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RESEARCH ARTICLE

The mechanism of phosphate solubilizing of *Pseudomonas* sp. TC952 and its solubilizing process on TC removal

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Abstract

Antibiotics undergo a series of complex transport and transformation route after entering the environment; however, there is scarce information about the effects of the bacterial phosphate-solubilizing process on tetracycline (TC) transformation. In this study, *Pseudomonas* sp. TC952 was identified as phosphate-solubilizing bacterium with high phosphate-solubilizing ability even under TC stress; it could solubilize maximum phosphate with a production of 400 mg/L soluble phosphate in 2 days. TC did not affect phosphate solubilizing in a short time incubation, but slightly promoted in a long incubation time. TC was adsorbed by inorganic phosphate with high efficiency of 53.09% within 1 day. Four tetracycline antibiotic resistance and sixteen inorganic phosphate-solubilizing-related genes were identified in the genome, which revealed the phosphate-solubilizing mechanism was that strain TC952 secrete organic acid to resolve inorganic phosphate and also secrete siderophore to chelate inorganic phosphate. So, during the inorganic phosphate-solubilizing process of strain TC952, TC was de-adsorbed from inorganic phosphate, and the solution was acidified into pH 4.3 through secreting organic acid to dissolve inorganic phosphorus, which resulted in Ca^{2+} and PO_4^{3-} releasing into the solution. Finally, the acidic condition and PO_4^{3-} enhanced TC hydrolysis. The mechanism of phosphate-solubilizing process on TC removal and genome analysis provides us new insight of the TC migration and transformation route in the environment.

Keywords *Pseudomonas* sp. TC952 · Phosphate solubilizing · Tetracycline · Genome analysis

Introduction

Phosphorus is an important macronutrient required for plant growth and development (Bechtaoui et al. 2021; Simpson et al. 2011). The account of phosphorus in top soil ranged at 50 to 3000 mg/kg soil, but only 0.1% phosphorus can be used or uptake by plants (Zhu et al. 2018). The most part of phosphorus was immobilized, adsorbed, precipitated by cations, or converted into organic form phosphorus, which restricted the usage of phosphorus in the environment (Azzi et al. 2017; Kishore et al. 2015). Consequently, phosphatic fertilizer was continuously supplied into the soil to meet the crop growth. But, a large part of phosphatic fertilizer were absorbed or precipitated in the soil, which cannot be utilized by plant, and lead to an environmental problem. Phosphate-solubilizing bacteria (PSB) can solubilize inorganic phosphate to soluble phosphate through different strategies, which could reduce the phosphoric fertilizer input (Kishore et al. 2015; Rawat et al. 2020). The major mechanism of phosphate solubilizing by PSB includes extracellular enzyme excretion, organic/inorganic acid production, siderophores and exopolysaccharide production, and proton releasing from NH_4^+ (Rawat et al. 2020). A large number of bacteria were isolated with the ability of inorganic phosphate solubilizing and identified as PSB (Soumare et al. 2020; Tian et al. 2021). For example, Liu et al. (2015) isolated twenty PSB from calcareous rhizosphere soils, which belong to *Bacillus megaterium*, *B. subtilis*, *Pseudomonas aeruginosa*, *Rhizobium* sp., *Acinetobacter* sp., and *P. oryzihabitans*. Li et al. (2018) isolated an efficient PSB, *Enterobacter* sp., from the soybean rhizosphere soil, and it could secrete organic acids to enhance the solubility of phosphates. So, PSB play an important role in soil phosphate cycle.

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As a broad-spectrum antibiotic, tetracycline (TC) was widely used as additives and veterinary medicine in livestock industry (Grossman 2016). But, TC cannot be absorbed or metabolized completely by animals, and 30–90% parent compounds or metabolites were excreted out into the environment via urine and feces, which induced adverse impacts on environment and human health in a long-term (Daghrir and Drogui 2013; Nguyen et al. 2014). Once entered into the environment, TC undergoes various abiotic and biotic processes, such as photolysis, hydrolysis, adsorption, microbial biodegradation, and plants uptake (Conde-Cid et al. 2018; Sengupta et al. 2016).

