AMB Express

ORIGINAL ARTICLE Open Access

Genome-based identification of phosphate-solubilizing capacities of soil bacterial isolates

Xiaoqing Chen[1,2], Yiting Zhao[1], Shasha Huang[1], Josep Peñuelas[3,4], Jordi Sardans[3,4], Lei Wang[1] and Bangxiao Zheng[1,2*]

Abstract
Identifying genomic markers for phosphate-solubilizing bacteria (PSB) is vital for advancing agricultural sustainability. This study utilizes whole-genome sequencing and comprehensive bioinformatics analysis, examining the genomes of 76 PSB strains with the aid of specialized genomic databases and analytical tools. We have identified the *pqq* gene cluster, particularly the *pqqC* gene, as a key marker for (P) solubilization capabilities. The *pqqC* gene encodes an enzyme that catalyzes the conversion of precursors to 2-keto-D-gluconic acid, which significantly enhances P solubilization in soil. This gene's importance lies not only in its biochemical function but also in its prevalence and effectiveness across various PSB strains, distinguishing it from other potential markers. Our study focuses on *Burkholderia cepacia* 51-Y1415, known for its potent solubilization activity, and demonstrates a direct correlation between the abundance of the *pqqC* gene, the quantitative release of P, and the production of 2-keto-D-gluconic acid over a standard 144-h cultivation period under standardized conditions. This research not only underscores the role of the *pqqC* gene as a universal marker for the rapid screening and functional annotation of PSB strains but also highlights its implications for enhancing soil fertility and crop yields, thereby contributing to more sustainable agricultural practices. Our findings provide a foundation for future research aimed at developing targeted strategies to optimize phosphate solubilization, suggesting areas for further investigation such as the integration of these genomic insights into practical agricultural applications to maximize the effectiveness of PSB strains in real-world soil environments.

Keywords Phosphate-solubilizing bacteria, *Burkholderia cepacia*, *Pqq* gene cluster, Genome sequence

Introduction

Phosphorus (P) is a critical macronutrient, essential for various physiological processes in plants including photosynthesis, energy conversion, and reproduction (Shen et al. 2011). Despite its abundance in soil, the bioavailable form of P is limited, leading to extensive use of phosphate fertilizers to enhance crop yields (Lekberg et al. 2021; Peñuelas et al. 2013). However, the inefficiency of these fertilizers, due to a substantial portion of P being bound in an insoluble form, poses economic and environmental challenges (Qiao et al. 2013; Peñuelas et al. 2020). These challenges include soil acidification, eutrophication, and depletion of high-quality P resources, accentuating the

*Correspondence:
Bangxiao Zheng
bangxiao.zheng@xmut.edu.cn
[1]School of Environmental Science and Engineering, Xiamen University of Technology, Xiamen 361024, People's Republic of China
[2]Xiamen Key Laboratory of Membrane Research and Application, Xiamen 361024, People's Republic of China
[3]CSIC, Global Ecology Unit CREAF-CSIC-UAB, Catalonia 08193 Bellaterra, Spain
[4]CREAF, 08193 Cerdanyola del Vallès, Catalonia, Spain

need for sustainable alternatives (Harvey et al. 2009). The role of phosphate-solubilizing bacteria (PSB) in converting insoluble P into forms accessible to plants offers a promising solution (Khan et al. 2009; Sackett et al. 1908). PSBs facilitate the solubilization process through the secretion of organic anions such as acetate, lactate, and gluconate, and by the activity of enzymes like acid phosphatases and phytases (Liu et al. 2022), targeting both inorganic and organic P compounds for solubilization and mineralization (Kour et al. 2021; Yu et al. 2022).

Central to the mechanism of P solubilization is the production of gluconic acid (GA) from glucose, a process significantly influenced by the gene *pqq* encoding the cofactor pyrroloquinoline quinone (PQQ) (Bhanja et al. 2021). PQQ plays a pivotal role in the direct oxidation pathway of glucose to GA, particularly under aerobic conditions or when substrate availability is high (Wagh et al. 2016). This metabolic pathway not only contributes to P solubilization but also provides a competitive advantage by limiting glucose availability to other microorganisms (An and Moe 2016; Cheng et al. 2023). Besides, PQQ serves as a cofactor for glucose dehydrogenase (GDH), which catalyzes the oxidation of glucose to gluconic acid (Wagh et al. 2016). This reaction is crucial for phosphate solubilization, as gluconic acid lowers the pH and chelates cations bound to phosphate, thereby increasing its availability to plants. Despite the absence of a direct linkage between *pqq* gene presence and P solubilization capabilities, PQQ is recognized as a key gene in the inorganic P solubilization pathway (Joshi et al. 2023). The complexity of the *pqq* gene family, including variations in gene clusters and synteny among different bacterial species, underscores the intricacies and difficulties involved in accurately predicting a bacterium's P-solubilization potential based solely on genome analysis (Wu et al. 2022).

The exploration of PSB mechanisms through genomics has been a focal point of recent studies (Li et al. 2023; Wu et al. 2022; Zeng et al. 2022), yet the identification of key genes associated with efficient P solubilization remains a challenge. This is attributed to the vast array of genes involved, especially within the *pqq* gene family, which complicates the rapid assessment of a newly isolated strain's potential for effective P solubilization. Our study aims to bridge this gap by establishing a correlation between the presence of specific genes and the ability to solubilize inorganic P efficiently by studying them at once. By analyzing the genomes of 76 known PSB strains (reported in Zheng et al. 2018) and conducting a comparative genomic study on a highly efficient PSB strain, *Burkholderia cepacia* 51-Y1415, we seek to develop a generalized approach to predict a strain's P-solubilizing capacity based on its *pqq* gene profile. This approach not only contributes to the understanding of microbial P solubilization mechanisms but also enhances the potential for utilizing PSBs in sustainable agriculture practices.

