Cloning and Functional Analysis of pqq Genes
Phosphorus Solubilizing From Bacillus Mycoides Gnyt1

yang xiaomei
Gansu Agricultural University

Tuo Yao (mailto:yaotuo@edu.com)
Gansu Agricultural University

Research article

Keywords: Bacillus mycoides, pqqgenes family, Phosphorus solubilization, Function verification

DOI: https://doi.org/10.21203/rs.3.rs-41467/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

[Background]

The \textit{pqq} genes which encodes phosphorus solubilizing from \textit{Bacillus mycoides} Gnyt1 has been cloned and characterized. The role of this action on the function of \textit{pqq} genes were detected. The phosphorus soluble \textit{pqq} genes can secrete some specific organic activity acids to work but don't destroy the living environment of plants such as soil. In contrast, this plants containing the \textit{pqq} genes grew well, even better than other plants with lower phosphorus solubilization, in the phosphorus free medium even though its \textit{pqq} genes activity and turned out phosphorus ring, while \textit{pqq} dependent expression was induced.

[Results]

This reaserch that five \textit{pqq} genes were cloned from \textit{Bacillus mycoides} Gnyt1, phylogenetic analysis revealed that pBI-\textit{pqq}A, pBI-\textit{pqq}B, pBI-\textit{pqq}C and pBI-\textit{pqq}E similarly cluster with Phosphorus-soluble protein. Under the action of five promoters, each coding region of \textit{pqq}A, \textit{pqq}B, \textit{pqq}C and \textit{pqq}E from \textit{Bacillus mycoides} Gnyt1 can directly participate in the phosphate solubilization of the strain, directly improving the phosphate solubilizing capacity of the plant, which have strong nitrogenase activity. Interestingly, changes in the phosphate solubilizing gene result in complete loss of the phosphate solubilization of the strain. However, the ability to dissolve the phosphorus ring and to dissolve the phosphorus was also exhibited in the medium having five phosphorus-dissolving genes.

[Conclusions]

The results indicated that the \textit{pqq} genes were successfully cloned and a new specific phosphate-dissolving strain was formed. In this research, \textit{pqq} genes were first cloned from \textit{Bacillus mycoides} Gnyt1.

1. Background

Phosphorus soluble gene was discovered in 1979 as a new type of phosphate-soluble cofactor for several prokaryotic dehydrogenases\cite{1-3}. The phenomenon of phosphorus deficiency in soil is widespread and should be given great attention. \textit{Pq} genes have been founded and characterized from bacteria, cloned and sequenced of the \textit{pqq} gene from \textit{Acetobacter aceti} \cite{4-5}. Several researches indicated that \textit{pqq} is present in a variety of plants and animals. For example, Kumazawa T \textit{et al.}\cite{6} and Mitchell et al. were found \textit{pqq} in human and rat tissues. Kumazawa \textit{et al.}\cite{7} research said that all kinds of food and vegetables contain \textit{pqq}. The recent research showed that PQQ-lack of mouse liver has low mitochondrial content and elevated plasma glucose concentration\cite{8}. In addition, PQQ is used as a scavenger for reactive oxygen species (ROS)\cite{9-10} and prevents damage to cells of DNA fragments\cite{11}. They are gram-negative of the bacteria famlily, which has a strong phosphorus dissolving ability\cite{12}. This enzyme can only the first
in our *Bacillus mycoides* Gnyt1, and they have been found in Acinetobacter sp. or Pseudomonas putida\textsuperscript{[13-14]}. Research progress showed the *pqq* gene most of the performance was *pqq*-adhB, which cloned *A. polyoxogenes*\textsuperscript{[5]}. Kosano *et al.*\textsuperscript{[15]} reported that PQQ inhibited a-MSH-stimulated melanin-producing B16 melanoma. This inhibition of melanin production is caused by PQQ, which inhibits the expression of tyrosinase mRNA. However, other roles of PQQ, such as the action of tyrosinase-related proteins and the action of Mitf transcription in the pigment system, are not well known\textsuperscript{[16]}. Whereas K. pneumonia is synthesized by gluconate dihydrogen dehydrogenase and coenzyme PQQ. Closely related intestinal bacteria and Salmonella typhimurium can only synthesize decoenzyme, while PQQ cannot\textsuperscript{[17]}. However, the addition of PQQ to *E. coli* and S. typhimurium cells produces active holoenzyme\textsuperscript{[18]}. Kumazawa *et al.*\textsuperscript{[19]} report said that all kinds of food and vegetables contained PQQ. Naito *et al.*\textsuperscript{[20]} reported that PQQ can stimulate DNA synthesis. PQQ is synthesized by a variety of microorganisms\textsuperscript{[21]} from the two amino acids glutamate and tyrosine encoded in the precursor peptide, and is shown as an effective growth factor for plants\textsuperscript{[22]}, bacteria\textsuperscript{[23]} or higher organisms\textsuperscript{[24]}. Although PQQ is an effective growth factor in both in vitro and in vivo models, the detailed mechanism of action remains unclear.

