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Screening of two efficient phosphorus-solubilizing bacteria, strain X42 (Bacillus_A_bombysepticus) and strain G62 (Bacillus velezensis) for their ability to promote Gan Nan navel orange growth

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Abstract

Background Due to inadequacies in the current management practices of navel orange orchards in southern Jiangxi, there is a deficiency in phosphorus content and a decline in overall soil quality. Therefore, developing microbial formulations that increase soil fertility while meeting green ecological standards is highly important. Rhizobacteria promote plant growth through various mechanisms, and given the critical role of phosphorus in plant growth and development, the development and application of such microbial agents offer an effective approach to address the aforementioned issues.

Results This study screened two strains of bacteria with high phosphate solubilization capabilities from the roots of navel oranges in southern Jiangxi. These strains were inoculated into potted plants to investigate their potential to promote plant growth. A comparison of the growth indicators of the experimental and control groups, as well as the enzyme activity indicators of navel orange leaves, revealed that both strains exhibited good growth-promoting effects. Furthermore, whole-genome sequencing of the two strains was conducted, and by comparing data from 31 housekeeping genes, strain X42 was preliminarily identified as *Bacillus bombysepticus*, and strain G62 was identified as *Bacillus velezensis*. The comparison also revealed the presence of phosphate solubilization-encoding genes in both strains, with strain G62 lacking the genes for phytate mineralization and inorganic phosphorus dissolution, which may prevent it from utilizing additional organic phosphorus sources.

Conclusion This study not only confirms the positive impact of two highly efficient phosphate-solubilizing *Bacillus* strains on the growth of navel oranges in southern Jiangxi but also deepens the understanding of the genetic basis of phosphate-solubilizing traits through whole-genome analysis. These findings are highly important for the development of biofertilizers and their application in sustainable agriculture, especially in terms of improving soil quality and increasing crop yields.

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Keywords Plant growth-promoting rhizobacteria (PGPR), Gan nan navel oranges, Promotive effects, Whole-genome sequencing, Phosphate-solubilizing genes

Introduction

The fame of Gannan navel oranges stems from their distinctive taste and high nutritional value, which has driven a growing demand for these fruits both domestically and internationally (Liu et al. 2022). However, existing nutrient management practices in Gannan navel orange orchards have led to significant soil degradation. Traditional fertilization methods often result in nutrient imbalances and can lead to environmental issues such as water pollution and reduced soil fertility. To address these challenges and improve both the quality of agricultural products and the health of the soil, there is a growing emphasis on the use of environmentally friendly biofertilizers. Biofertilizers, which are derived from beneficial microorganisms, offer a sustainable alternative to chemical fertilizers by enhancing soil health and promoting natural nutrient cycling. They can help restore soil microbial diversity, increase plant resilience, and reduce reliance on synthetic inputs. By adopting these eco-friendly practices, Gannan navel orange orchards can maintain their reputation for producing high-quality fruit while also ensuring long-term sustainability and ecological balance.

Rhizosphere-promoting bacteria colonize plant roots and promote plant growth through different mechanisms, increasing soil fertility and enhancing plant absorption and utilization of mineral nutrients(Cherni et al. 2019; Ling et al. 2022). Phosphorus is an essential element for plant growth, and among many rhizosphere-promoting bacteria, many microorganisms, including bacteria, fungi, actinomycetes, and cyanobacteria, have phosphorus solubilization abilities (Kour et al. 2021). Research has shown that phosphorus-solubilizing microorganisms can promote the growth of rice in calcareous paddy soils and increase the concentrations of phosphorus and trace elements(Beheshti et al. 2022). Among many phosphorus-solubilizing microorganisms, bacteria make up the largest proportion, being 2–150 times more common than fungi(Broeckling et al. 2008; Kalayu 2019). Although fungi are present in relatively low proportions, this does not mean that their role is smaller than that of bacteria; research by Sharma has proven the importance of fungi(Sharma et al. 2013).

Among the various bacterial genera, Bacillus has received particular attention because of its ability to thrive under harsh conditions. Bacillus spp. are known for their robustness and versatility, making them ideal candidates for developing biofertilizers and improving soil health(Danilova and Sharipova 2020). These microorganisms not only increase nutrient availability but also contribute to the overall resilience of agricultural systems, thereby supporting sustainable farming practices. Bacillus species have shown promising research prospects in the field of agriculture. Research has shown that Bacillus bacteria can promote the growth of plants, such as corn, soybeans, and tomatoes, and assist in the absorption of phosphorus by these plants (Song et al. 2022; Lu et al. 2022; Luo et al. 2024). In addition to enhancing plant growth, Bacillus spp. can also inhibit the growth of certain pathogenic bacteria, thereby reducing the incidence of plant diseases and indirectly increasing crop yields. Studies aimed at controlling rice pathogens have revealed the potential of Bacillus velezensis as a biocontrol agent against fungal diseases (Su et al. 2024). Bacillus offers multiple benefits to plants. They can colonize the rhizosphere through chemotaxis and increase plant growth (Morales-Cedeño et al. 2021). These bacteria use a combination of direct and indirect mechanisms to establish themselves in their host environment. The direct mechanisms include nitrogen fixation, siderophore production, phytohormone synthesis, and nutrient solubilization. The indirect mechanisms include the production of hydrogen cyanide (HCN), extracellular polysaccharide (EPS), biofilm formation, and lytic enzymes. These strategies aim to promote plant growth and production under various environmental conditions (Chaudhary et al. 2022; Etesami et al. 2023). In addition to their promising applications in agriculture, Bacillus species also have potential in livestock farming. They can be used as feed additives to improve gut health, increase feed conversion efficiency, and reduce the occurrence of diseases (Grant et al. 2018; Zhou et al. 2020).

This study focused on two highly efficient phosphate-solubilizing bacteria, *Bacillus bombysepticus* and *Bacillus velezensis*, which were isolated from the rhizosphere soil of navel oranges in southern Jiangxi. The primary objective of this study was to investigate the potential of these two strains to promote the growth of navel orange seedlings. To gain a deeper understanding of their phosphate-solubilizing characteristics, whole-genome sequencing analysis will be conducted on these strains, providing essential foundational data for related research. *Bacillus bombysepticus* and *Bacillus velezensis*, both of which belong to the genus *Bacillus*, exhibit significant differences in their biological characteristics and application fields. Initially, *Bacillus bombysepticus* gained attention as the primary pathogen causing bacterial septicemia in silkworms, with its *PlcR* virulence regulatory mechanism resembling that of the well-known *Bacillus thuringiensis*. This feature positions *Bacillus bombysepticus* as

a valuable subject in agricultural pest control and environmental management, making it a research hotspot (Gohar et al. 2008; Cheng et al. 2014).

In contrast, *Bacillus velezensis* has a broader range of applications. This strain demonstrates excellent degradation capability for polyurethane in materials science and plays a crucial role in animal husbandry, particularly in aquaculture. For example, *Bacillus velezensis MB01B* exhibits potent inhibitory effects against *Vibrio cholerae GXFL1-4*, a key pathogen responsible for diseases in *Macrobrachium rosenbergii*, highlighting its potential as a probiotic agent for enhancing animal health (Zeng et al. 2024). Moreover, *Bacillus velezensis* is widely applied in plant disease management because of its outstanding biocontrol efficacy. Studies indicate that when used in conjunction with potassium sorbate, *Bacillus velezensis* can effectively reduce the incidence of banana Fusarium wilt (Gao et al. 2025). Additionally, *Bacillus velezensis* significantly promoted crop growth. According to the report by Hao Cao, this strain significantly enhances the growth performance of strawberries, including increasing the leaf number; increasing the fresh weight of stems, leaves, and roots; expanding the leaf area; and increasing the plant height (Cao et al. 2024).