Furthermore, many factors could influence TC fate due to the complicated environment of soil, such as metal ions, organic matters, soil minerals, pH, and microorganism community. Now researches on the fate of TC biotransformation and migration in the environment mainly focused on TC adsorption and biodegradation by bacterial communities or pure bacteria (Lin et al. 2021; Peng et al. 2020; Yin et al. 2020), while studies on the influence of phosphate on TC fate were mainly focus on its influence on soil or mineral adsorption. For example, Wang et al. (2010) found that the addition of phosphate on aluminum and iron oxides rich soils decreased the adsorption of TC on soils. Zhu et al. (2020) found that phosphate can strongly interact with iron oxides/goethite surface and increase the negative charges of their surface; thus, the electrostatic repulsion between iron oxide particles and negatively charged TC species inhibits TC adsorption. But, studies on the inorganic phosphate-solubilizing process by PSB (soluble phosphate) on TC fate in the environment are scarce and need further study.

This study aimed to explore the influence of inorganic phosphate-solubilizing process on TC removal by a TC resistance strain *Pseudomonas* sp. TC952. Firstly, the inorganic phosphate-solubilizing ability by strain TC952 was investigated with/without TC stress. Then, TC removal efficiency under different characters on the inorganic phosphate solubilizing was studied. Finally, genome analysis was performed to identify TC resistance genes and inorganic phosphate-solubilizing genes. The effects of inorganic phosphate-solubilizing process on TC removal by strain TC952 filled a gap in TC immigration and transformation process.

Materials and methods

Strain and chemicals

Pseudomonas sp. TC952 was isolated with TC removal ability in our previous work (Tan et al. 2022) and was used in this study. Lysogeny broth (LB) medium and National Botanical Research Institute's phosphate (NBRIP) growth medium was prepared in this study. NBRIP growth medium contain as follows: $Ca_3(PO_4)_2$, 5.0 g/L; glucose, 10 g/L; $(NH_4)_2SO_4$, 0.5 g/L; NaCl, 0.3 g/L; KCl, 0.3 g/L; $MgSO_4{\cdot}7H_2O$, 0.3 g/L; $FeSO_4$, 0.03 g/L; and $MnSO_4$, 1.0 g/L, pH 7. Tetracycline (TC, CAS#60–54-8) stock solution was prepared in 1 g/L and sterilized using a 0.22-μm sterile nylon filter.

Inorganic phosphate-solubilizing kinetics with/without TC stress

The stock bacterial suspensions were prepared as follows: strain TC952 was cultured in LB medium and grow to mid-logarithmic phase at 30 °C and 150 rpm. The bacterial pellet was obtained at 5000 rpm in 10 min and washed three times using sterile ultrapure water. Then, the bacterial pellet was suspended in sterile water and OD_{600} was adjusted at 1.0. Unless otherwise statement, all experiments were performed by adding 2% stock bacterial suspensions into the medium, and all the experiments were carried out in triplicates.

For the inorganic phosphate-solubilizing kinetics by strain TC952, stock bacterial suspensions were inoculated into NBRIP medium with/without 10 mg/L TC at 30 °C and 150 rpm. Samples were collected at day 0, 0.5, 1, 2, 3, 4, 5, and 6. The collected samples were centrifugated at 3000 rpm in 10 min; then, the supernatant was filtered through a 0.22-μm filter. Soluble phosphate concentration was detected using the phosphomolybdate method according to Biswas et al. (2018). TC concentration was detected by HPLC according to our previous work (Tan et al. 2022). Cellular biomass (OD_{600}) and the solution pH was tested.