*Bacillus mycoides* Gnyt1 is a Gram-positive normal isolate from the rhizosphere of the alpine steppe plant of the alpine steppe, which is isolated and named in our laboratory, and has a high ability to dissolve phosphorus\textsuperscript{[25]}. At present, there is no known about the mechanism of phosphorus solubilization and the phosphorus-dissolving gene of this kind of bacteria. In this study, we aimed to clarify the functions of *Bacillus mycoides* Gnyt1 of *pqq* genes family, the four *pqq* genes were cloned and their activities and properties were characterized. We further validated the cloning of the phosphate-dissolving gene and its effect on the ability of the fraction to dissolve phosphorus.

### 2. Methods

#### 2.1 Media and growth conditions

*Bacillus mycoides* Gnyt1 was cultured in medium, which contains: 5g yeast powder, 10g Peptone, 10g NaCl, PH 7.0. Optimal growth occurs at 28°C in aerobic conditions.

#### 2.2 Cloning of *pqq* genes

The complete sequence of the *pqq* genes were selected from the baseline genome-wide database\textsuperscript{[26]} in our laboratory, all of which have accession numbers in NCBI. Primers are designed from conserved regions. All primers for this study were listed in Table 1. Primer pairs *pqqF* and *pqqR* were performed to obtain partially conserved regions. The amplified PCR product was purified and cloned into pMD-T19 vector (Takara, Japan). The expression vector pBI-121 was obtained from the wheat laboratory of Gansu Agricultural University, and after transformation in bacterial DH5α competent cells, the plasmid was
isolated from the positive clone and inserted. DNA was obtained by PCR, and the remaining four clones were cloned by conventional PCR.

Table 1 List of primers used in the study

| Primers | Oligonucleotide sequence (5’→3’) | Purpose       |
|---------|---------------------------------|---------------|
| pqq1F   | AACAGGACGAGATTGGAGCA             | pqq1 amplification |
| pqq1R   | TTGCGTGGATGCCCAAAAG              | pqq1 amplification |
| pqq2F   | GTAGCGATGGTTGGTCAGTAGGG          | pqq2 amplification |
| pqq2R   | TGCTGGAGCCGGATTCAACATAAC         | pqq2 amplification |
| pqq3F   | AGGCGGAGCGCCTGATACATTCC         | pqq3 amplification |
| pqq3R   | TTCTGCCGCAAATCCAG               | pqq3 amplification |
| pqq4F   | AGCTCTTCGTGTCGCAGGAATTAC        | pqq4 amplification |
| pqq4R   | ACTGCACCGCTCGTACCAATTG          | pqq4 amplification |
| pqq5F   | TCATTCCCGACAGATGCCTTCAG         | pqq5 amplification |
| pqq5R   | CGGATCGGTTCTAGCTTCCATTG         | pqq5 amplification |

2.3 Sequence analysis and phylogeny prediction

ORF Finder found ORF (open reading frame)(http://www.ncbi.nlm.nih.gov/g-orf/gorf.html). The conserved domain of the pqq gene was detected by the Conservative Domain Database (CDD) search tool on the NCBI server (http://www.ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi). The physical and chemical parameters of the protein were determined using the ProtParam tool (http://us.expasy.org/tools/protparam.html).

2.4 Expression of pqq genes in E. Coli

The forward primer pqq with the built-in QuickCutTM Smal cleavage site and the pqq clone ORF with the QuickCutTM XbaI site and five pqq genes (Table 2) were used. The PCR product was digested with Smal and XbaI and ligated into the PBI-121 expression vector. Positive transformants were identified by PCR. The culture was cultured in Luria-Bertani (LB) medium with a positive concentration of 50 μg/mL. When the OD600 reached 0.5-0.7 at 37 °C, the culture (5 mL) was transferred to an alternative 500 mL LB medium. In the 2000 mL flask, a 50 μg/mL pseudonym was added to the medium. When the OD600 reached about 0.6, the antibiotic thio-β-galactoside (IPTG) was added to the medium at a final concentration of 0.1 mmol / L. The bacteria were then centrifuged at 5,000 g for 30 minutes at 16 °C, washed with PBS, and then cultured with binding buffer (20 mmol / L imidazole, 500 mmol / L NaCl, 20).
Hours to induce expression of the DfSQS1 protein. Methylene / L Na3PO4, pH 7.4) resuspended. Then, a certain amount of lysozyme (0.1 mg / mL) and RNAses (20 μg / mL) were added to the cell suspension. The cell suspension was stirred and placed at 4 ° C for 30 minutes. The cell suspension was disrupted by sonication 20 and the supernatant was harvested by centrifugation at 4200 rpm for 20 minutes at 4 ° C and replaced by a 750 kDa hollow fiber column. After washing with binding buffer (20 mmol / L imidazole, 500 mmol / L NaCl, 20 mmol / L Na3PO4, pH 7.4), protein and exchange buffer (20 mmol / L Na3PO4, 250 mmol / L imidazole, 500 mmol) ) combined / L NaCl, pH 7.4). All collected samples were analyzed by SDS-PAGE.