In summary, *Bacillus bombysepticus* and *Bacillus velezensis* serve not only as ideal models for studying microbial phosphorus solubilization mechanisms but also as important resources for developing new agricultural biotechnology products. In-depth research on these two strains holds promise for increasing crop yields, improving soil health, and achieving sustainable agricultural development.

Materials and methods

Materials

Collection of rhizosphere soil

Soil was collected from Nixian County, Ganzhou city, Jiangxi Province, in a navel orange orchard (25°14'43" N, 114°58'13" E) and from another navel orange orchard in Ganxian County (25°48'82" N, 115°09'99" E). Soil samples were taken from near the root hairs of navel orange plants that were at least five years old. The soil was dug 10–30 cm below the drip line of the plants, and the soil near the root hairs was collected along with the root hairs, which were then placed in sterile sealed bags and brought back to the laboratory for storage at 4 °C.

Seedlings and soil

For the selection of navel orange seedlings, we selected samples from a local nursery in Ganzhou. Importantly, the seedlings presented a consistent growth status and appeared healthy overall. Specifically, the chosen seedlings should be free from any signs of disease or pest infestation, possess vibrant green leaves, and have a robust root system without any damage.

Main reagents

Preparation of LB medium: To prepare 1 L of LB medium, 10 g of peptone, 5 g of yeast extract, 10 g of NaCl, and 20 g of agar were added, with a pH of 7.0.

Preparation of inorganic phosphate medium (Chai et al. 2011; Liu et al. 2014; Wang et al. 2020): To prepare 1 L of inorganic phosphate medium, 10 g of glucose, 5 g of tricalcium phosphate, 0.5 g of ammonium sulfate, 0.3 g of sodium chloride, 0.3 g of potassium chloride, 0.3 g of magnesium sulfate heptahydrate, 0.03 g of ferrous sulfate, and 0.03 g of manganese sulfate tetrahydrate were needed, with a pH of 7.0–7.5.

Catalase (CAT) activity assay: The CAT activity assay kit was purchased from KeYiZhe Science Laboratory.

Peroxidase (POD) activity assay: The Solarbio Peroxidase (POD) Activity Assay Kit was purchased from KeYiZhe Science Laboratory.

Superoxide dismutase (SOD) assay: A total superoxide dismutase (SOD) assay kit purchased from Nanjing Jiancheng Bioengineering Research Institute was used.

Methods

Screening, purification, and identification of phosphate-solubilizing bacteria

Stones, weeds, dead branches, etc., were removed from the collected soil, and the soil attached to the root hairs was brushed off. The soil was thoroughly mixed, and 5 g of the soil suspension was prepared and heated to 80 °C in a water bath to obtain heat-resistant strains. The soil suspension was then diluted via gradient dilution (dilution factors of 10^5, 10^6, and 10^7). One hundred microliters of suspension was then spread on LB media. Single bacterial colonies on LB media were selected and purified via the streaking method for 2–3 generations. Purified colonies were then inoculated on slant media and stored at 4 °C.

The purified strains were inoculated on inorganic phosphate media and cultured at 37 °C for 7 days. Bacteria with transparent circles have phosphate solubilization ability, and a quantitative experiment was conducted to select strains with strong phosphate solubilization ability. The selected strains were then purified and stored.

The strains were morphologically and physiologically identified according to Bergey's Manual of Systematic Bacteriology and the Common Bacterial Systematic Identification Manual, and the bacterial samples were sent to the Shanghai Meiji Biological Company for molecular physiological and biochemical identification.

Whole-genome sequencing of phosphate-solubilizing bacteria

The strains to be tested were sent to Shanghai Majorbio Bio-Pharm Technology Co., Ltd., for whole-genome sequencing. The specific experimental procedures are as follows:

Library construction The company starts with purified bacterial genomic DNA, fragments it via a Covaris device, and then adds adapter sequences A and B to both ends of the DNA fragments. Only DNA fragments correctly ligated with these two adapters are retained, minimizing interference from nonspecific ligation.

Bridge PCR Suitably sized DNA fragments are selected via agarose gel electrophoresis and treated with sodium hydroxide to denature them into single strands under alkaline conditions. These single-stranded DNA fragments are then hybridized to specific primers immobilized on the surface of the sequencing chip, forming bridge structures. PCR amplification subsequently generates DNA clusters. To prepare for sequencing, the double-stranded DNA within the clusters is converted to single strands. During sequencing, specialized DNA polymerases and fluorescently labeled dNTPs are used; the fluorescence is excited by a laser, and the nucleotide type added in each cycle is determined based on the color emitted. The original DNA sequence is gradually deciphered by collecting fluorescence signals from each sequencing round.

Illumina sequencing The entire process utilized the Illumina sequencing platform, constructing a library with approximately 400 bp insert fragments and performing PE150 paired-end sequencing. This ensures that each sample achieves at least 100-fold genome coverage in the raw data volume, ultimately completing the assembly of the genome scaffolds.

To identify the 19 strains most closely related to the target strains at the species level, we selected 31 commonly used housekeeping genes (*dnaG*, *frr*, *infC*, *nusA*, *pgk*, *pyrG*, *rplA*, *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplK*, *rplL*, *rplM*, *rplN*, *rplP*, *rplS*, *rplT*, *rpmA*, *rpoB*, *rpsB*, *rpsC*, *rpsE*, *rpsI*, *rpsJ*, *rpsK*, *rpsM*, *rpsS*, *smpB*, *and tsf*) as references. By aligning and analyzing the sequences of these genes, we identified 19 strains most closely related to the target strains at the species level. We subsequently constructed phylogenetic trees via the neighbor–joining (NJ) method in MEGA 6.0 software. The steps included first, performing multiple sequence alignments of the 31 housekeeping genes from the 19 selected strains to ensure the accuracy and consistency of the alignments; then, constructing the phylogenetic tree in MEGA 6.0 via the NJ method; setting appropriate parameters such as the evolutionary model and starting tree to ensure the reliability and accuracy of the tree; and finally, evaluating the confidence and support of the phylogenetic tree via bootstrap analysis (1000 replicates).

To comprehensively annotate the genomes of the target strains, we employed various software tools for different predictions and analyses. First, we used GeneMarkS to predict coding sequences (CDSs) in the genome. GeneMarkS is an efficient prokaryotic gene prediction tool capable of accurately identifying gene start and stop sites. Second, we used barrnap to predict rRNA sequences in the genome. Barrnap is a fast and accurate rRNA prediction tool suitable for multiple prokaryotes. Additionally, we used tRNAscan-SE to predict tRNA sequences in the genome. tRNAscan-SE is a widely used tRNA prediction tool with high sensitivity and specificity.

To visually present the structure and characteristics of the genome, we used GCView software to draw genome circle diagrams. GCView can generate high-quality genome visualization diagrams, displaying information such as GC content, gene distribution, and repetitive sequences.

