phosphate. J Microbiol Biotechnol. 27(4):844–855.
Zeng Q, Wu X, Wen X. 2017. Identification and characterization of the rhizosphere phosphate-solubilizing bacterium Pseudomonas frederiksbergensis JW-SD2 and its plant growth-promoting effects on poplar seedlings. Ann Microbiol. 67 (3):219–230.
Zhang J, Wang P, Fang L, Zhang Q, Yan C, Jingyi C. 2017. Isolation and characterization of phosphate-solubilizing bacteria from mushroom residues and their effect on tomato plant growth promotion. Pol J Microbiol. 66(1):47–55.
Zhang D, Zhang C, Tang X, Li H, Zhang F, Rengel Z, Whalley WR, Davies WJ, Shen J. 2016. Increased soil phosphorus availability induced by faba bean root exudation stimulates root growth and phosphorus uptake in neighbouring maize. New Phytol. 209(2):823–831.
Zhu J, Li M, Whelan M. 2018. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. Sci Total Environ. 612:522–537.