Table 2. List of enzyme cleavage site primers used in the study

| Primers | Oligonucleotide sequence (5′→3′) | Purpose   |
|---------|---------------------------------|-----------|
| pqq1F   | GCTCTAGAAACAGGACGAGATTGGAGCA   | pqq1 amplification |
| pqq1R   | CGACCCGGGTGCTGGATGCCCACAAG      | pqq1 amplification |
| pqq2F   | GCTCTAGAGTAGCGATGGTTGGTGTCAGTAGG | pqq2 amplification |
| pqq2R   | CGACCCGGGTGCTGGAGCCGATTCAACATAAC | pqq2 amplification |
| pqq3F   | GCTCTAGAAGCGGAGCTGACTACATTTC   | pqq3 amplification |
| pqq3R   | CGACCCGGGTGTTCTGCCCACAATCCAG   | pqq3 amplification |
| pqq4F   | GCTCTAGAAGCTCTTCTGTCGAGAATTAC  | pqq4 amplification |
| pqq4R   | CGACCCGGAGCTGCACCGACTACATTTC   | pqq4 amplification |
| pqq5F   | GCTCTAGATCATTCCGCGACAGTATGCTTCAG | pqq5 amplification |
| pqq5R   | CGACCCGGGCGGATCGGTCTAGCTCCATTG | pqq5 amplification |

3. Results

3.1 Cloning of five pqq genes from B. mycoides Gnyt1

The five pqq genes clusters were isolated from Bacillus mycoides Gnyt1. Five phosphorus soluble genes were cloned by conventional PCR using individual two genes primers pqqF and pqqR, as the figure shows that the pqq genes related to phosphorus soluble gene after cloning and screening. Moreover, BLAST analysis and sequence comparison revealed that the size of pqqE is 1539bp, which is the longest gene fragment, pqqA is 924bp, pqqB is 897bp, and pqqC is 645bp, which is presumed to be the target fragment. The sequencing datas obtained in this study have been deposited in the GenBank database under the following accession numbers by CP020743. The total of five genes sequence involving the putative open reading frame(ORF) was obtained, the ORF was amplified by using primer pair.
3.2 Construction of cloned phospholysis gene and plasmid for expression vector

The plasmid for expression vector PBI-121 has been successfully constructed and stored in a laboratory cryogenic refrigerator, which measurement was far more than 15000bp (Fig.2.A). Four plasmids of four pqq genes were successfully extracted using a plasmid kit, and the sizes were all less than 2500 bp (Fig.2.B). As can be seen from the figure, the agarose gel electrophoresis showed that the band was clear and single, and it was proved that the plasmid of the gene was successfully extracted, and the next experiment can be carried out.

3.3 Double digestion of plasmid

The enzymatic digestion system was as follows: 10×QuickCut Buffer 3ul, plasmid DNA 6ul, QuickCutTM SmaI 1ul, QuickCutTM XbaI 1ul, five genes were successfully extracted, and the expression vector pBI121 plasmid DNA was added to the rapidly cleaved enzymes SmaI and XbaI for double digestion, ddH2O is up to 30ul. The enzyme was digested at 37 °C for 6 hours, electrophoresis was detected, and the target fragment was recovered. It can be cut from the figure and has successfully recovered 6 fragments of plasmid DNA (Fig.3).

3.4 Linked expression vector

The four pqq genes and the expression vector PBI121 were ligated, and the ligation system was: 10×T4 DNA Ligase Buffer 1ul, pqqA / pqqB / pqqC / pqqE 6ul, pBI121 DNA 1ul, T4 DNA Ligase 1ul, dd H2O 10ul. Except T4 DNA Ligase, the reaction was incubated at 65 °C for 3 minutes, then rapidly transferred to ice for a few seconds, then T4 DNA Ligase was ligated for 16 hours or more and stored at 4 degrees for conversion.

3.5 Phylogenetic analysis

The amino acid sequences corresponding to the four cloned gene products of the Gnyt1 variable are compared with similar sequences already in GenBank, and the software MEGU is used to generate the phylogenetic tree using the conversion method. The results show that the pqq genes family is derived from a single Gnyt1 pqqA phosphorus dissolving gene that is closer to bacterial phospholytic protein (WP 003194766.1) and clustered together with other distributions (Figure 5.5), and the phosphorus dissolving gene pqqB is closer to PqqB Thiazoline antibiotic biosynthetic protein (WP 018985147.1) (Figure 5.6), while pqqC is clustered closer to the Bacillus pqqC family protein group (Figure 5.7), and pqqE is closer to the Bacillus and Bacillus cereus dissolved phosphorus family protein cluster (Figure 5.8).

3.6 E. coli transformation

Using competent cells, each product was transformed into E. coli, and E. coli was used as a vector for propagation and growth, and the constructed PBI-pqqA, PBI-pqqB, PBI-pqqC, PBI-pqqE vectors were transformed into Escherichia coli DH5α in the large intestine. A large number of bacteria are amplified and positively transformed by bacterial liquid phase PCR.
White colonies cultured overnight were selected, and individual colonies were picked and cultured in LB liquid medium for about 13 hours. At the same time, blue spots were picked as a negative control (Fig.8), and bacterial cells were detected by PCR, and the target bands were amplified and correctly sequenced. It is used to detect the phosphate-dissolving activity of phosphate-dissolving bacteria.