To further understand the functions of the predicted genes, the annotated genes were compared against six major databases. The comparison was conducted via Diamond software for the NR (nonredundant protein) database, Swiss-Prot database, COG (Clusters of Orthologous Groups of Proteins) database, and KEGG (Kyoto Encyclopedia of Genes and Genomes) database. For the Pfam database and GO (Gene Ontology) database, the comparison tools used were hmmer3 and blast2go, respectively. 2.2.3 Measurement of plant growth indicators.

To prepare a bacterial suspension of phosphate-solubilizing bacteria (PSB) with an optical density (OD_{600}) of 1, we followed the following steps. First, the PSB were inoculated into LB liquid medium and incubated on a shaker at a temperature between 30 °C and 37 °C until the bacterial concentration reached the desired level. Next, we measured the OD_{600} value of the bacterial culture via a spectrophotometer and diluted it to the required OD_{600} of 1 by adding an appropriate amount of sterile water. This concentration corresponded to approximately 10^8 colony-forming units (CFU/mL).

The inoculation treatment was subsequently performed as follows: once a week, the prepared PSB suspension was applied to the roots of navel orange seedlings through irrigation to promote microbial colonization in the rhizosphere. For the control group, sterilized LB medium was used and applied in the same manner as the PSB treatment was not applied to the seedlings. This approach eliminated the potential influence of the medium on the experimental results. Six seedlings were used as parallel controls for each treatment group.

After the seedlings were cultivated for nine weeks, they were harvested, and their growth indicators were

measured. At the end of the cultivation period, all the experimental plants were collected, and a series of growth parameters were measured. These parameters included plant height, stem diameter, leaf number, leaf color, dry weight, and fresh weight. By analyzing these data, we were able to evaluate the specific promoting effect of PSB on the growth of navel orange seedlings.

Measurement of plant physiological indicators

After nine weeks of growth, we collected the leaves of the plants for physiological indicator testing. By measuring enzyme activity, we aimed to assess the ability of the bacterial strain to promote leaf growth. In this study, we determined the activities of catalase (CAT), peroxidase (POD), and total superoxide dismutase (SOD), as well as the chlorophyll and carotenoid contents.

The measurement of these enzyme activities and pigment contents helps us understand the specific promoting effects of phosphate-solubilizing bacteria (PSB) on the growth of navel orange seedlings. Catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) are important antioxidant enzymes that participate in the removal of reactive oxygen species (ROS) and protect cells from oxidative damage. Chlorophyll and carotenoids are key pigments involved in photosynthesis, and changes in their contents reflect the strength of a plant's photosynthetic capacity. The extraction method for determining the chlorophyll content in the leaves of young navel orange seedlings via ethanol followed the procedure described by Lichtenthaler and Wellburn (Lichtenthaler and Wellburn 1983).

Statistical methods

Initial data collection and subsequent analysis were completed via Microsoft Excel 2019. The data were analyzed via SPSS software via t tests ($P \leq 0.05$). GraphPad Prism was used for data processing and graph generation, with error bars and the symbol "*" used to indicate statistical significance.

Table 1 Physiological and biochemical characteristics of strain X42 and strain G62

Experiment Name		Experimental results (negative is "-", positive is "+")	
		X42	G62
Catalase		+	-
Sugar and alcohol fermentation	Glucose	-	+
	Lactose	-	-
	Maltose	-	-
	Sucrose	-	+
	Mannose	-	-
Methyl Red		-	-
Voges-Proskauer (V-P)		-	+
Gram Staining		+	+
Starch Hydrolysis		+	+
Ammonia Production		+	+

Results and analysis

Screening and identification of phosphate solubilization bacteria (PSB)

Morphological identification of strains

In this study, 34 strains were isolated from the soil. By inoculating them into inorganic phosphate media, it was found that all of them possessed certain phosphate solubilization abilities. Two strains with strong phosphate solubilization effects, X42 and G62, were selected through a secondary screening process on inorganic phosphate media. The amount of phosphate solubilized by these strains reached 146.8 mg/L and 139.47 mg/L, respectively, after 7 days. Table 1 shows that the two strains of PSB exhibit different morphological characteristics.

Genome overview of the strains

As shown in Fig. 1. Genomic DNA was extracted from the strains and subjected to PCR amplification. Comparative analysis revealed that strain X42 is highly similar to *Bacillus_A_bombysepticus*, with 100% sequence identity and alignment coverage. Similarly, strain X42 also presented 100% sequence identity and alignment coverage with *Bacillus cereus AZ*. However, the score ratio of strain X42 to *Bacillus_A_bombysepticus* was 36,902, whereas the score ratio to *Bacillus cereus AZ* was 36,899. Higher scores indicate greater homology between sequences. However, the possibility that it was *Bacillus cereus* AZ cannot be ruled out. Consequently, strain X42 was preliminarily identified as *Bacillus_A_bombysepticus*. Strain G62 shares 99.6% sequence identity with *Bacillus velezensis* and 99.4% sequence identity with *Bacillus amyloliquefaciens*, leading to its preliminary identification as *Bacillus velezensis*.

Whole-genome sequencing of phosphate-solubilizing Bacteria

Basic genomic features and functional analysis of the two PSB strains

The basic genomic features of the strains X42 and G62 are presented in Table 2. The genome size of strain X42 was 6,051,156 bp, with a GC content of 34.7%, and it contained 41 chromosomal scaffolds. The genome size of strain G62 was 4,026,981 bp, with a GC content of 46.11%, and it contained 24 chromosomal scaffolds. Additionally, the genome of strain X42 included 6,001 CDSs, 99 tRNAs, and 11 rRNAs, whereas the genome of strain G62 contained 3,965 CDSs, 87 tRNAs, and 9 rRNAs. The genomic circles for strains X42 and G62 are shown in Fig. 2.

In addition, analysis of mobile elements revealed that the genomic island analysis identified 6 and 10 predicted