Materials and methods

Biochemical characterization and gene identification

The 76 phosphate-solubilizing bacteria (PSBs) were initially isolated from agricultural field soils near Hailun in Heilongjiang Province (47′ 26″ N, 126′ 38″ E) and Yingtan in Jiangxi Province (28′ 14″ N, 116′ 54″ E), China (Zheng et al. 2018). To evaluate the phosphate-solubilizing capabilities of the 76 soil bacterial isolates, the pH and soluble phosphate concentration were measured post-incubation. Each isolate was cultivated in 50 mL of the modified PVK medium at 30 °C for 72 h. After cultivation, supernatants were harvested via centrifugation at 4200 g for 10 min. The pH was assessed using an XL60 pH meter (Fisher Scientific, USA), and phosphate concentrations were quantified using the molybdate-blue method (Olsen et al. 1954). The presence of various *pqq* gene clusters within these isolates was also examined through quantitative PCR (qPCR), details of which are provided in the Supplementary Materials and Methods.

Universal primers targeting five distinct *pqq* gene clusters were used (Table S1, referenced from An and Moe 2016; Meyer et al. 2011; Zheng et al. 2017). qPCR assays were prepared with SYBR premix Ex Taq, BSA, and respective primers, and conducted on a LightCycler 480 System (Roche, Basel, Switzerland) following a protocol of initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The specificity of reactions was confirmed via melting-curve analysis. Although the primers were designed based on conserved regions across species, it is important to note that not all species may possess the targeted gene fragments, and as such, not all might be detectable by qPCR. The presence of *pqq* genes in this study was determined based on a threshold of $C_T < 30$, to ensure a more tolerant detection rate for qPCR assays.

The PSB strains were isolated from agricultural field soils with varying phosphate levels in Hailun, Heilongjiang, and Yingtan, Jiangxi, China. Strains were selected based on their phosphate-solubilizing activity, determined by the molybdate-blue method, and their ability to decrease the pH of the medium. While these criteria aimed to capture a diverse range of phosphate-solubilizing capabilities, they may introduce biases by favoring strains adapted to specific soil conditions.

Genome sequencing and annotation of *Burkholderia cepacian* 51-Y1415

Among the 73 strains assessed for phosphate-solubilizing capabilities, *Burkholderia cepacia* 51-Y1415 (CCTCC AB 2017151) was identified as one of the strains exhibiting

a high phosphate-solubilizing capacity. Significantly, similar to classical P-solubilizing *Pseudomonas* strains (Meyer et al. 2011), it possesses a complete set of five *pqq* gene clusters. This feature makes *Burkholderia cepacia* 51-Y1415 an ideal candidate for detailed genomic analysis to explore the genetic basis of its phosphate-solubilizing ability. Therefore, this strain was subjected to whole-genome sequencing and comparative genomic analysis, serving as a representative example to investigate the role of *pqq* genes in the phosphate-solubilization process.

Detailed protocols for DNA extraction, sequencing, and assembly, as well as comparative genomic analyses, are available in the Supplementary Materials and Methods. Briefly, the genome of *Burkholderia cepacia* 51-Y1415 was sequenced to provide insights into its phosphate-solubilizing genetic mechanisms. Following 24-h incubation in PVK medium at 30 °C, genomic DNA was extracted and sequenced using the Illumina HiSeq 2000 platform. Assembly was performed with the SOAPdenovo v2.01 software, producing 233 contigs across 229 scaffolds. Gene prediction was conducted using GeneMarkS v 4.28, with coding DNA sequences (CDSs) annotated through submission to various databases including GenBank and KEGG for functional gene analysis. The assembled genome sequence and associated data were deposited in DDBJ/ENA/GenBank and the Sequence Read Archive, respectively. For the comparative genomic analysis, *Burkholderia cepacia* 1178_BCEN, *B. ubonensis* MSMB1138, *B. ambifaria* FDAARGOS_419, and other 7 *Burkholderia* strains were selected based on phylogenetic proximity and known P-solubilizing capabilities (Deng et al. 2016; Riera et al. 2017). Besides, digital DNA–DNA hybridization (dDDH) values were estimated using the Genome–Genome Distance Calculator 2.1 (GGDC) to assess genomic relatedness among the strains (Meier-Kolthoff et al. 2013, 2014). Orthologous genes were identified using OrthoFinder, which facilitated the identification of shared and unique genes related to P solubilization (Emms and Kelly 2015).

Statistical analyses

Statistical analyses were conducted to establish correlations between genetic profiles and phosphate-solubilization capabilities of the studied bacterial strains. Sequence alignments and phylogenetic trees were generated using Clustal X 2.0 (Larkin et al. 2007) and MEGA 6.0 (Tamura et al. 2013). The statistical significance of differences in gene presence and phosphate solubilization efficiencies were determined through variance analyses (ANOVAs) performed with IBM SPSS Statistics 21. The specificity of PCR products was verified by melting-curve analysis, and changes in *pqq* gene expression were quantified using the $2^{-\Delta\Delta CT}$ method, with LB medium conditions serving as the control (Livak and Schmittgen 2001). Each experiment was replicated thrice to ensure the reliability of the results.

Results

The relationship between *pqq* genes and bacterial phosphate-solubilizing capabilities

Analysis of 76 phosphate-solubilizing bacteria (PSB) strains demonstrated a clear link between phosphate solubilization abilities and the presence of *pqq* gene clusters, which correlated with decreased medium pH levels (Table 1). Strains such as *Bacillus megaterium* exhibited high phosphate-solubilizing capacities (96.32±27.05 ug mL^{-1}) with medium pH values ranging from 4.2 to 5.2. These strains almost invariably possessed a comprehensive set of *pqqABCD* genes. In contrast, strains with lower solubilization capabilities, including *Arthrobacter oxydans* 08-OY02 and *Rhodanobacter* sp. 21-Y7, displayed incomplete *pqq* gene clusters, which coincided with their higher medium pH levels. This pattern underscores the potential role of the *pqq* gene cluster in enhancing the biochemical processes that reduce pH and increase phosphate solubilization.