3.7 Identification of phosphate-dissolving activity

Qualitative experiments have shown (Fig.9) that the strains of recombinant phosphorus-solubilizing genes can produce small phosphorus-solubilizing circles on the culture medium, and the phosphorus dissolving characteristics of PBI-ppqA, PBI-ppqB, PBI-ppqC, and PBI-ppqE were identified by liquid chromatography. Secreted organic acid content, standard solutions of different concentrations are purified and enriched by C18 column mobile phase, and analyzed under specific chromatographic conditions. The results showed that the four recombinant phosphorus-solubilizing gene strains can all secrete organic acids, and thus have a role in phosphorus dissolution (Fig. 10). The concentration peak of secreted organic acids can be clearly seen from the figure, successfully verifying that the four clones linked to the previous clones Phosphorus gene, indicating that the phosphorus dissolution gene dissolves phosphorus Among them, the peak of oxalic acid is the highest, and the peak of citric acid is the lowest. In addition (Table 3), PBI-ppqA can secrete 6 kinds of organic acids, with the strongest phosphorus-dissolving effect, followed by PBI-ppqB and PBI-ppqC, and can secrete 4 kinds of organic acids, followed by phosphorus-dissolving effect. PBI-ppqA and PBI-ppqC secreted the highest lactate concentration, while PBI-ppqB and PBI-ppqD secreted the highest malate concentration.

Table 3 Content of organic acids in recombinant strains

| Stains | Oxalic acid | Tartaric acid | Malic acid | Citric acid | Lactic acid | Acetic acid |
|--------|-------------|---------------|------------|-------------|-------------|------------|
| pqqA   | 14.93±0.41  | 14.10±0.90    | 14.81±0.69 | 14.39±0.46  | 18.23±0.94  | 18.18±0.05 |
| pqqB   | 3.21±0.15   | 5.12±0.64     | 106.82±2.75| 13.94±0.76  |_            |_          |
| pqqC   | 24.24±0.34  |               | 20.17±0.97 | 0.48±0.01   | 0.29±0.01   |_          |
| pqqD   | 32.09±0.77  | _             | 119.58±7.30| 0.63±0.14   |_            |_          |

4. Discussion

In the present study, we found that the same pathway, Bacillus was used as beneficial bacteria in our research because it has high phosphorus dissolving ability\[^{27-28}\]. PQQ genes stably catalyzes sustained redox cycling as a cofactor and does so more efficiently than most other redox active cofactors\[^{29}\]. However, like all redox active compounds, PQQ can still promote oxidation under certain conditions and induce oxidative protein modification, including oxidation of cysteamine thiol\[^{30-32}\]. It has been demonstrated that terpenoids including PQQ undergo an electron redox cycle in the presence of ascorbate, NAD(P)H and thiol compounds (such as glutathione), thereby forming corresponding
semiquinone free radicals\cite{33-36}. In addition, PQQ oxidizes the redox regulatory site of the N-methyl-D-aspartate (NMDA) receptor, thereby protecting NMDA or glutamate-mediated neuronal cell damage\cite{37}. Therefore, the permanent phosphorus solubilization gene may have a certain basic significance for supplementing the ability of plants to dissolve phosphorus.

In this report, we successfully identified the four \textit{pqq} genes in \textit{Bacillus} for use as a phosphorus solubilization. We obtained conventional PCR clone of \textit{pqqA}, \textit{pqqB}, \textit{pqqC} and \textit{pqqE}. Compared with other known \textit{pqq} genes, three key genes were found in our strain\cite{38-39}. The phosphorus-dissolving family is an important gene for bacterial phosphorus solubilization. We identified the \textit{pqq} genes of \textit{Bacillus mysoides} Gnyt1. This is the first clone and complete sequence description of the phosphate solubilizing gene in \textit{Bacillus mysoides} Gnyt1. The plasmids of the four genes extracted were all within 15000 bp, and were well aligned with the partial sequence of \textit{Bacillus} (partial sequence 94% identical). A high level of protection indicates that the Gnyt1 strain has a phosphate soluble gene similar to the reported corresponding phosphate-dissolving family gene\cite{40-41}.

The cloning of the \textit{pqq} genes of \textit{Bacillus} provides interesting and important suggestions for the regulation of phosphorus and phosphorus-soluble organic acid metabolites. \textit{Bacillus mysoides} Gnyt1 has the largest total number of acidic and basic residues for each protein. Explaining the mechanism of phosphorus solubilization is of great significance for the biotechnological application of such proteins. In this regard, the \textit{Bacillus} Gnyt1 \textit{pqq} genes may be a good model for research. Studies have shown that micro-spatial analysis of gene expression shows that changes in different PQQ genes can significantly affect metabolites, cell signaling and immune function-related genes\cite{42}. Strict control of the activity and expression of the phosphate-dissolving gene, transcription or post-translational regulation depends on the specific environment of the host organism. B. Gnyt1 is at ambient temperature under aerobic and anaerobic conditions and uses hydrogen hydride as the electron donor. The amino-acid sequences of \textit{pqqA} and \textit{pqqB} of B. Gnyt1 are more similar to the corresponding proteins in bacterial phospholytic protein and PqqB quinolinone biosynthetic protein (WP 003194766.1, WP 018985147.1).