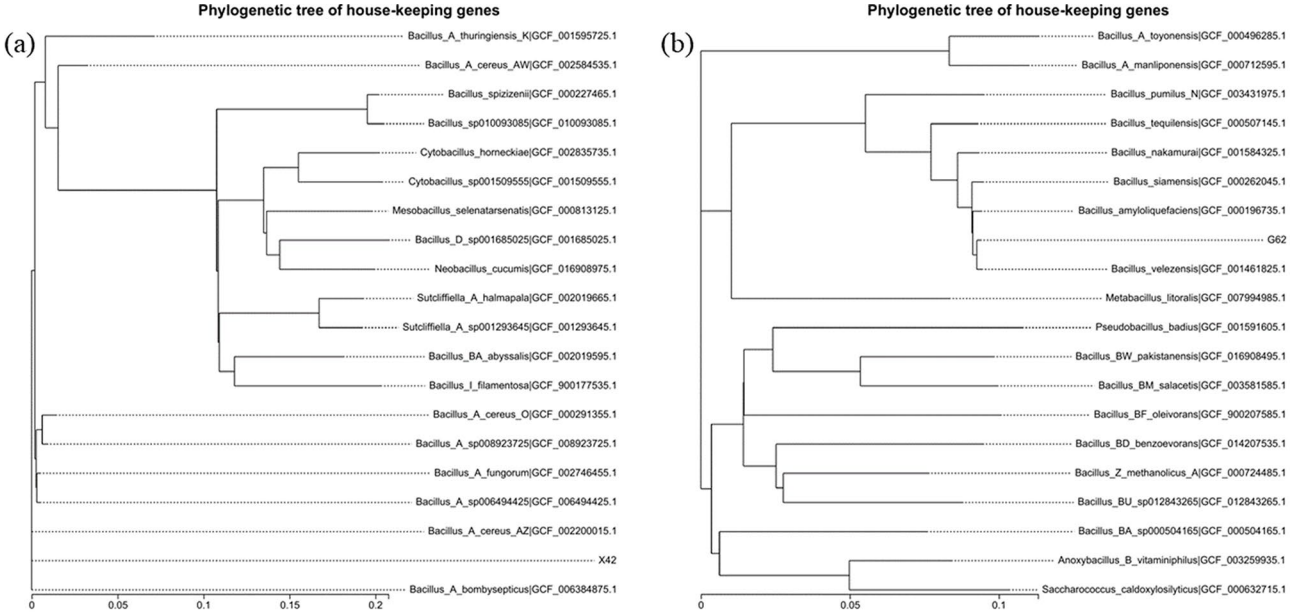

Fig. 1 Phylogenetic trees of the housekeeping genes of strain X42 (**a**) and strain G62 (**b**)

Table 2 Basic genomic features of strain X42 and strain G62

Sample name	Genome size (bp)	Scaffold no.	GC content (%)	CDS no.	tRNA no.	rRNA No.
X42	6,051,156	41	34.7	6001	99	11
G62	4,026,981	24	46.11	3965	87	9

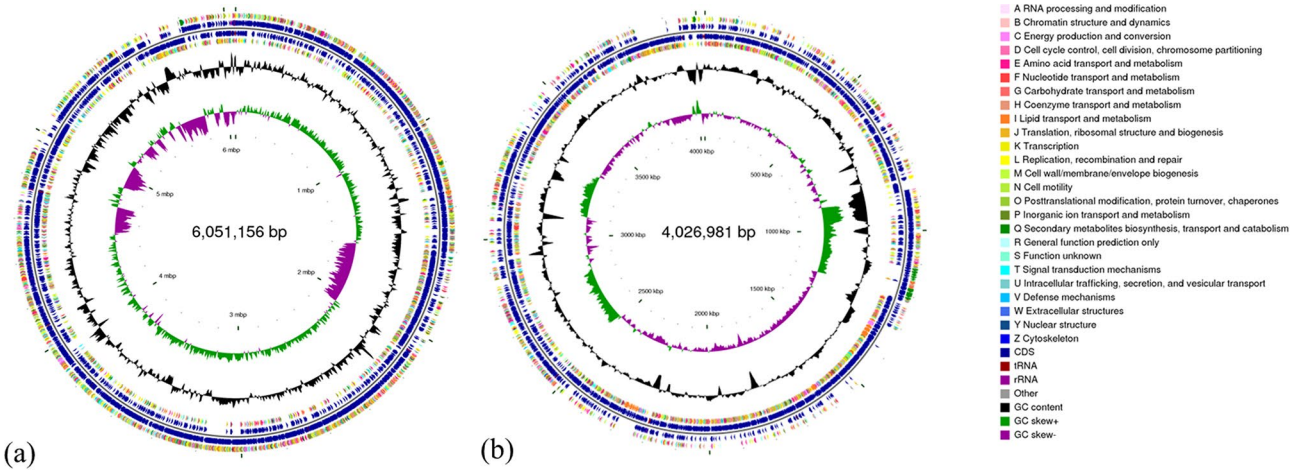

Fig. 2 Circular genome maps of strain X42 (**a**) and strain G62 (**b**)

genomic islands in strains X42 and G62, respectively. CRISPR–Cas analysis revealed that strain G62 contains two CRISPR arrays. Transposon analysis revealed that strain X42 contained 30 transposons, whereas strain G62 contained only four transposons.

Gene annotation of two phosphate-solubilizing Bacteria strains

In this study, gene annotations were obtained by comparing six major databases (NR, Swiss-Prot, Pfam, COG, GO, and KEGG). The number of genes annotated in strain X42 across these databases was 5,982, 4,275, 4,879, 4,406, 2,185, and 3,891, respectively. The percentages of these genes out of the total genes were 99.68%, 71.24%, 81.30%, 73.42%, 36.41%, and 64.84%, respectively. For strain G62, 3,959, 3,633, 3,401, 3,114, 1,765, and 2,846 genes were annotated in these databases, respectively. The percentages of these genes out of the total genes were 99.85%, 91.62%, 85.78%, 78.54%, 44.51%, and 71.78%, respectively (Table 3).

Table 3 Gene annotation statistics of strain X42 and strain G62

Sample name	Gene no.	NR	Swiss-Prot	Pfam	COG	GO	KEGG
X42	6001	5982	4275	4879	4406	2185	3891
G62	3965	3959	3633	3401	3114	1765	2846