Genetic insights from *Burkholderia Cepacia* 51-Y1415

Further investigation into the genus *Burkholderia* revealed that, similar to classical *Pseudomonas* strains, *Burkholderia* spp. also harbored complete *pqqABCDE* gene clusters. A prime example is *Burkholderia cepacia* 51-Y1415, which demonstrated high phosphate-solubilizing capability (92.03 ug mL^{-1}) and a medium pH of 5.00. This strain was selected for whole-genome sequencing, providing comprehensive insights into the genetic basis of its solubilization capacity (comprehensive details on the whole-genome sequencing, analysis, functional annotation, and exploration of *pqq* genes for strain 51-Y1415 providing in-depth insights into the genetic basis of its solubilization capacity refer to the Supplementary Materials and Methods and Supplementary Results, Table S2–S4, Figures S1–S5). The presence of *pqq* gene clusters was identified and compared with *Pseudomonas kilonensis* and ten other *Burkholderia* strains (Figure S6) to analyze the conserved nature of *pqq* genes across the genus (Fig. 1). Comparisons of *pqq* gene clusters across eleven *Burkholderia* strains highlighted the conservation of these genes within the genus. However, it was noted that the presence of complete *pqq* gene clusters is not universally characteristic across all strains, as evidenced by strains like *B. phytofirmans* 56-OY3 (P release of 3.85 ug mL^{-1}, Table 1), which lacked a complete *pqq* gene cluster and showed low P-solubilization activity.

Strain 51-Y1415 distinguishes itself by possessing a complete series of enzymes essential for the direct oxidative pathway of glucose, allowing for efficient conversion

Table 1 Biochemical characterization and *pqq* gene identification of 76 PSB strains

Strain	Medium pH	*P* concentration (ug mL^{-1})	Presence of pqq genes				
			pqqA	pqqB	pqqC	pqqD	pqqE
Bacillus megaterium 01-A3	4.80	85.57	+	+	+	+	
Bacillus megaterium 02-A7	4.59	89.08	+	+	+	+	
Pseudomonas frederiksbergensis 03-D2	5.21	64.28	+	+	+	+	+
Rhodococcus opacus 04-OD7	5.17	28.06		+		+	
Arthrobacter phenanthrenivorans 05-OD11	5.89	12.24		+			
Arthrobacter defluvii 06-OD12	8.34	59.11		+			
Arthrobacter chlorophenolicus 07-OD13	5.58	20.84		+			
Arthrobacter oxydans 08-OY2	6.64	3.85					
Arthrobacter sp. 09-OY5	5.11	43.00		+			
Bacillus megaterium 10-Y11	4.77	106.46	+	+	+	+	
Pseudomonas frederiksbergensis 11-D3	5.25	81.76	+	+	+	+	+
Massilia putida 12-OD1	4.63	97.29	+	+	+	+	+
Duganella sp. 13-D4	5.69	10.78					+
Bacillus megaterium 14-Y2	4.75	101.58	+	+	+	+	
Pseudoduganella sp. 15-Y6	5.29	49.64	+			+	
Bacillus megaterium 16-Y9	4.66	80.20	+	+	+	+	
Bacillus megaterium 17-Y5	4.85	80.39	+	+	+	+	
Variovorax paradoxus 19-D4	5.42	55.69		+	+	+	
Rhizobium leguminosarum 20-OD2	5.69	10.78		+			+
Rhodanobacter sp. 21-Y7	7.72	2.58		+			
Bacillus megaterium 22-A1	5.00	100.51	+	+	+	+	
Pseudomonas frederiksbergensis 23-D2	5.20	63.41	+	+	+	+	+
Bacillus megaterium 24-Y916	4.79	109.39	+	+	+	+	
Rhodanobacter sp. 25-Y8	4.82	18.20		+			+
Bacillus megaterium 26-Y91	4.63	46.61	+	+	+	+	
Bacillus megaterium 27-Y93	4.37	117.30	+	+	+	+	
Bacillus megaterium 28-Y911	4.54	126.48	+	+	+	+	
Bacillus megaterium 29-Y924	4.55	136.83	+	+	+	+	
Bacillus megaterium 30-Y1411	4.48	134.39	+	+	+	+	
Bacillus megaterium 31-Y142	4.71	97.29	+	+	+	+	
Arthrobacter sp. 32-OD9	5.31	43.19		+			
Streptomyces tumescens 33-X1	8.02	2.77	+				
Streptomyces prasinopilosus 34-Y1	7.75	3.07	+				
Streptomyces rishiriensis 35-Y3	5.76	44.37	+				
Kurthia zopfii 36-Y7	4.52	81.57		+	+	+	
Rhodanobacter sp. 37-Y8	4.88	32.55		+		+	
Bacillus megaterium 38-Y92	4.51	91.04	+	+	+	+	
Bacillus megaterium 39-Y94	4.43	91.62	+	+	+	+	
Bacillus megaterium 40-Y95	4.44	134.49	+	+	+	+	
Bacillus megaterium 41-Y99	4.41	159.48	+	+	+	+	
Bacillus megaterium 42-Y910	4.58	75.22	+	+	+	+	
Bacillus megaterium 43-Y912	4.58	72.39	+	+	+	+	
Bacillus megaterium 44-Y913	4.50	46.51	+	+	+	+	
Bacillus megaterium 45-Y914	4.65	94.26	+	+	+	+	
Bacillus megaterium 46-Y923	4.62	81.57	+	+	+	+	
Bacillus megaterium 47-Y141	4.62	70.73	+	+	+	+	
Rhizobium sp. 48-Y930	7.86	3.75	+				
Bacillus megaterium 49-Y1412	4.60	138.68	+	+	+	+	
Rhizobium sp. 50-Y1414	8.02	5.60	+				
Burkholderia cepacia 51-Y1415	5.00	92.03	+	+	+	+	+
Arthrobacter defluvii 52-OD12	4.59	76.10		+			
Bacillus acidiceler 53-Q11	4.39	127.07	+	+	+	+	

Table 1 (continued)