Although the research was carried out in antibiotics, the results showed important information. The expression of four phosphorus-dissolving genes increased the expression rates of PBI-\textit{pqq}, PBI-\textit{pqq}, PBI-\textit{pqq}, and PBI-\textit{pqq} by increasing the temperature to 37 °C. Finally, we will obtain a single, expressible phosphate soluble gene that is recombined. In one study, the phosphate solubilizing gene was also present in other plants or phosphate solubilizing bacteria. However, other studies have shown that the phosphorus-dissolving family includes many genes, and different ones contain different phosphorus-dissolving genes. Among them, \textit{pqqA}, \textit{pqqC} and other reports have the same length\cite{43-45}, and \textit{pqqE} are rarely reported in other studies and can be used as a resource for further research.

There are few reports on phosphorus solubilization in \textit{Bacillus mysoides}, our research will enrich the knowledge of phosphorus solubilization in these bacteria.
In the present study, we found that the same pathway, *Bacillus* was used as beneficial bacteria in our research because it has high phosphorus dissolving ability\(^{[27-28]}\). PQQ genes stably catalyzes sustained redox cycling as a cofactor and does so more efficiently than most other redox active cofactors\(^{[29]}\). However, like all redox active compounds, PQQ can still promote oxidation under certain conditions and induce oxidative protein modification, including oxidation of cysteamine thiol\(^{[30-32]}\). It has been demonstrated that terpenoids including PQQ undergo an electron redox cycle in the presence of ascorbate, NAD(P)H and thiol compounds (such as glutathione), thereby forming corresponding semiquinone free radicals\(^{[33-36]}\). In addition, PQQ oxidizes the redox regulatory site of the N-methyl-D-aspartate (NMDA) receptor, thereby protecting NMDA or glutamate-mediated neuronal cell damage\(^{[37]}\). Therefore, the permanent phosphorus solubilization gene may have a certain basic significance for supplementing the ability of plants to dissolve phosphorus.

In this report, we successfully identified the four *pqq* genes in *Bacillus* for use as a phosphorus solubilization. We obtained conventional PCR clone of *pqqA*, *pqqB*, *pqqC* and *pqqE*. Compared with other known *pqq* genes, three key genes were found in our strain\(^{[38-39]}\). The phosphorus-dissolving family is an important gene for bacterial phosphorus solubilization. We identified the *pqq* genes of *Bacillus mysoide* Gnyt1. This is the first clone and complete sequence description of the phosphate solubilizing gene in *Bacillus mysoide* Gnyt1. The plasmids of the four genes extracted were all within 15000 bp, and were well aligned with the partial sequence of *Bacillus* (partial sequence 94% identical). A high level of protection indicates that the Gnyt1 strain has a phosphate soluble gene similar to the reported corresponding phosphate-dissolving family gene\(^{[40-41]}\).

The cloning of the *pqq* genes of *Bacillus* provides interesting and important suggestions for the regulation of phosphorus and phosphorus-soluble organic acid metabolites. *Bacillus mysoide* Gnyt1 has the largest total number of acidic and basic residues for each protein. Explaining the mechanism of phosphorus solubilization is of great significance for the biotechnological application of such proteins. In this regard, the *Bacillus* Gnyt1 *pqq* genes may be a good model for research. Studies have shown that micro-spatial analysis of gene expression shows that changes in different PQQ genes can significantly affect metabolites, cell signaling and immune function-related genes\(^{[42]}\). Strict control of the activity and expression of the phosphate-dissolving gene, transcription or post-translational regulation depends on the specific environment of the host organism. B. Gnyt1 is at ambient temperature under aerobic and anaerobic conditions and uses hydrogen hydride as the electron donor. The amino-acid sequences of *pqqA* and *pqqB* of B. Gnyt1 are more similar to the corresponding proteins in bacterial phospholytic protein and PqqB quinolinone biosynthetic protein (WP 003194766.1, WP 018985147.1).

Although the research was carried out in antibiotics, the results showed important information. The expression of four phosphorus-dissolving genes increased the expression rates of PBI-*pqq*, PBI-*pqq*, PBI-*pqq*, and PBI-*pqq* by increasing the temperature to 37 °C. Finally, we will obtain a single, expressible phosphate soluble gene that is recombined. In one study, the phosphate solubilizing gene was also present in other plants or phosphate solubilizing bacteria. However, other studies have shown that the
phosphorus-dissolving family includes many genes, and different ones contain different phosphorus-dissolving genes. Among them, $pqqA$, $pqqC$ and other reports have the same length$^{[43-45]}$, and $pqqE$ are rarely reported in other studies and can be used as a resource for further research.

There are few reports on phosphorus solubilization in *Bacillus mycoides*, our research will enrich the knowledge of phosphorus solubilization in these bacteria.