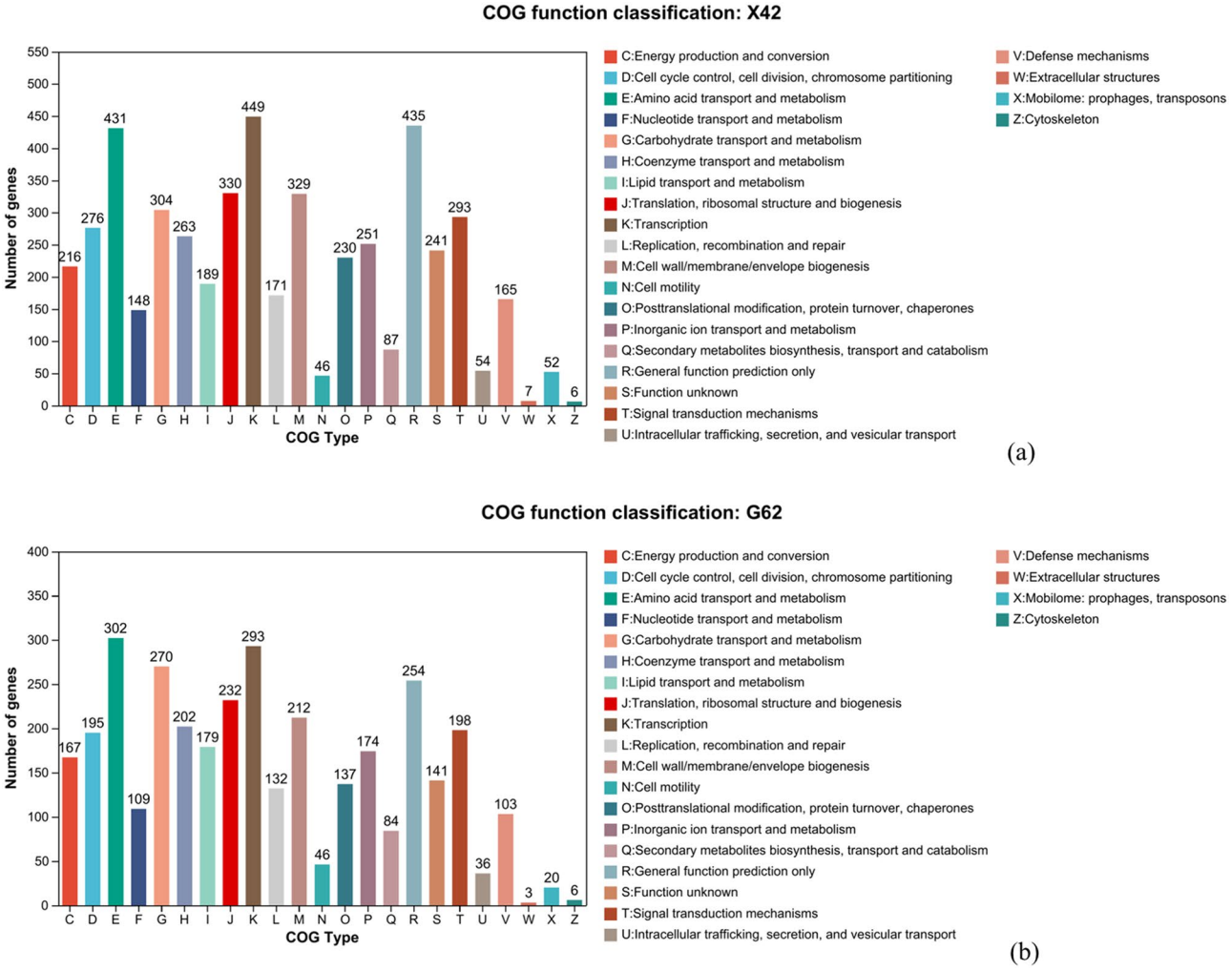

Fig. 3 COG annotation charts of strain X42 (**a**) and strain G62 (**b**)

Among the six databases, the NR database is comprehensive in its content; however, its drawback is that many entries are not experimentally validated, making it less reliable. This information can be used as a reference for other annotation results. The Swiss-Prot and Pfam databases focus on protein annotation and are suitable for studying the functional features and detailed information of proteins. Because this study focused on the functional characterization of genes in the strains, we referred primarily to the COG, GO, and KEGG databases.

As shown in Fig. 3, genes from both strains were categorized into 23 classes in the COG database. For strain X42, the category with the greatest number of genes was 'Transcription', with 449 genes, accounting for 10.19% of the COG-annotated genes. The second-highest number was for genes related to 'Amino acid transport and metabolism,' with 431 genes, accounting for 9.78% of the COG-annotated genes. The categories with the least number of genes are 'Cytoskeleton' and 'Extracellular structures,' with 0.136% and 0.159% of the COG-annotated genes, respectively.

For strain G62, the category with the greatest number of genes was 'Amino acid transport and metabolism,' with 302 genes, accounting for 9.69% of the COG-annotated genes. The second-highest number was for genes related to transcription, with 293 genes accounting for 9.41% of the COG-annotated genes. The third highest number was for genes related to carbohydrate transport and metabolism, with 270 genes, accounting for 8.67% of the COG-annotated genes. The categories with the least number

of genes are 'Cytoskeleton' and 'Extracellular structures,' with 0.193% and 0.096% of the COG-annotated genes, respectively.

This analysis provides insights into the functional distribution of genes in both strains, highlighting the predominant and least represented functions in terms of gene counts.

As shown in Fig. 4, strains X42 and G62 were categorized into three main groups within the GO database. Strain X42 had 1,282 genes related to cellular components, whereas strain G62 had 915 genes in this category.

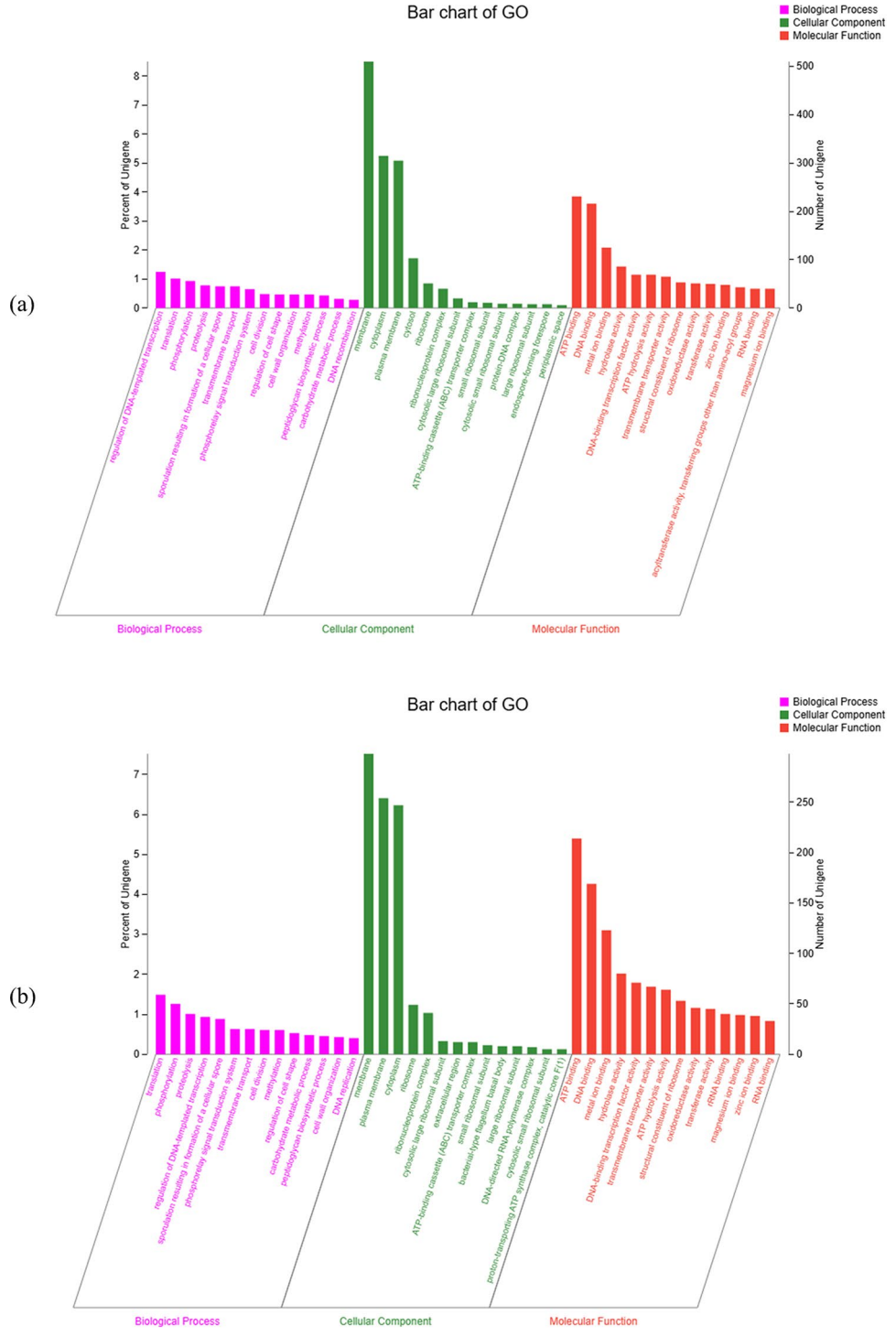

Fig. 4 GO annotation diagram of strains X42 (**a**) and G62 (**b**)

For molecular function, strain X42 had 1,607 genes, whereas strain G62 had 1,401 genes. Finally, for biological processes, strain X42 had 1,130 genes, whereas strain G62 had 953 genes.