TC removal in different conditions produced in the phosphate-solubilizing process

According to the results of inorganic phosphate-solubilizing kinetics, the effects of different factors conditions (pH 4.3, Ca^{2+}, and PO_4^{3-} conditions) which were produced in the phosphate-solubilizing process by strain TC952 on TC removal was investigated. Ten milligram per liter TC was added into NBRIP medium in pH 4.3 without adding $Ca_3(PO_4)_2$ and supplemented with/without 1.84 g/L Na_2HPO_4 (400 mg/L P) or 2.15 g/L $CaCl_2$ (776.46 mg/L Ca^{2+}). Samples were collected at day 0, 0.5, 1, 2, 3, 4, 5, and 6, and filtered through a 0.22-μm filter. TC concentration was detected by HPLC.

Quantification of TC by HPLC

The concentration of TC was determined by high-performance liquid chromatograph (HPLC; Agilent 1260 II, USA) as described in our previous study (Tan et al. 2022).

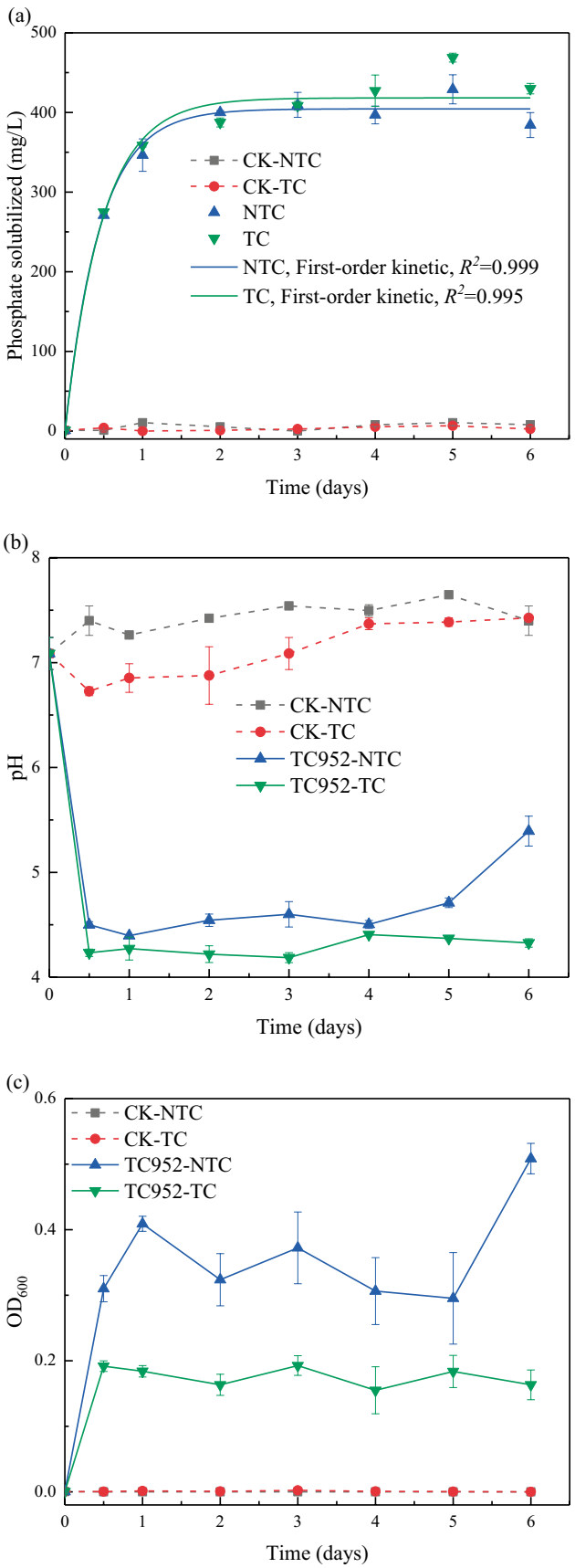

Fig. 1 Phosphate-solubilizing ability (**a**), solution pH (**b**), and bacterial growth (**c**) of strain TC952 under TC stress or not. CK-TC, CK-NTC means with the absence of strain TC952 in TC stress or in no TC stress conditions, respectively. TC952-TC, TC952-NTC means with the presence of strain TC952 in TC stress or in no TC stress conditions, respectively