Strain	Medium pH	*P* concentration (ug mL^{-1})	Presence of pqq genes				
			pqqA	pqqB	pqqC	pqqD	pqqE
Streptomyces prasinopilosus 54-Y1	5.29	49.64	+				
Pseudomonas frederiksbergensis 55-D3	4.96	35.87	+	+	+	+	+
Burkholderia phytofirmans 56-OY3	8.20	3.85	+				+
Variovorax paradoxus 57-Y925	5.30	10.88					+
Telluria mixta 58-Y97	4.62	106.85		+	+	+	
Sphingomonas koreensis 59-Y96	7.31	2.77					
Streptomyces flaveolus 60-OD3	7.95	2.19	+				
Rhodanobacter sp. 61-Y8	4.49	62.23		+	+		+
Streptomyces sp. 62-Y930	6.50	3.46	+				
Rhodococcus cercidiphylli 63-OD5	6.77	3.07		+			
Bacillus megaterium 64-Y98	4.53	107.44	+	+	+	+	
Bacillus megaterium 65-Y918	4.71	69.75	+	+	+	+	
Bacillus megaterium 66-Y143	4.55	82.84	+	+	+	+	
Rhodococcus sp. 67-OD10	5.45	52.67			+	+	
Arthrobacter oxydans 68-OY1	6.15	16.44					
Pseudomonas sp. 69-Y94	4.87	71.51	+	+	+	+	+
Bacillus megaterium 70-Y917	4.43	76.10	+	+	+	+	
Pseudomonas sp. 71-Y928	5.41	37.82	+	+	+	+	+
Bacillus megaterium 72-Y13	4.61	112.03	+	+	+	+	
Bacillus megaterium 73-Y142	4.77	106.46	+	+	+	+	
Streptomyces sp. 74-Y144	5.00	22.50	+				
Leifsonia shinshuensis 75-Y145	4.54	27.08	+				
Bacillus megaterium 76-Y149	4.78	59.70	+	+	+	+	
Streptomyces sp. 77-Y1410	5.25	34.41	+				

"+" indicates the presence of *pqq* genes by qPCR assays based on a threshold of $C_T < 30$

to gluconate and gluconate-6-phosphate (Figures S7 and S8). During the 144-h cultivation period, strain 51-Y1415 demonstrated the crucial role of 2-keto-D-gluconic acid in phosphate solubilization. The concentration of this metabolite increased alongside enhanced phosphate release and was significantly correlated with the abundance and expression levels of the *pqqC* gene and the entire *pqqABCDE* cluster (Fig. 2). Despite the presence of other various organic acids (lactic acid, acetic acid, succinic acid, oxalic acid and citric acid), only 2-keto-D-gluconic acid demonstrated a significant positive correlation with P release in the medium ($P<0.05$, Table 2). The secretion of 2-keto-D-gluconic acid was also significantly correlated with the expression levels of the entire *pqqABCDE* gene cluster ($P<0.01$, Table 2), confirming the indispensability of *pqq* genes in the solubilization mechanism.

Discussions

To elucidate the role of *pqq* gene clusters in bacterial phosphate-solubilizing capabilities, our examination reveals that these clusters are pivotal, not merely for their functional attributes but also for the regulatory mechanisms they impose on environmental pH modifications. Central to this discussion, the correlation between the integrity of *pqq* gene clusters and phosphate solubilization efficiency has been substantiated by comparing strains such as *Bacillus megaterium* and *Arthrobacter oxydans*. Notably, *Bacillus megaterium* strains, characterized by a robust assembly of *pqqABCD* genes, exhibit significantly enhanced phosphate solubilization, typically accompanied by acidification of their milieu (An and Moe 2016; Miller et al. 2010). Contrastingly, strains like *Arthrobacter oxydans*, which possess fragmented *pqq* gene clusters, show a concomitant rise in medium pH, underscoring a diminished solubilizing activity (Magnusson et al. 2004). This dichotomy not only highlights the functional imperative of these genes but also emphasizes their role in ecological adaptations, mediating microbial interactions with their abiotic environment (Cheng et al. 2023; Zheng et al. 2019).

Further stratification within the genus *Burkholderia* illustrates this concept robustly. For instance, *Burkholderia cepacia*, distinguished by its complete *pqqABCDE* gene assembly, showcases significant phosphate-solubilization capacities. This phenotype is mirrored across strains within the genus that share a similar genetic architecture, suggesting a conserved evolutionary strategy aimed at optimizing nutrient acquisition (Rodríguez and Fraga 1999; Sashidhar and Podile 2010). The synthesis and regulatory functions of the PQQ (pyrroloquinoline quinone) cofactor, facilitated by the orchestration of