As shown in Fig. 5, when strains X42 and G62 were compared with the KEGG database, 3,891 and 2,846 genes with functional annotations were found, respectively. In strains X42, 340, 71, 327, 165, 125, 118, 254, 94,

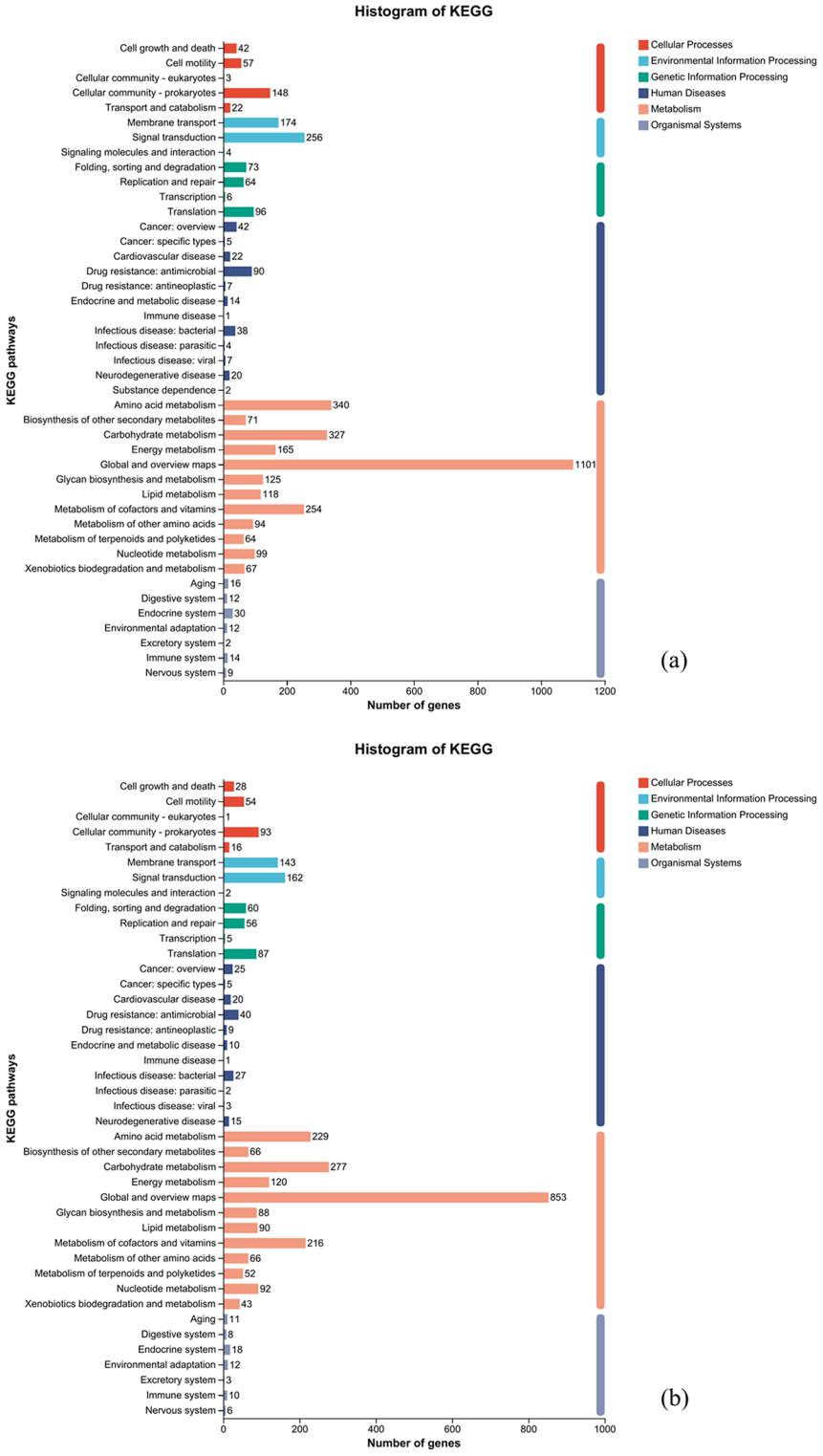

Fig. 5 KEGG annotation diagram of strains X42 (**a**) and G62 (**b**)

64, and 99, the genes were related to amino acid metabolism; the biosynthesis of other secondary metabolites; carbohydrate metabolism; energy metabolism; polysaccharide biosynthesis and metabolism; lipid metabolism; cofactor and vitamin metabolism; other amino acid metabolism; terpenoid and polyketide metabolism; nucleotide metabolism; and the degradation and metabolism of exogenous organisms, respectively. In strain G62, the numbers of genes annotated in these categories were 229, 66, 277, 120, 88, 90, 216, 66, 52, 92, and 43.

Phosphate-solubilizing genes of the two phosphate-solubilizing bacteria

This study examined the phosphate solubilization-encoding genes associated with the phosphate solubilization abilities of strains X42 and G62 through gene annotation (Table 4). On the basis of previous literature (Tang et al. 2020), genes linked to phosphorus activation were identified. A comparison with the KEGG database revealed that strain X42 possessed 31 genes related to phosphorus activation, whereas strain G62 presented 16 such genes.

Specifically, strain X42 contained five genes involved in phosphate ester mineralization (*glpA*, *glpQ*, *glpK*, *phoA*, and glycerol uptake facilitator protein). In contrast, strain G62 had seven genes involved in the same process (*glpA*, *glpQ*, *glpK*, *phoA*, *phoD*, glycerol uptake facilitator protein, and phytase 3). Both strains share common genes involved in phosphate ester transport (*glpP*, *glpT*, *pstA*, *pstB*, *pstC*, *pstS*, and inorganic phosphate transporters). Strain X42 presented additional genes related to phosphate ester transport (*phnC*, *phnD*, *phnE*, *ugpA*, *ugpB*, *ugpC, and ugpE*).

Both strains also possessed the ability to respond to phosphorus deficiency regulation through the *phoR* gene. Strain X42 contains an additional gene related to the regulation of phosphorus deficiency (*phoU*). Furthermore, strain X42 contained additional genes involved in phosphate ester mineralization and inorganic phosphate.

Plant growth indicators

Changes in plant fresh weight, dry weight, and height

As shown in Table 5, the height, aboveground fresh weight, belowground fresh weight, aboveground dry weight, and belowground dry weight of the young citrus plants inoculated with the bacterial solutions were greater than those of the control group without inoculation. Specifically, compared with the control strain, strain X42 increased these parameters by 28.07%, 17.42%, 22.38%, 28.06%, and 34.36%, respectively. Compared with the control, strain G62 improved the same parameters by 32.62%, 28.10%, 70.13%, 26.70%, and 61.63%, respectively.

Statistical analysis via IBM SPSS Statistics 26 software revealed significant differences between the experimental and control groups ($p < 0.05$).

Changes in chlorophyll and carotenoid contents in navel orange leaves

As shown in Fig. 6, inoculation with the strains increased the chlorophyll content in the leaves of the potato orange seedlings compared with that of the blank control group without inoculation. Specifically, the mean chlorophyll a content in the potted navel orange seedlings inoculated with the X42 strain was 0.98 mg/g, which was greater than the 0.83 mg/g recorded in the blank group. The mean chlorophyll b content (0.46 mg/g) was also greater than that in the blank group (0.34 mg/g), but the difference was not statistically significant. However, the total chlorophyll content (1.44 mg/g) was still notably greater than that of the blank group (1.17 mg/g).