Genome analysis

Two percent stock bacterial suspensions were inoculated into LB medium and incubated at 30 °C and 150 rpm. Bacteria cultures were collected after 1-day incubation, and samples were centrifugated at 10,000 rpm 4 °C in 2 min; then, the bacterial pellet was obtained through discarding the supernatant and used to extract genome DNA. The genome DNA of *Pseudomonas* sp. TC952 was extracted and sequenced at NextOmics Inc. (Wuhan, China). The obtained DNA sequence was analyzed and CDS were predicted through prodial. The function of CDS were blasted in six different function databases, the clusters of orthologous groups (COG) database, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the Gene Ontology (GO) database, reference sequence (Refseq) database, Pfam database, and TIGRFAMs. Furthermore, the antibiotics resistance genes were identified through blasting in the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al. 2017; Zhang et al. 2020).

Statistical analysis

The mean values and corresponding standard deviations of the triplicates were calculated. The software of IBM SPSS statistics 26 was used to calculate statistical significance, and it was assumed when the p value < 0.05.

Results and discussion

TC stress on phosphate solubilizing ability and bacterial growth of strain TC952

Pseudomonas sp. TC952 was isolated from soil in our previous work, and it had strong ability to remove tetracycline through extracellular polymeric substance (EPS) adsorption (Tan et al. 2022). In this study, strain TC952 was identified with inorganic phosphate-solubilizing ability, and the inorganic phosphate-solubilizing ability with/without TC stress was investigated (Fig. 1a). In *Pseudomonas* genus, many species were isolated with the ability of solubilizing inorganic phosphate, such as *P. aeruginosa*, *P. prosekii* YLYP6, and *Pseudomonas* sp. YLYP29 (Gupta et al. 2022; Yu et al. 2019). During the experiment, soluble phosphate was not detected in the medium with the absence of strain TC952.

After inoculation of strain TC952 into the medium, the soluble phosphate concentration increased sharply with/without TC stress. The soluble phosphate concentration increased from 0 to 400.06 mg/L at day 2 without TC stress, and then fluctuated in a range of 384.25 to 429.05 mg/L at day 3 to day 6. Under TC stress condition, the soluble phosphate concentration increased from 0 to 468.87 mg/L at day 5, and then decreased to 429.91 mg/L at day 6. At the beginning of incubation time at day 0 to day 4, the soluble phosphate concentration did not have difference between without TC stress and with TC stress conditions, but the soluble phosphate concentration under TC stress was higher than that under no TC stress at day 5 to day 6, which mean that TC promoted inorganic phosphate solubilizing at days 5–6. The phosphate solubilization of strain TC952 under no TC or TC stress fitted well with the first-order kinetics model, and the correlation coefficient R^2 was 0.999 and 0.995, respectively (Table 1). The solubilizing constant under TC stress (416 mg/L day^{-1}) was higher than that under no TC condition (400 mg/L day^{-1}) (Table 1), which indicated that 10 mg/L TC promoted phosphate solubilizing of strain TC952 compared with no TC stress.

As shown in Fig. 1b, the solution pH remained nearly at pH 7.4 with the absence of strain TC952. But with the inoculation of strain TC952 into the medium, the solution pH decreased sharply, and the solution pH value under TC stress was lower than that under no TC stress significantly, but the decreasing trend of pH was similar. Under no TC stress condition, the solution pH decreased from pH 7 to pH 4.4 at day 1 and then fluctuated in a pH range of pH 4.4 to 4.7 at day 2 to day 5, but increased to pH 5.4 at day 6. Under TC stress condition, the solution pH decreased to pH 4.23 at day 0.5 and then fluctuated in a pH range of pH 4.19 to 4.41 at day 1 to day 6. It was reported that the acidification of medium by PSB is one of the principal causes of inorganic phosphate solubilization through secreting organic acids, such as gluconic acid, oxalic acid, and citric acid (Biswas et al. 2018; Teng et al. 2019). So, acidification is one of the strategies to solubilize inorganic phosphate by strain TC952.