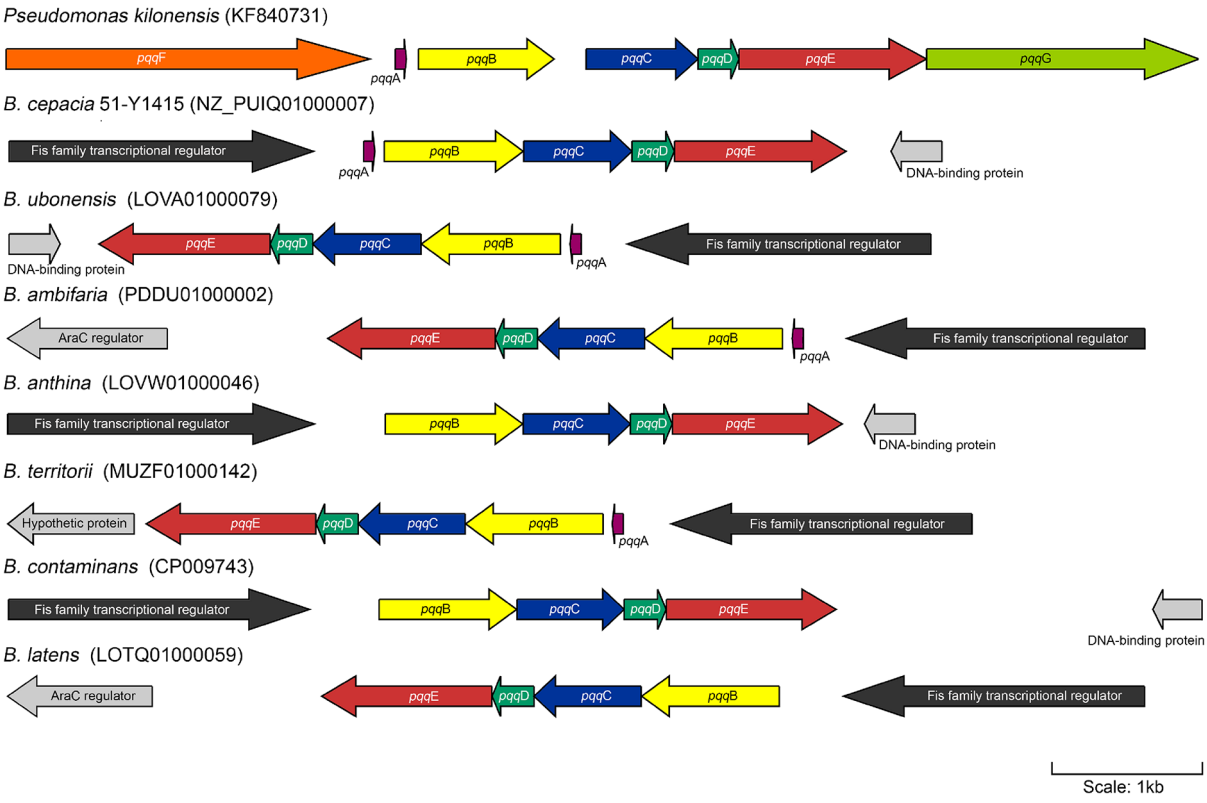

Fig. 1 The genetic structures of the*pqq*operon in*Burkholderia cepacia*51-Y1415 and other strains, showing the conserved nature of these genes across different species.Genetic structures of *pqq* operon of *Burkholderia cepacian* 51-Y1415, *B. ubonensis*, *B. ambifaria*, *B. anthina*, *B. territorii*, *B. contaminans* and *B. latens* with a *Pesudomonas* species as reference. The location and polarity of genes are showed with arrows

pqqD/*pqqE*, *pqqB*, and *pqqC*, underline a complex biochemical pathway critical for effective phosphate solubilization (Fig. 2, Latham et al. 2015; Magnusson et al. 2004; Velterop et al. 1995). These findings not only refine our understanding of the biochemical pathways involved but also highlight the ecological significance of these gene clusters in microbial population dynamics and nutrient cycling.

This nuanced comprehension extends beyond the operational mechanics to suggest that the presence and configuration of *pqq* genes are indicative of a broader ecological strategy, enabling certain microbial taxa to thrive under nutrient-limited conditions. The ecological significance of pqq gene clusters extends beyond individual strains, influencing microbial community dynamics and nutrient cycling. The disparate presence of these genes across different strains further illustrates the evolutionary plasticity and ecological ramifications of phosphate solubilization capabilities within microbial communities. These insights, supported by comprehensive genomic data available in the Supplementary materials, offer profound implications for microbial ecology and the management of biogeochemical cycles.

The pivotal role of the *pqq* gene cluster in enhancing phosphate solubilization efficiency emerges distinctly in our findings, particularly illustrated by strain 51-Y1415. The presence of a complete *pqqABCDE* gene cluster underpins the biosynthesis of the cofactor PQQ, which is crucial for the activity of PQQ-dependent glucose dehydrogenase (PQQ-GDH) (Fig. 3). This enzyme facilitates the transformation of glucose into gluconic acid, a key step in the direct oxidative pathway of glucose metabolism leading to enhanced phosphate solubilization. This pathway, predominant in strain 51-Y1415 and more effective than the alternative Entner-Doudoroff pathway, exemplifies the biochemical precision with which these bacteria adapt to phosphorus-limited environments (Holden et al. 2004; Nierman et al. 2004).

The role of 2-keto-D-gluconic acid during a 144-h cultivation period further accentuates the efficacy of this metabolic pathway. This acid, significantly correlated with phosphate release, marks a critical phase in P solubilization, overshadowing the contribution of other organic acids detected. The robust expression of the *pqqABCDE* cluster, particularly *pqqC*, during this period underscores the genetic basis for this enhanced solubilization capacity. Notably, *pqqC*'s function as an oxidase in the redox reactions vital for effective phosphate solubilization highlights its indispensability in this biochemical process (An and Moe 2016; Zheng et al. 2018, 2019).