In comparison, the potted seedlings inoculated with the G62 strain presented an even greater chlorophyll content, reaching 1.53 mg/g, which was significantly different from that of the blank group. After inoculation with the G62 strain, the chlorophyll a content (0.99 mg/g) and chlorophyll b content (0.55 mg/g) in the navel orange seedlings were both significantly greater than those in the blank group.

In terms of carotenoid content, no significant differences were noted between inoculated and noninoculated navel orange seedlings. The carotenoid content in the leaves of potted plants inoculated with the X42 strain was 0.36 mg/g, and for those inoculated with the G62 strain, it was 0.39 mg/g, whereas it was 0.35 mg/g in the blank group.

Changes in the CAT, SOD, and POD contents of navel orange leaves

Catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) are crucial redox enzymes in plant cells. These enzymes play pivotal roles in redox reactions, help maintain cellular redox homeostasis, and protect plants from oxidative stress-induced damage.

After the strains X42 and G62 were inoculated into separate pots and cultivated for nine weeks, the CAT, SOD, and POD activities of the navel orange plants were measured. As shown in Fig. 7, the CAT, SOD, and POD activities in navel orange seedlings treated with both strains were significantly greater than those in the control group. These findings indicated that both Bacillus strains increased the activities of CAT, SOD, and POD.

Specifically, strain X42 increased the CAT, SOD, and POD activities in navel orange leaves by 61.03%, 22.74%, and 53.07%, respectively. Moreover, strain G62 increased the CAT, SOD, and POD activities in navel orange leaves by 77.37%, 30.33%, and 88.79%, respectively.

For example, hydrogen peroxide (H_2O_2) is an important signaling molecule in plant development and stress responses. While H_2O_2 aids in maintaining cellular redox regulation, excessive accumulation can damage plant cells

Table 4 Phosphate solubilization-encoding genes of strains X42 and G62

Functional grouping and encoded proteins		Gene	KEGG NO. KO	Enzyme NO. EC	Sample Name
Phosphate ester mineralization					
Periplasmic glycerophosphoryl diester phosphodiesterase		*glpQ*	K01126	3.1.4.46	X42、G62
Glycerol-3-phosphate dehydrogenase		*glpA*	K00111	1.1.5.3	X42、G62
Glycerol kinase		*glpK*	K00864	2.7.1.30	X42、G62
Glycerol uptake facilitator protein			K02440		X42、G62
Alkaline phosphatase		*phoA*	K01077	3.1.3.1	X42、G62
Alkaline phosphatase D		*phoD*	K01113	3.1.3.1	G62
3-phytase			K01083	3.1.3.8	G62
Phosphonate mineralization					
Alkylphosphonate utilization operon protein PhnA		*phnA*	K06193		X42
Zinc ribbon domain-containing protein YjdM		*phnA*	K06193		X42
C-P lyase multienzyme complex/Aminoalkylphosphonate N-acetyltransferase		*phnO*	K09994	2.3.1.280	X42
2-aminoethylphosphonate-pyruvate transaminase		*phnW*	K03430	2.6.1.37	X42
Phosphonoacetaldehyde hydrolase		*phnX*	K05306	3.11.1.1	X42
Inorganic phosphorus dissolution					
Polyphosphate kinase			K01507	2.7.4.1	X42
Exopolyphosphatase		*ppx-gppA*	K01524	3.6.1.11. 3.6.1.40	X42
Phosphorus uptake					
Phosphonate transportation					
Phosphonate transport system ATP-binding protein		*phnc*	K02041	7.3.2.2	X42
Phosphonate transport system substrate-binding protein		*phnD*	K02044		X42
Phosphonate transport system permease protein		*phnE*	K02042		X42
Sn-glycerol 3-phosphate transport system permease protein		*ugpA*	K05814		X42
Sn-glycerol 3-phosphate transport system substrate-binding protein		*ugpB*	K05813		X42
Sn-glycerol 3-phosphate transport system ATP-binding protein		*ugpC*	K05816	7.6.2.10	X42
Sn-glycerol 3-phosphate transport system permease protein		*ugpE*	K05815		X42
Glyceruptake operon antiterminator		*glpP*	K02443		X42、G62
Glycerol-3-phosphate transporte		*glpT*	K02445		X42、G62
Phosphate transport system permease protein		*pstA*	K02038		X42、G62
Phosphate transport system ATP-binding protein		*pstB*	K02036	7.3.2.1	X42、G62
Phosphate transport system permease protein		*pstC*	K02037		X42、G62
Phosphate transport system substrate-binding protein		*pstS*	K02040		X42、G62
Inorganic phosphate transporter			K03306		X42、G62
P-starvation response regulation					
Phosphate regulon sensor histidine kinase	Sensory box histidine kinase PhoR	*phoR*	K07636	2.7.13.3	X42
	HAMP domain-containing sensor histidine kinase	*phoR*	K07636	2.7.13.3	G62
	ATP-binding protein	*phoR*	K07636	2.7.13.3	X42、G62
	Envelope stress sensor histidine kinase HitS	*phoR*	K07636	2.7.13.3	X42
	Two component system histidine kinase	*phoR*	K07636	2.7.13.3	X42
	Phosphate signaling complex protein PhoU	*phoU*	K02039		X42

Table 5 Plant growth indicators of young Citrus plants inoculated with strain X42 or strain G62

Sample name	**Plant height (cm)**		
	Mean ± standard deviation	**Significance**	**Increase**
CK	46.17 ± 7.37	-	-
X42	59.13 ± 3.01	0.024	28.07%
G62	61.23 ± 0.37	0.025	32.62%
Sample name	**Fresh weight aboveground (g)**		
	Mean ± standard deviation	**Significance**	**Increase**
CK	42.70 ± 3.04	-	-
X42	50.14 ± 1.40	0.018	17.42%
G62	54.70 ± 3.64	0.012	32.62%
Sample name	**Fresh weight belowground (g)**		
	Mean ± standard deviation	**Significance**	**Increase**
CK	23.37 ± 2.48	-	-
X42	28.60 ± 1.29	0.032	22.38%
G62	39.76 ± 2.53	0.001	70.13%
Sample name	**Dry weight aboveground (g)**		
	Mean ± standard deviation	Significance	Increase
CK	16.18 ± 2.09	-	-
X42	20.72 ± 1.84	0.048	28.06%
G62	20.50 ± 1.14	0.035	26.70%
Sample name	**Dry weight belowground (g)**		
	Mean ± standard deviation	Significance	Increase
CK	11.00 ± 0.82	-	-
X42	14.78 ± 1.46	0.017	34.36%
G62	17.58 ± 3.47	0.033	61.63%