5. Conclusion

$PqqA$, $pqqB$, $pqqC$ and $pqqE$ genes were the first clone analysis and *E. coli* transformation. In this case, four genes cloned from B. Gnyt1 were full-length DNA encoding $pqqA$, $pqqB$, $pqqC$ and $pqqE$, and were named pBI-$pqqA$, pBI-$pqqB$, pBI-$pqqC$ and pBI-$pqqE$. Bioinformatics analysis of pBI-$pqqA$, pBI-$pqqB$, pBI-$pqqC$ and pBI-$pqqE$ have been completed. Phylogenetic analysis trees reflect the accumulation of the $pqq$ genes in higher clade, slightly closer to Phosphorus-soluble protein. Four $pqq$ genes were expressed in *E. coli*, and four recombinant phospholytic genes were obtained, which proved that PBI-$pqqA$, PBI-$pqqB$, PBI-$pqqC$ and PBI-$pqqE$ have phosphorus-dissolving properties. This is the first report on the cloning and identification of the phosphate solubilizing gene from *Bacillus mycoides* Gnyt1. Finally, the B. Gnyt1 phosphate-dissolving gene a promising model for studying the mechanism of phosphorus solubilization by specific phosphorus-dissolving bacteria, a feature that makes it an attractive candidate for biotechnology applications.

Abbreviations

B. Gnyt1—*Bacillus mycoides*Gnyt1

Declarations

Declarations

Not applicable

Ethics approval and consent to participate

Approve and agree to participate

Consent for Publication

All authors agree to publish content

Availability of data and material

Not applicable

Competing interests
The authors declare that there are no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (31660688) and the Provincial Department of Organizations “Development of Key Technologies and New Products for Microbial Fertilizers” (LYRC2019-113). The funder determines the research topic and content, revises the paper and finalizes the paper.

Authors’ contributions

Xiaomei Yang cloned the gene and drafted the manuscript. Tuo Yao designed the research and project outline and finalized the manual. Xiaomei Yang was genetically analyzed. All authors have read and approved the final manuscript.

Acknowledgements

Thanks for the funded project, for the training and teaching of the tutor, for the hard work of the editor teachers, and for the opportunity given by the review teachers.

References

1. Salisbury SA, Forrest HS, Cruse WB, Kennard O. 1979. A novel coenzyme from bacterial primary alcohol dehydrogenases. *Nature*, 280, 843-844.
2. Stites, T. E., Mitchell, A. E., Rucker, R. B. 2000. Physiological importance of quinoenzymes and the o-quinone family of cofactors. *Journal of Nutrition* 130, 719-727.
3. McIntire WS. 1998. Newly discovered redox cofactors: possible nutritional, medical, and pharmacological relevance to higher animals. *Annual Review of Nutrition*, 18, 145-177.
4. Inoue T., Sunagawa M., Mori A., Imai C., Fukuda M., Takagi M., Yano K. 1979. Cloning and sequencing of the gene encoding the 72-kilodalton dehy-drogenase subunit of alcohol dehydrogenase form Acetobacter aceti. *Journal of Bacteriology*, 171, 3115-3122.
5. Tamaki T., Fukaya M., Takemura H., Tayama K., Okumura H., Kawamura Y., Nishiy-ama M., Horinouchi, S., Beppu, T., 1991. Cloning and sequencing of the gene cluster en-coding two subunits of membrane-bound alcohol dehydrogenase from Acetobacter polyoxygenes. *Biochemistry Biophysics Acta*, 1088, 292-300.
6. Kumazawa T, Seno H, Urakami T, Matsumoto T, Suzuki O. 1992. Trace levels of pyrroloquinoline quinone in human and rat samples detected by gas chromatography/mass spectrometry. *Biochim Biophys Acta*, 1156, 62-66.
7. Kumazawa T, Sato K, Seno H, Ishii A, Suzuki O. 1995. Levels of pyrroloquinoline quinone in various foods. *Biochemical Journal*, 307, 331-3.
8. Stites T, Storms D, Bauerly K, Mah J, Harris C, Fascetti A, et al. Pyrroloquinoline quinone modulates mitochondrial quantity and function in mice. *Journal of Nutrition*, 136, 390-396.

9. Misra HS, Khairnar NP, Barik A, Indira Priyadarsini K, Mohan H. 2004. Apte SKPyr roloquinoline-quinone: a reactive oxygen species scavenger in bacteria. *FEBS Lett*, 578, 26-30.

10. He K., Nukada H., Urakami T., Murphy M.P. 2003. Antioxidant and prooxidant properties of pyrroloquinoline quinone (PQQ): implications for its function in biological systems. *Biochem Pharmacol*, 65, 67-74.

11. Hara H, Hiramatsu H, Adachi T. 2007. Pyrroloquinoline quinone is a potent euro-protective nutrient against 6-hydroxydopamine-induced neurotoxicity. *Neurochem Res*, 32, 489-495.

12. Adachi, N. Yoshihara, S. Tanasupawat, H. Toyama, K. Matsushita, 2003. Purification and characterization of membrane-bound quinoprotein quinate dehydrogenase, *Biotechnol Biochem*. 67, 2115-2123.

13. A. Elsemore, L.N. Ornston. 1994. The pca-pob supraoperonic cluster of Acinetobacter calcoaceticus contains quiA, the structural gene for quinate-shikimate dehy- drogenase, *J. Bacteriol*, 176, 7659-7666.

14. I. Jiménez, B. Miñambres, J.L. García, E. Díaz. 2002. Genomic analysis of the aromatic catabolic pathways from Pseudomonas putida KT2440, *Environment Microbiol*. 4, 824-841.