The bacteria growth of strain TC952 was also tested during the experiment (Fig. 1c), no bacteria growth was detected in the medium without the inoculation of strain TC952. The bacteria grow fast when strain TC952 was inoculated into the medium, and strain TC952 grow to OD_{600} at 0.41 at day 1, and then fluctuated in an OD_{600} range at 0.3 to 0.51 under no TC stress. Under TC stress, the growth of strain TC952 increased to OD_{600} at 0.19 at day 0.5 and then fluctuated in an OD_{600} range at 0.16 to 0.19 at day 1 to day 6 (Fig. 1c). From the result, 10 mg/L TC inhibits the growth of strain TC952, which was different with our previous result that 0–50 mg/L TC did not affect the growth of strain TC952 (Tan et al. 2022). It can be explained by that the ability of tolerating TC stress of strain TC952 under oligotrophic conditions was weak than under nutrient-rich conditions. Furthermore, the bacterial growth variation trends were different with pH changes, which means that TC stress-promoted pH decreasing, but inhibited bacterial growth of strain TC952. These results suggested that TC stress regulating the bacterial growth and pH decreasing is divergently affected.

Table 1 Fitting phosphate solubilizing kinetics with strain TC952 under TC/no TC conditions

Treatment	First-order kinetic model fitting equation	k (mg/Ld^{-1})[a]	b	R^2
NTC	$c = k \cdot (1\text{-}\exp(-bt))$	400	2.12	0.999
TC		416	2	0.995

[a]Rate constant

Phosphate-solubilizing process on TC removal

Next, the effect of phosphate-solubilizing process on TC removal by strain TC952 was investigated (Fig. 2). With the absence of strain TC952, the concentration of TC did not change without adding inorganic phosphate ($Ca_3(PO_4)_2$) at day 1 to day 6, which mean that TC did not hydrolyze in no phosphate NBRIP medium. The reason is that the presence of high concentration of divalent cations in no phosphate NBRIP medium (Mg^{2+}, Fe^{2+}, and Mn^{2+}) inhibited TC hydrolysis. It was reported that TC can chelate with cation

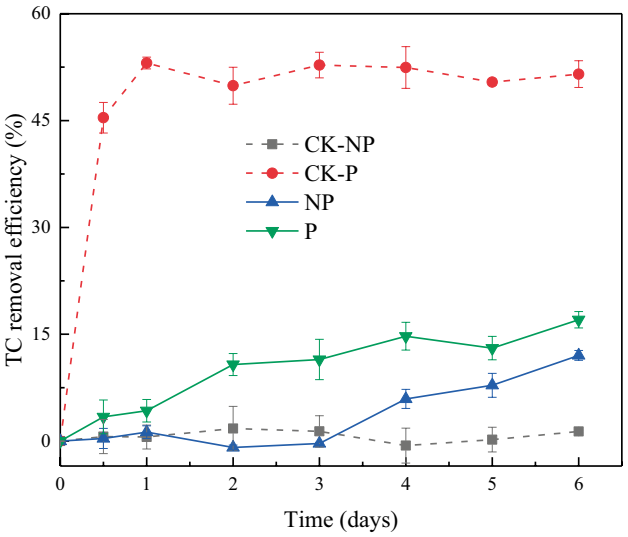

Fig. 2 The effects of phosphate solubilizing process on TC removal efficiency by strain TC952. CK-NP, CK-P means with the absence of strain TC952 in no inorganic phosphate or in inorganic phosphate conditions, respectively. TC952-NP, TC952-P means with the presence of strain TC952 in no inorganic phosphate or in inorganic phosphate conditions, respectively