(Mhamdi et al. 2010). CAT neutralizes oxidative threats by converting H_2O_2 produced during photosynthesis into harmless water and oxygen (Hackenberg et al. 2011). Moreover, CAT plays a role in plant immunity; plants can regulate CAT activity to accumulate ROS, thereby activating programmed cell death (PCD) to defend against pathogen invasion or directly kill pathogens via accumulated ROS (Gao et al. 2018; Liu et al. 2023). Additionally, CAT participates in nonbiotic stress responses, hormone signal transduction, and the metabolism of reactive nitrogen species within the plant (Liu et al. 2023)Peroxidase (POD) is an oxidoreductase enzyme distributed throughout plant organs that reacts with H_2O_2 to produce oxidized products and water, thus eliminating excess H_2O_2 in plants (Lv et al. 2024). POD exhibits antioxidant defense properties, clearing out oxidative stress-inducing substances within cells and maintaining the cellular redox balance (Zhan et al. 2003). Furthermore, POD contributes to the lignification of plant cell walls, the synthesis of pigments, and the response to adverse conditions (Ma et al. 2012). Superoxide dismutase (SOD) exists in various forms, with different types of SOD collectively catalyzing the dismutation of ROS (Wei et al. 2020). Excessive ROS levels can lead to membrane damage, altered gene expression, damage to photosynthetic proteins, and chlorophyll degradation (Sarkar et al. 2015). Additionally,

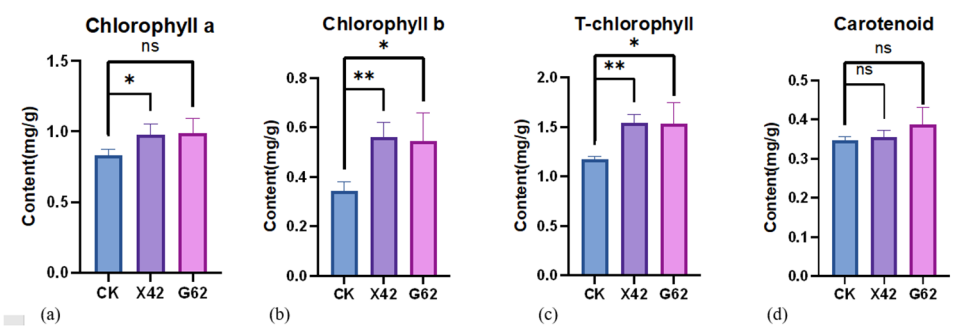

Fig. 6 Chlorophyll and carotenoid contents in citrus leaves

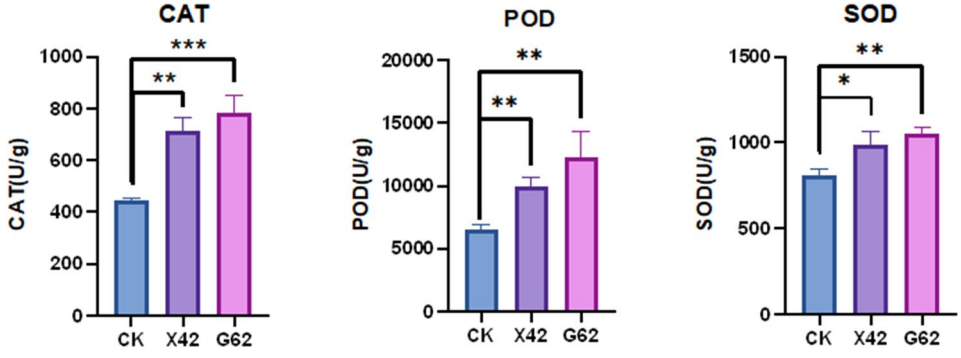

Fig. 7 Changes in CAT, SOD, and POD activities in plants

SOD plays a significant role in the response to nonbiotic stress. Studies have shown that SOD activity is positively correlated with salt concentration under static experimental conditions, indicating that SOD helps plants cope with salt stress (Sekmen et al. 2012; Minxuan et al. 2015). In addition to assisting with salt stress, evidence suggests that SOD can alleviate stress caused by drought and temperature changes (As et al. 1993; Wang et al. 2009; Kim et al. 2010; Qing-Chen et al. 2013). Therefore, the determination of CAT, SOD, and POD activities can help determine whether the inoculated strains promote the growth of navel oranges.

Discussion and conclusion

The genus *Bacillus* represents a common group of spore-forming bacteria that occupy significant ecological niches in the soil environment. It is estimated that *Bacillus* species account for approximately 5–10% of the bacterial population in the soil. The ability of these bacteria to form spores allows them to enter a dormant state under harsh conditions, such as nutrient or water scarcity, ensuring their long-term survival and widespread distribution in soil ecosystems. Research has shown that *Bacillus spp.* can increase crop performance in the field (Hossain et al. 2015). In this study, we isolated highly efficient phosphate-solubilizing strains from the rhizospheres of citrus fruits. Through quantitative screening of their phosphate solubilization ability, two strains with notable phosphate solubilization abilities were identified. Whole-genome sequencing of these strains was performed to further understand their genetic makeup. Understanding the function of genes involved in phosphate solubilization is crucial for elucidating how microorganisms convert insoluble phosphorus in the soil into forms that can be absorbed by plants. Phosphate-solubilizing genes encode proteins, such as phosphatases and enzymes involved in the synthesis of organic acids, aiding in the breakdown of diverse phosphorus sources (Rodríguez et al. 2006).

Our results showed that strains X42 and G62 were capable of solubilizing phosphate. Whole-genome sequencing revealed that X42 contained genes related to the mineralization of phosphonates and phosphates, solubilization of inorganic phosphates, transport of phosphonates, and regulation of phosphorus deficiency responses. In contrast, strain G62 harbors genes involved in the mineralization of phosphonates, transport of phosphonates, and regulation of phosphorus deficiency responses, potentially limiting its ability to utilize additional organic phosphorus sources.

Catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) are critical antioxidant enzymes found in plant cells. Collectively, these enzymes form part of the plant's antioxidant defense system, helping the plant resist oxidative stress caused by environmental pressures, such as UV radiation, pollutants, and drought. By scavenging reactive oxygen species (ROS), these enzymes maintain the cellular redox balance, which is essential for healthy plant growth. In pot trials, X42 and G62 promoted the growth of citrus seedlings. Compared with those in the control group, the citrus seedlings in the experimental group presented greater growth in terms of height, fresh weight, and dry weight. Significant differences in enzyme activity were detected between the experimental and control groups. Our findings indicate that strains X42 and G62 increase the activities of CAT, POD, and SOD in citrus seedlings, thereby alleviating the various environmental stresses experienced by these plants.

This study confirmed the effectiveness of strains X42 and G62 in improving the growth of citrus seedlings and enhancing their stress resistance. These strains increase the activity of antioxidant enzymes, assisting plants in coping with diverse environmental stresses and thus promoting health and growth. These findings provide theoretical support for the use of *Bacillus* strains to improve crops and lay the groundwork for future research.

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Author contributions
Wenyuan Zhao led the implementation of most of the experiments, conducted the data analysis, and drafted the initial manuscript. Haojie Cao was responsible for the experimental design and provided technical support. Tao Peng assisted with the experiments and participated in the data analysis. Huimin Huang contributed to data interpretation and offered critical feedback on the manuscript. Shuijing Yu, as the corresponding author, supervised the entire project, designed the experiments, and revised the manuscript.

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Data availability
The research data supporting this publication are available through the data platform of Majorbio Biopharm Technology Co., Ltd. at https://www.majorbio.com/. All other relevant data supporting the findings of this study are available from the corresponding author upon reasonable request.

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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