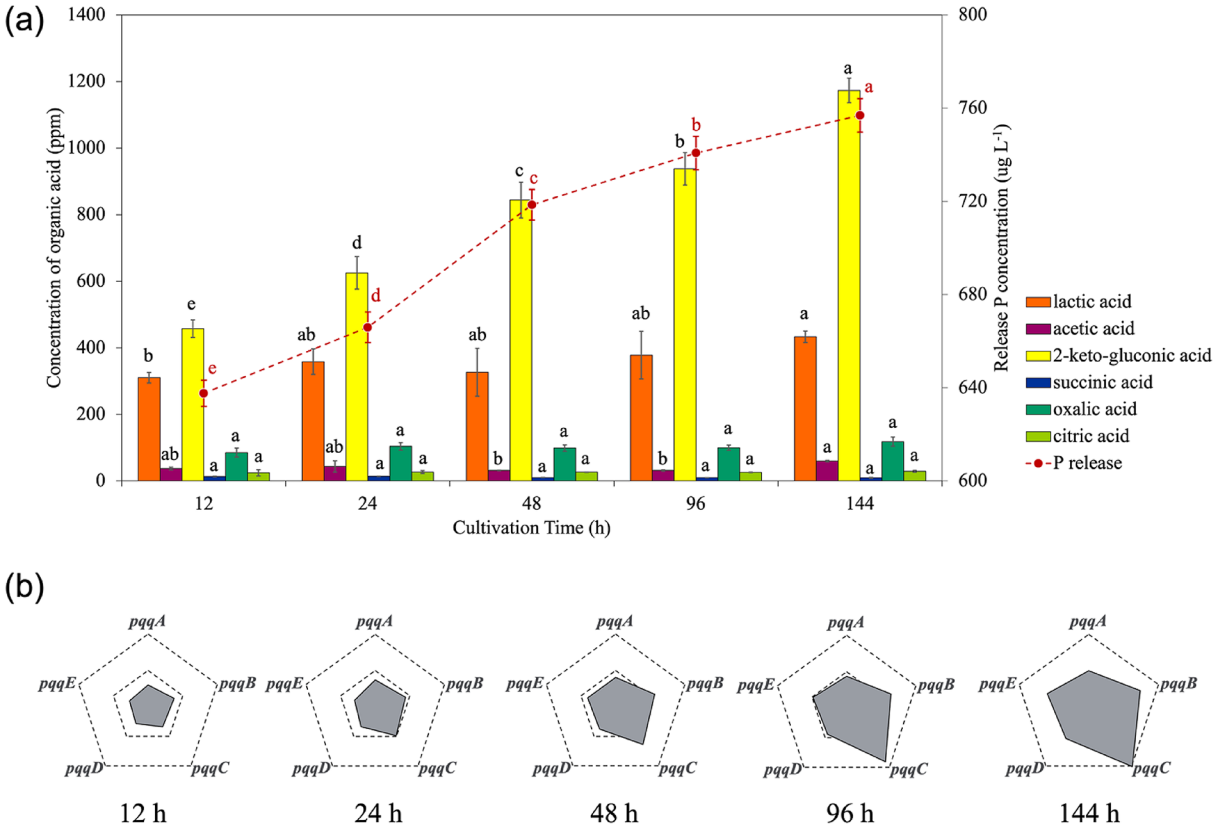

Fig. 2 Biochemical and genetic analysis of strain 51-Y1415 cultivated in PVK medium over 144 h. **a** Average values of triplicate measurements with standard deviations. The line graph illustrates P release into the medium, while the bar chart quantifies the concentrations of various organic acids produced. Significant differences between groups indicated by different letters were determined by ANOVA ($P < 0.05$). **b** Radar charts depicting the relative expression levels (fold change) of the *pqq* gene cluster in strain 51-Y1415, with expression normalized to conditions in LB medium over 144 h

Table 2 Pearson's correlation analysis between P release and organic acid produced by strain 51-Y1415 and its *pqq* gene abundance based on qPCR results

	***P* release**	**pqqA**	**pqqB**	**pqqC**	**pqqD**	**pqqE**
P release	–	**0.946***	**0.902***	**0.940***	**0.897***	0.872
Lactic acid	0.848	**0.897***	0.836	0.868	**0.914***	0.866
Acetic acid	0.313	0.497	0.414	0.390	0.546	0.485
2-keto-D-gluconic acid	**0.903***	**0.988****	**0.995****	**0.973****	**0.985****	**0.985****
Succinic acid	− 0.792	− 0.826	**− 0.921***	− 0.878	− 0.853	**− 0.903***
Oxalic acid	0.811	**0.895***	0.794	0.776	0.848	0.792
Citric acid	0.679	0.818	0.718	0.661	0.763	0.712

Significant values are indicated in bold type, and asterisk * and ** indicates the significance level $P < 0.05$ and $P < 0.01$, respectively

Our analysis also reveals a significant variability in the presence of complete *pqq* gene clusters among different strains, reflecting a sophisticated regulatory mechanism that correlates gene expression with phosphate solubilization efficiency. The distinct expression dynamics of *pqqC*, directly associated with solubilization capacity, underscores its potential as a valuable marker for identifying efficient PSB. This gene's expression is not only stimulated by glucose and phosphorus availability but also appears to be upregulated in response to phosphate release, suggesting a feedback mechanism that enhances its solubilization potential (Magnusson et al. 2004).

Moreover, our findings elucidate that despite variations in the biosynthetic pathways for PQQ, the essential redox reactions mediated by *pqqC* are critical for phosphate solubilization across diverse ecological niches. This realization expands our understanding of microbial solubilization capabilities and highlights the potential of genomic insights to inform bioaugmentation strategies aimed at improving soil fertility and plant phosphorus uptake. As such, the comprehensive genetic framework

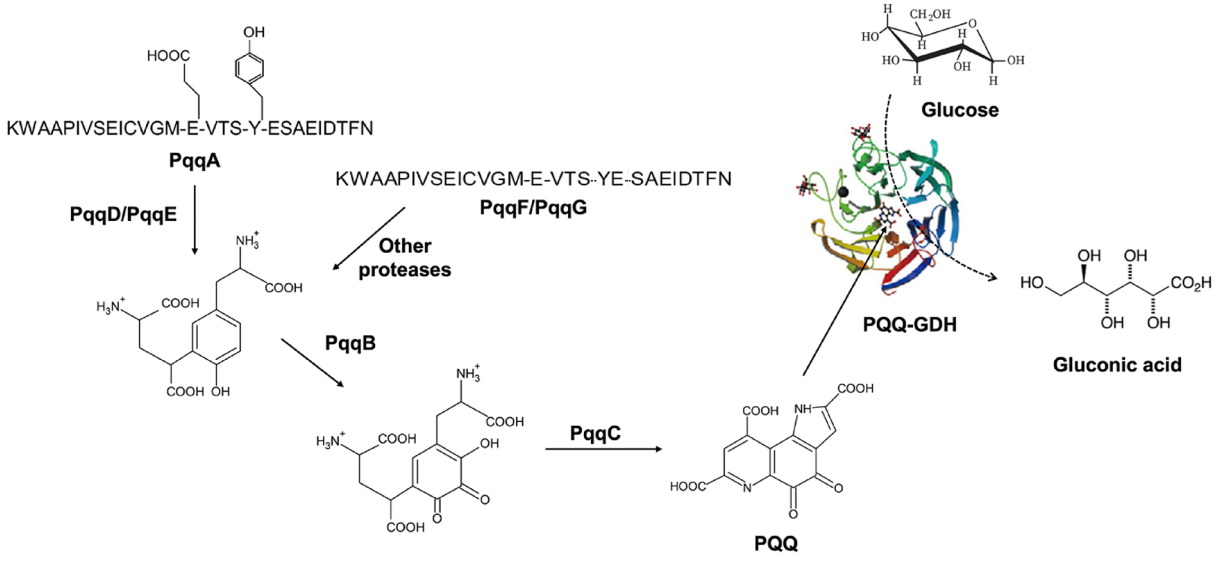

Fig. 3 The schematic diagram of the proposed pyrroloquinoline quinone (PQQ) biosynthetic pathway and glucose to gluconic acid conversion via PQQ-GDH (PQQ-dependent glucose dehydrogenase). (Adapted from Martins et al. 2019; Mi et al. 2020). Depicts the biosynthesis of PQQ from the peptide PqqA through four conserved enzymes: PqqE (radical SAM enzyme), PqqD (peptide chaperone), PqqB (dual hydroxylase), and PqqC (eight-electron, eight-proton oxidase), alongside an alternative pathway involving PqqF/G. This process underscores PQQ's role in the conversion of glucose to gluconic acid

provided by the *pqqC* gene offers a strategic target for enhancing microbial interventions in nutrient cycling, emphasizing the need for continued exploration into the regulatory networks that govern *pqq* gene expression and activity.