metals (ionic radius between 0.55 and 0.86 or > 0.7 Å) to form metal-TC complex (Zhang et al. 2014). Same phenomenon was also observed in our previous study that 0.1–2 μg/L Zn^{2+} inhibited TC hydrolysis (Tan et al. 2022). But, TC concentration decreased sharply when inorganic phosphate was added into the medium, and the TC removal efficiency reached to 53.09% at day 1, then fluctuated in a range at 49.9% to 52.8% at day 2 to day 6, which mean that TC was adsorbed by inorganic phosphorus. When strain TC952 was inoculated into medium with the absence of inorganic phosphate, TC concentration did not change at the beginning of day 0 to day 3. But, TC concentration decreased slightly at day 4 to day 6, and the removal efficiency was 5.92%, 7.84%, and 12.03%, respectively. However, when strain TC952 was inoculated into medium with the presence of inorganic phosphate, TC removal efficiency increased to 10.75% at day 2, and then fluctuated in a range of 11.46 to 17.04 at day 3 to day6. So, the phosphate-solubilizing process by strain TC952 removed the amount of insoluble phosphate and reduced the amount of adsorbed TC by inorganic phosphorus.

Mechanism of TC removal under the phosphate-solubilizing process

As depicted above, inorganic phosphate can remove TC through adsorption, and strain TC952 was capable of solubilizing inorganic phosphate which inhibited TC removal significantly (Fig. 2). Under the inorganic phosphate-solubilizing process of strain TC952, the solution pH decreased to nearly pH 4.3, and many soluble phosphorus and Ca^{2+} were produced and released into the solution. So, the effects of different factors (acidic condition (pH 4.3), PO_4^{3-}, and Ca^{2+}) produced on the inorganic phosphate-solubilizing process by strain TC952 on TC removal was investigated (Fig. 3).

On pH 4.3 conditions, TC hydrolyzed slightly in 3 days (hydrolysis efficiency 6.5%); then, the hydrolysis efficiency plateaued at day 3 to day 6, which indicated that acidic condition (pH 4.3) induced TC hydrolysis comparing with under pH 7 conditions (Fig. 2). It is reported that the dimethyl amine group of TC protonated under the action of H^+ at acidic condition; then, the protonated TC or the parent compound could be oxidized through oxygenation under aerobic conditions (Pulicharla et al. 2017; Shao et al. 2019). The amount of H^+ in acidic conditions determined the protonation of TC, which mean that acidic condition promote TC hydrolysis. With the presence of Ca^{2+} on pH 4.3 solution, the hydrolysis of TC was inhibited significantly, and the hydrolysis efficiency was nearly 0 at day 0 to day 6, which indicated that Ca^{2+} strongly inhibited TC hydrolysis. It was reported that TCs could bind with some metal ions and form TCs-metal (TCs-M) complex according to the size of metal ion, metal ions with radius of 0.55–0.86 Å

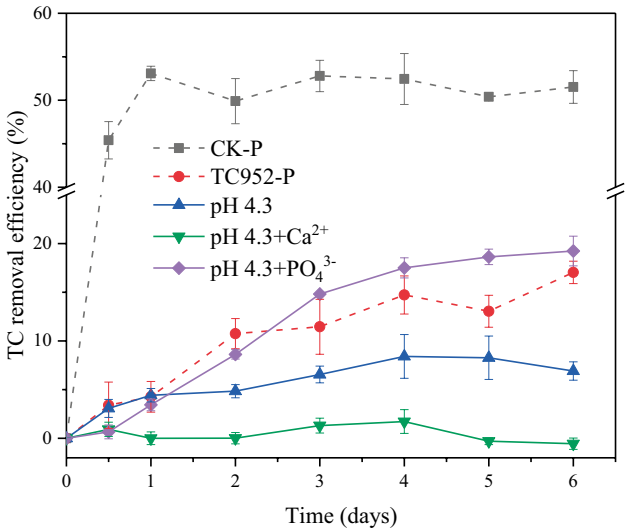

Fig. 3 The effects of different solution factors on TC removal efficiency CK-P and TC952-P means without/with the presence of strain TC952 in organic phosphate conditions, respectively