15. Kosano H, Setogawa T, Kobayashi K, Nishigori H. 1995. Pyrroloquinoline quinone (PQQ) inhibits the expression of tyrosinase mRNA by α-melanocyte stimulating hormone in murine B16 melanoma cells. *Life Sci*, 56, 1707-13.

16. Tsuboi T, Kondoh H, Hiratsuka J, Mishima Y. 1998. Enhanced melano genesis induced by tyrosinase gene-transfer increases boron-uptake and killing effect of boron neutron capture therapy for amelanotic melanoma. *Pigment Cell Res*, 11, 275-82.

17. Hommes R.W.J., Postma, P.W., Neijssel O.M., Tempest, D.W., Dokter,P. and Duine, J.A. 1984. The genetics of PQQ and PQQ-containing enzymes. *FEMS Microbiol Letters*, 24, 329-333.

18. J.M. Meulenberg, E.Sellink, W.A.M. Loenen, N.H. Riegman, M. van Kleef, P.W.Postma. 1990. Cloning of *Klebsiella pneumoniae pqq* genes and PQQ biosynthesis in *Escherichia coli*. *FEMS Microbiology Letters*, 71, 337-344.

19. Kumazawa T, Sato K, Seno H, Ishii A, Suzuki O. 1995. Levels of pyrroloquinoline quinone in various foods. *Biochemical Journal*, 307, 331-333.

20. Naito Y, Kumazawa T, Kino I, Suzuki O. 1993. Effects of pyrroloquinoline quinine (PQQ) and PQQ-oxazole on DNA synthesis of cultured human fibroblasts. *Life Sci*, 52, 1909-1915.

21. Goodwin, P. M., Anthony, C. 1998. The biochemistry, physiology and genetics of PQQ and PQQ-containing enzymes. *Advances in Microbial Physiology*, 40, 1-80.

22. Ameyama M., Matsushita K., Shinagawa E., Hayashi M., Adachi O. 1988. Pyrroloquinoline quinone: excretion by methylotrophs and growth stimula- tion for microorganisms, *Biofactors*, 1, 51-53.
23. Choi O., Kim J., Kim J.G., Jeong Y., Moon J.S., Park C.S., Hwang I. 2008. Pyrroloquinoline quinone is a plant growth promotion factor produced by Pseudomonas fluorescens B16. *Plant Physiol*, 146, 657-668.

24. Steinberg, F. M., Gershwin, M. E., Rucker, R. B. 1994. Dietary pyrroloquinoline quinone: growth and immune response in BALB/c mice. *Journal of Nutrition*, 124, 744-753.

25. Liu T, Yao T, Chen JG, Ma WB, Liu H, Ma CY, Jiang YM. 2016. Identification and study on the effects of plant growth promoting rhizobacteria of Carexenervis. *Acta Prataculturae Sinica*, 12, 130-139.

26. Li JH. 2017. The characteristics of a fine plant growth-promoting rhizobacteria *Bacillus mycoides* Gnyt1 and its whole genome sequencing analysis. Gansu agricultural university. Doctoral dissertation.

27. Ma Y, Xia Z, Liu X, Chen S. 2007. Paenibacillus sabinae sp. nov., a nitrogen-fixing species isolated from the rhizosphere soils of shrubs. *Int J Syst Evol Microbiol*. 57, 6-11.

28. Salisbury SA, Forrest HS, Cruse WB. 1976. Kennard O: A novel coenzyme from bacterial primary alcohol dehydrogenases. *Nature*, 280, 843-844.

29. Stites TE, Mitchell AE, Rucker RB. 2000. Physiological importance of quinoenzymes and the oquinone family of cofactors. *Journal of Nutrition*, 130, 719-27.

30. Ishii, T., Akagawa, M., Naito, Y., Handa, O., Takagi, T., Mori, T., Kumazawa, S., Yoshikawa, T., Nakayama, T. 2010. Pro-oxidant action of pyrroloquinoline quinone: characterization of protein oxidative modifications. *Biosci Biotechnol Biochem*, 74, 663-666.

31. He K, Nukada H, Urakami T, Murphy MP. 2003. Antioxidant and pro-oxidant properties of pyrroloquinoline quinone (PQQ): implications for its function in biological systems. *Biochem Pharmacol*, 65, 67-74.

32. Park, J., Churchich, J. E. 1992. Pyrroloquinoline quinone (coenzyme PQQ) and the oxidation of SH residues in proteins. *Biofactors*, 3, 257-260.

33. Itoh, S., Kato, N., Ohshiro, Y., Agawa, T. 1985. Catalytic oxidation of thiols by coenzyme PQQ. *Lett.* 14, 135-136.

34. Itoh S., Kato N., Mure M., Ohshiro Y. 1987. Kinetic studies on the oxidation of thiols by coenzyme PQQ. *Bulletin of the Chemical Society of Japan*, 60, 420-422.

35. Itoh S., Kinugawa M., Mita N., Ohshiro Y. 1989. Efficient NAD regeneration system with heteroaromatic o-quinones and molecular oxygen. *Journal of the Chemical Society*, 35, 694-695.