This research advances our understanding of microbial contributions to soil phosphorus cycling and underscores the potential of leveraging genetic insights to optimize microbial capabilities for sustainable agricultural practices. Future studies should focus on the precise regulatory networks governing *pqq* gene expression and activity, particularly the unique contributions of *pqqC*, to harness these insights for enhancing soil health and crop productivity. Besides, our study's primary limitation lies in its focus on the *pqq* gene cluster and the use of whole-genome sequencing, which, while precise, does not capture the full complexity of microbial community interactions. Additionally, the selection criteria for PSB strains may introduce biases, affecting the study's conclusions. Future research should incorporate metagenomic and metatranscriptomic analyses to explore functional interactions within microbial communities and conduct field trials to assess the practical applications of our findings in diverse agricultural settings.

Abbreviations

P	Phosphorus
PSB	Phosphate solubilizing bacteria
PQQ	Pyrroloquinoline quinone
GDH	Glucose dehydrogenase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13568-024-01745-w.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements

None.

Author contributions

XQC, LW, and BXZ conceived and designed research. XQC, YTZ, and SSH conducted experiments. XQC, LW, and BXZ analyzed the data. XQC, JP, and JS wrote the manuscript. All authors have read and approved the manuscript.

Funding

The study was supported by the Xiamen Natural Science Foundation Youth Project (3502Z202372040). We also acknowledge the funding provided to Bangxiao Zheng by the National Foreign Expert Project (Grant No. G2023021001L). The Spanish Government funded the research contributions of Josep Peñuelas and Jordi Sardans through Grants PID2020115770RB-I, TED2021-132627 B–I00, and PID2022-140808NB-I00.G2023021001L.

Availability of data and materials

The 16 S sequence of stain 51-Y1415 was submitted to the NCBI Sequence Read Archive (KU647244). The genome sequence as part of this study was deposited at DDBJ/ENA/GenBank under accession PUIQ00000000. The raw sequence was deposited in Sequence Read Archive under accession SRR6785072. The biochemical properties and other analyzed data have been fully stated in this study.

Declarations

Ethics approval and consent to participate

Nonapplicable.

Competing interests

The authors declare that they have no financial or other conflicts of interest.

Received: 7 May 2024 / Accepted: 17 July 2024
Published online: 29 July 2024

References

An R, Moe LA (2016) Regulation of pyrroloquinoline quinone-dependent glucose dehydrogenase activity in the model rhizosphere-dwelling bacterium *Pseudomonas putida* KT2440. Appl Environ Microbiol 82:4955–4964

Bhanja E, Das R, Begum Y, Mondal S (2021) Study of pyrroloquinoline quinine from phosphate-solubilizing microbes responsible for plant growth: in silico approach. Front Agron 3:667339

Cheng Y, Narayanan M, Shi X, Chen X, Li Z, Ma Y (2023) Phosphate-solubilizing bacteria: their agroecological function and optimistic application for enhancing agro-productivity. Sci Total Environ 901:166468

Deng P, Wang X, Baird SM, Showmaker KC, Smith L, Peterson DG, Lu S (2016) Comparative genome-wide analysis reveals that *Burkholderia contaminans* MS14 possesses multiple antimicrobial biosynthesis genes but not major genetic loci required for pathogenesis. Microbiol Open 5:353–369

Emms DM, Kelly S (2015) OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol 16:157

Harvey PR, Warren RA, Wakelin S (2009) Potential to improve root access to phosphorus: the role of non-symbiotic microbial inoculants in the rhizosphere. Crop Pasture Sci 60:144–151

Holden MT, Titball RW, Peacock SJ, Cerdeno-Tarraga AM, Atkins T, Crossman LC, Pitt T, Churcher C, Mungall K, Bentley SD, Sebaihia M, Thomson NR, Bason N, Beacham IR, Brooks K, Brown KA, Brown NF, Challis GL, Cherevach I, Chillingworth T, Cronin A, Crossett B, Davis P, DeShazer D, Feltwell T, Fraser A, Hance Z, Hauser H, Holroyd S, Jagels K, Keith KE, Maddison M, Moule S, Price C, Quail MA, Rabbinowitsch E, Rutherford K, Sanders M, Simmonds M, Songsivilai S, Stevens K, Tumapa S, Vesaratchavest M, Whitehead S, Yeats C, Barrell BG, Oyston PC, Parkhill J (2004) Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei*. Proc Natl Acad Sci USA 101:14240–14245

Joshi S, Gangola S, Jaggi V, Sahgal M (2023) Functional characterization and molecular fingerprinting of potential phosphate solubilizing bacterial candidates from Shisham Rhizosphere. Sci Rep 13:7003

Khan MS, Zaidi A, Wani PA (2009) Role of phosphate solubilizing microorganisms in sustainable agriculture—a review. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C (eds) Sustainable agriculture. Springer, Dordrecht, pp 551–570

Kour D, Rana KL, Kaur T, Yadav N, Yadav AN, Kumar M, Kumar V, Dhaliwal HS, Saxena AK (2021) Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and -mobilizing microbes: a review. Pedosphere 31:43–75

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Latham JA, Iavarone AT, Barr I, Juthani PV, Klinman JP (2015) PqqD is a novel peptide chaperone that forms a ternary complex with the radical S-Adenosylmethionine protein PqqE in the pyrroloquinoline quinone biosynthetic pathway. J Biol Chem 290:12908–12918

Lekberg Y, Arnillas CA, Borer E, Bullington LS, Fierer N, Kennedy PG, Leff JW, Luis AD, Seabloom EW, Henning JA (2021) Nitrogen and phosphorus fertilization consistently favor pathogenic over mutualistic fungi in grassland soils. Nat Commun 12:3484

Li XL, Lv XY, Ji JB, Wang WD, Wang J, Wang C, He HB, Ben AL, Liu TL (2023) Complete genome sequence of *Nguyenibacter* sp. L1, a phosphate solubilizing bacterium isolated from *Lespedeza bicolor* rhizosphere. Front Microbiol 14:1257442