and > 0.7 Å preferably bind with site "a" (C2 and C3 position of A ring) and site "b" (B, C, and D ring), respectively (Pulicharla et al. 2017). Ca^{2+} (ionic radii 1.12 Å) could bind with TC site "b," which induced TC extended and inhibited H^+/OH^- attacking TC compound and finally inhibited TC hydrolysis (Chen and Huang 2011). With the presence of PO_4^{3-} in pH 4.3 solution, TC hydrolysis did not occur in 1 day (TC hydrolysis efficiency was 0), but the hydrolysis efficiency increased significantly at day 1 to day 6, which were higher than that in pH 4.3 solution and also higher than that in inorganic phosphate medium with the presence of strain TC952 (Fig. 3). So, PO_4^{3-} promoted TC hydrolysis significantly. Few researches have reported the interaction between anions and TC, especially phosphate anions. However, it was reported that when pH > 4, the predominant species of phosphate were HPO_4^{2-} (phosphate was added as NaH_2PO_4) (Wang et al. 2010), which decreased the zeta-potential of iron oxide surface significantly and resulted in weakening TC adsorption on three iron oxides minerals (Zhu et al. 2020). Same phenomenon of zeta-potential decreasing was observed by Shao et al. (2019) that zeta-potential decreased rapidly to minimum value (27 to − 20.3 mV) during TC biodegradation of strain *Klebsiella* sp. SQY5. So, the addition of phosphate at pH 4.3 condition may influence the redox state of the solution, which finally promoted TC hydrolysis. From these results, TC hydrolysis was promoted by acidic condition (pH 4.3) and PO_4^{3-} comparing with that under pH 7 condition (Fig. 2), but inhibited by divalent ion (Ca^{2+}). In conclusion, in the process of inorganic phosphate solubilizing, strain TC952 could acidify the solution to pH 4.3 (secrete organic/inorganic acid) to dissolve inorganic phosphorus and also released Ca^{2+} and PO_4^{3-} into the

solution, which mean that acidic condition and PO_4^{3-} promoted TC hydrolysis. And, the phosphate (PO_4^{3-}) in the solution also released the adsorbed TC into the solution and then promoted TC hydrolysis.

Genome analysis

The genome of *Pseudomonas* sp. TC952 was sequenced and analyzed (Table 2). The genome length of strain TC952 was 5,564,089 bp with a GC content of 61.93%, and no plasmid was detected in strain TC952. There are 80 tRNA, 22 rRNA, and other 32 ncRNA in the genome of strain TC952. 3 GIs and 0 CRISPR were detected in the genome of strain TC952. And, 5023 protein coding genes was annotated by blasting in 6 protein functional annotating database, which account for 89% of the whole genome sequence.

Among 5023 genes, 3377 genes were annotated by COG database (Cluster of Orthologous Groups), which included 24 category functional classification (Fig. 4a). Abundant genes participated in amino acid transport and metabolism (E, 452, ~ 9%); general function prediction (R, 359, ~ 7.15%); transcription (K, 382, ~ 7.61%), signal transduction mechanisms (T, 301, ~ 5.99%), translation, ribosomal structure and biogenesis (J, 273, ~ 5.43%); energy production and conversion (C, 251, ~ 5%); cell wall/envelope/membrane biogenesis (M, 233, ~ 4.64%); lipid transport and metabolism (I, 204, ~ 4.06%); inorganic ion transport and metabolism (P, 204, ~ 4.06%); and coenzyme transport and metabolism (H, 201, ~ 4%) (Fig. 4a). Meanwhile, 2769 genes were annotated by KEGG database, including metabolism (955), environmental information processing (411), cellular processing (277), genetic information processing (206), human disease (101, including 31 antimicrobial drug resistance genes and 14 antineoplastic drug resistance genes), and organism systems (46) (Fig. 4b). Furthermore, 3018 genes were annotated by GO database, which were classified into three categories: cellular component, molecular function, and biological process (Fig. 4c).