36. Casini A., Finazzi-Agro A., Sabatini S.; El-Sherbini E. S.; Tortorella S.; Scipione L. 1999. Role of calcium in the reaction between pyrroloquinoline quinone and pyridine nucleotides monomers and dimers. *Archives Of Biochemistry And Biophysics*, 368, 385-393.

37. Ray, P. D., Huang, B. W., Tsuji Y. 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal*, 24, 981-990.

38. Paulsen C.E., Truong T.H., Garcia F.J., Homann A., Gupta V., Leonard S.E., Carroll K.S. 2011. Peroxide-dependent sulfenylation of the EGFR catalytic site enhances kinase activity. *Nature*
39. Thannickal V.J., Fanburg B.L. 2000. Reactive oxygen species in cell signaling. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 279, 1005-1028.

40. Kenner K. A., Anyanwu E., Olefsky J.M., Kusari J. 1996. Protein-tyrosine phosphatase 1B is a negative regulator of insulin and insulin-like growth factor-I-stimulated signaling. *Journal of Biological Inorganic Chemistry*, 271, 19810-19816.

41. Liu F., Chernoff J. 1997. Protein tyrosine phosphatase 1B interacts with and is tyrosine phosphorylated by the epidermal growth factor receptor. *Biochemical Journal*, 327, 139-145.

42. Chang Y., Ceacareanu B., Zhuang D., Zhang C., Pu Q., Ceacareanu A.C., Hassid A. 2006. Counter-regulatory function of protein tyrosine phosphatase 1B in platelet-derived growth factor- or fibroblast growth factor-induced motility and proliferation of cultured smooth muscle cells and in neointima formation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 26, 501-507.

43. Tchaparian E., Marshal L., Cutler G., Bauerly K., Chowanadisai W., Satre M., Harris C., Rucker R.B. 2010. Identification of transcriptional networks responding to pyrroloquinoline quinone dietary supplementation and their influence on thioredoxin expression, and the JAK/STAT and MAPK pathways. *Biochemical Journal*, 429, 515-526.

44. Sidney Aquino Neto, David P. Hickey, Ross D. Milton, Adalgisa R. De Andrade, Shelley D. Minteer. 2015. High current density PQQ-dependent alcohol and aldehyde dehydrogenase bioanodes, *Biosensors and Bioelectronics*, 72, 247-254.

45. Paulsen C.E., Truong T.H., Garcia F.J., Homann A., Gupta V., Leonard S.E., Carroll K.S. 2011. Peroxide-dependent sulfonylation of the EGFR catalytic site enhances kinase activity. *Nature Chemical Biology*, 8, 57-64.

46. Noji N., Nakamura T., Kitahata N., Taguchi K., Kudo T., Yoshida S., Tsujimoto M., Sugiyama T. Asami T. 2007. Simple and sensitive method for pyrroloquinoline quinone (PQQ) analysis in various foods using liquid chromatography/electrospray-ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 55, 7258-7263.

**Figures**
Figure 1

pqqA, pqqB, pqqC and pqqE of clone B. mycoidesGnyt1 strain. M: Marker 2000, 1: pqqA 924bp, 2: pqqB 897bp, 3: pqqC 645bp, 4: pqqE 1539bp

Figure 2

PCR detection of PBI-121, pqqA, pqqB, pqqC and pqqE plasmids. A: PCR detection of PBI-121 plasmid. M: marker 15000, 1: PBI-121 plasmid B: PCR detection of pqqA, pqqB, pqqC and pqqD plasmids. M: marker 15000, 1: pqqA plasmid, 2: pqqB plasmid, 3: pqqC plasmid, 4: pqqE plasmid
Figure 3
Double digestion of PBI-121, pqqA, pqqB, pqqC and pqqE plasmids. A: Double-digested plasmid recovery of plasmid PBI-121. M: Marker 15000, 1: PBI-121 Plasmid B: PCR detection of pqqA, pqqB, pqqC and pqqE double digested plasmids. M: marker 15000, 1: double digestion of pqqA plasmid, 2: double digestion of pqqB plasmid, 3: double digestion of pqqC plasmid, 4: double digestion of pqqE plasmid

Figure 4
PBI-121, pqqA, pqqB, pqqC and pqqE plasmid ligation
Figure 5

B. mycoides Gnyt1-pqqA of the phylogenetic tree

Figure 6

B. mycoides Gnyt1-pqqB of the phylogenetic tree
Figure 7

B. mycoides Gnyt1-pqqC of the phylogenetic tree

Figure 8

B. mycoides Gnyt1-pqqE of the phylogenetic tree
Figure 9

Blue and white spots of recombinant genes transformed into E. coli. A: PBI-pqqA blue and white spots. B: Blue and white speckle diagram of PBI-pqqB. C: Blue and white speckle diagram of PBI-pqqC. D: Blue and white speckle diagram of PBI-pqqE.

Figure 10

Qualitative determination of phosphorus-solubilizing gene strains
Figure 11

Determination of the content of organic acids produced by the phosphorus-dissolving gene strains. A: Determination of oxalic acid production of phosphorus-solubility gene strains. B: Determination of six organic acids produced by the phosphate-dissolving gene strain's dissolution characteristics.