Liu X, Han R, Cao Y, Turner BL, Ma LQ (2022) Enhancing phytase availability in soils and phytate-P acquisition by plants: a review. Environ Sci Technol 56:9196–9219

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Magnusson OT, Toyama H, Saeki M, Rojas A, Reed JC, Liddington RC, Klinman JP, Schwarzenbacher R (2004) Quinone biogenesis: structure and mechanism of PqqC, the final catalyst in the production of pyrroloquinoline quinone. Proc Natl Acad Sci USA 101:7913–7918

Martins AM, Latham JA, Martel PJ, Barr I, Iavarone AT, Klinman JP (2019) A two-component protease in *Methylorubrum extorquens* with high activity toward the peptide precursor of the redox cofactor pyrroloquinoline quinone. J Biol Chem 294:15025–15036

Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60

Meier-Kolthoff JP, Klenk HP, Goker M (2014) Taxonomic use of DNA G plus C content and DNA–DNA hybridization in the genomic age. Int J Syst Evol Microbiol 64:352–356

Meyer JB, Frapolli M, Keel C, Maurhofer M (2011) Pyrroloquinoline quinone biosynthesis gene *pqqC*, a novel molecular marker for studying the phylogeny and diversity of phosphate-solubilizing *pseudomonads*. Appl Environ Microbiol 77:7345–7354

Mi Z, Cheng J, Zhao P, Tian P, Tan T (2020) Improved production of pyrroloquinoline quinone by simultaneous augmentation of its synthesis gene expression and glucose metabolism in *Klebsiella pneumoniae*. Curr Microbiol 77:1174–1183

Miller SH, Browne P, Prigent-Combaret C, Combes-Meynet E, Morrissey JP, O'Gara F (2010) Biochemical and genomic comparison of inorganic phosphate solubilization in *Pseudomonas* species. Environ Microbiol Rep 2:403–411

Nierman WC, DeShazer D, Kim HS, Tettelin H, Nelson KE, Feldblyum T, Ulrich RL, Ronning CM, Brinkac LM, Daugherty SC, Davidsen TD, Deboy RT, Dimitrov G, Dodson RJ, Durkin AS, Gwinn ML, Haft DH, Khouri H, Kolonay JF, Madupu R, Mohammoud Y, Nelson WC, Radune D, Romero CM, Sarria S, Selengut J, Shamblin C, Sullivan SA, White O, Yu Y, Zafar N, Zhou L, Fraser CM (2004) Structural flexibility in the *Burkholderia mallei* genome. Proc Natl Acad Sci USA 101:14246–14251

Olsen SR, Cole CV, Wantanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Washington USDA

Peñuelas J, Poulter B, Sardans J, Ciais P, van der Velde M, Bopp L, Boucher O, Godderis Y, Hinsinger P, Llusia J, Nardin E, Vicca S, Obersteiner M, Janssens IA (2013) Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nat Commun 4:2934

Peñuelas J, Janssen IA, Clais P, Obersteiner M, Sardans J (2020) Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. Glob Chang Biol 26:1962–1985

Qiao J, Yang L, Yan T, Xue F, Zhao D (2013) Rice dry matter and nitrogen accumulation, soil mineral N around root and N leaching, with increasing application rates of fertilizer. Eur J Agron 49:93–103

Riera N, Handique U, Zhang Y, Dewdney M, Wang N (2017) Characterization of antimicrobial-producing beneficial bacteria isolated from Huanglongbing escape citrus trees. Front Microbiol 8:2415

Rodŕiguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Sackett W, Patten A, Brown C (1908) The solvent action of soil bacteria upon the insoluble phosphates of raw bone meal and natural raw rock phosphate. Centralbl Bakteriol 202:688–703

Sashidhar B, Podile AR (2010) Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. J Appl Microbiol 109:1–12

Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F (2011) Phosphorus dynamics: from soil to plant. Plant Physiol 156:997–1005

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Velterop JS, Sellink E, Meulenberg JJ, David S, Bulder I, Postma PW (1995) Synthesis of pyrroloquinoline quinone in vivo and in vitro and detection of an intermediate in the biosynthetic pathway. J Bacteriol 177:5088–5098

Wagh J, Chanchal K, Sonal S, Praveena B, Archana G, Kumar GN (2016) Inoculation of genetically modified endophytic *Herbaspirillum seropedicae* Z67 endowed with gluconic and 2-ketogluconic acid secretion, confers beneficial effects on rice (*Oriza sativa*) plants. Plant Soil 409:51–64

Wu X, Cui Z, Peng J, Zhang F, Liesack W (2022) Genome-resolved metagenomics identifies the particular genetic traits of phosphate-solubilizing bacteria in agricultural soil. ISME Commun 2:17

Yu H, Wu X, Zhang G, Zhou F, Harvey PR, Wang L, Fan S, Xie X, Li F, Zhou H, Zhao X, Zhang X (2022) Identification of the phosphorus-solubilizing bacteria strain JP233 and its effects on soil phosphorus leaching loss and crop growth. Front Microbiol 13:892533

Zeng J, Tu Q, Yu X, Qian L, Wang C, Shu L, Liu F, Liu S, Huang Z, He J, Yan Q, He Z (2022) PCycDB: a comprehensive and accurate database for fast analysis of phosphorus cycling genes. Microbiome 10:101

Zheng BX, Hao XL, Ding K, Zhou GW, Chen QL, Zhang JB, Zhu YG (2017) Long-term nitrogen fertilization decreased the abundance of inorganic phosphate solubilizing bacteria in an alkaline soil. Sci Rep 7:42284
Zheng BX, Ibrahim M, Zhang DP, Bi QF, Li HZ, Zhou GW, Ding K, Peñuelas J, Zhu Y-G, Yang X-R (2018) Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. AMB Express 8:47
Zheng BX, Zhang DP, Wang Y, Hao XL, Wadaan MAM, Hozzein WN, Peñuelas J, Zhu YG, Yang XR (2019) Responses to soil pH gradients of inorganic phosphate solubilizing bacteria community. Sci Rep 9:25

Publisher's Note