Table 2 Genome statistical results of filtered sequencing data and characteristics of the newly sequenced genome of *Pseudomonas* sp. TC952

Issue	Number	Issue	Number
Genome size (bp):	5,564,089	Total base length	898,594,520
GC content (%):	61.93	Gap total length (bp)	0
CDS number:	5023	Contig number	1
CDS length (bp):	4,952,265	Contig length (bp)	5,564,089
CDS length/genome (%):	89	Contig N50 (bp)	5,564,089
tRNA number:	80	Contig N90 (bp)	5,564,089
tRNA length (bp):	6,253	Contig Max (bp)	5,564,089
rRNA number:	22	Plasmid number	0
rRNA length:	31,833	GIs number:	3

As shown in Tables 3 and 4 antibiotics resistance genes were annotated in the chromosome after blasting the genome sequences of strain TC952 on the Comprehensive Antibiotic Research Database (CARD), including 3 copies of *adeF* and 1copy of *tet(A)*. *adeF* coding gene (a resistance-nodulation division (RND) antibiotic efflux pump coding gene) was responsible for fluoroquinolone antibiotic and tetracycline antibiotic resistance. It was reported that RND antibiotic efflux pump broadly existed in bacteria genome and can pump out many kinds of antibiotic substrates from bacterial cell (Leng et al. 2017). *tet(A)* was a major facilitator superfamily (MFS) antibiotic efflux pump coding gene, which was responsible for tetracycline antibiotic resistance. Through blasting the sequence of *tet(A)* in NCBI, it is found that *tet(A)* gene also existed in other *Pseudomonas* species genome, such as *P. aeruginosa* GIMC5034:PA52Ts32 (QLJ86063), *P. putida* NCTC10936 (SUD75896), and *P. oleocorans* NCTC10860 (SUD60024), which indicated that those *Pseudomonas* species may capable of resistance TC to grow.

It is reported that bacteria had many ways to solubilize inorganic phosphate, such as production of organic acids, production of inorganic acid and H_2S, proton releasing from NH_4^+ (assimilation/respiration), direct oxidation pathway, and siderophore production (Rawat et al. 2020). As shown in Table 4, 16 genes were identified and related with inorganic phosphate solubilizing, including 1 *pqqD*, 2 *gcd*, 1 *eno*, 1 f*epC*, 9 *cirA*, and 2 *fepD*. Among them, *gcd* gene encoded glucose dehydrogenase enzyme (a quinoprotein), which mediated secretion of gluconic acid (Suleman et al. 2018). And, *gcd* gene plays an important role in direct oxidation pathway, gluconic acid, and further oxidized 2-ketogluconic acid act as chelators of minerals. *pqqD* gene encoded a small, redox-active molecule, and a cofactor for glucose dehydrogenase (Shahid et al. 2012), which is responsible for dehydrogenase activity and mineral phosphate solubilization. *eno* gene encoded enolase and function as phosphopyruvate hydratase, which involved in phosphorus solubilization (Liu et al. 2019). *fepC* gene encoded an iron complex transport system ATP-binding protein, which act as ABC-type cobalamin/Fe^{3+}-siderophores transport (Kaur and Reddy, 2014; Schubert et al. 1999). *cirA* gene encoded a TonB-dependent siderophore receptor protein and act as iron complex outer membrane receptor (Pinkert et al. 2021). *fepD* was another siderophore coding gene; it is an ABC-type Fe^{3+}-siderophore transport system and acts as permease component (Schubert et al. 1999). According to the genome analysis, strain TC952 can secrete organic acid to resolve inorganic phosphate and also secrete siderophore to chelate inorganic phosphate and help it to enter into the cell.

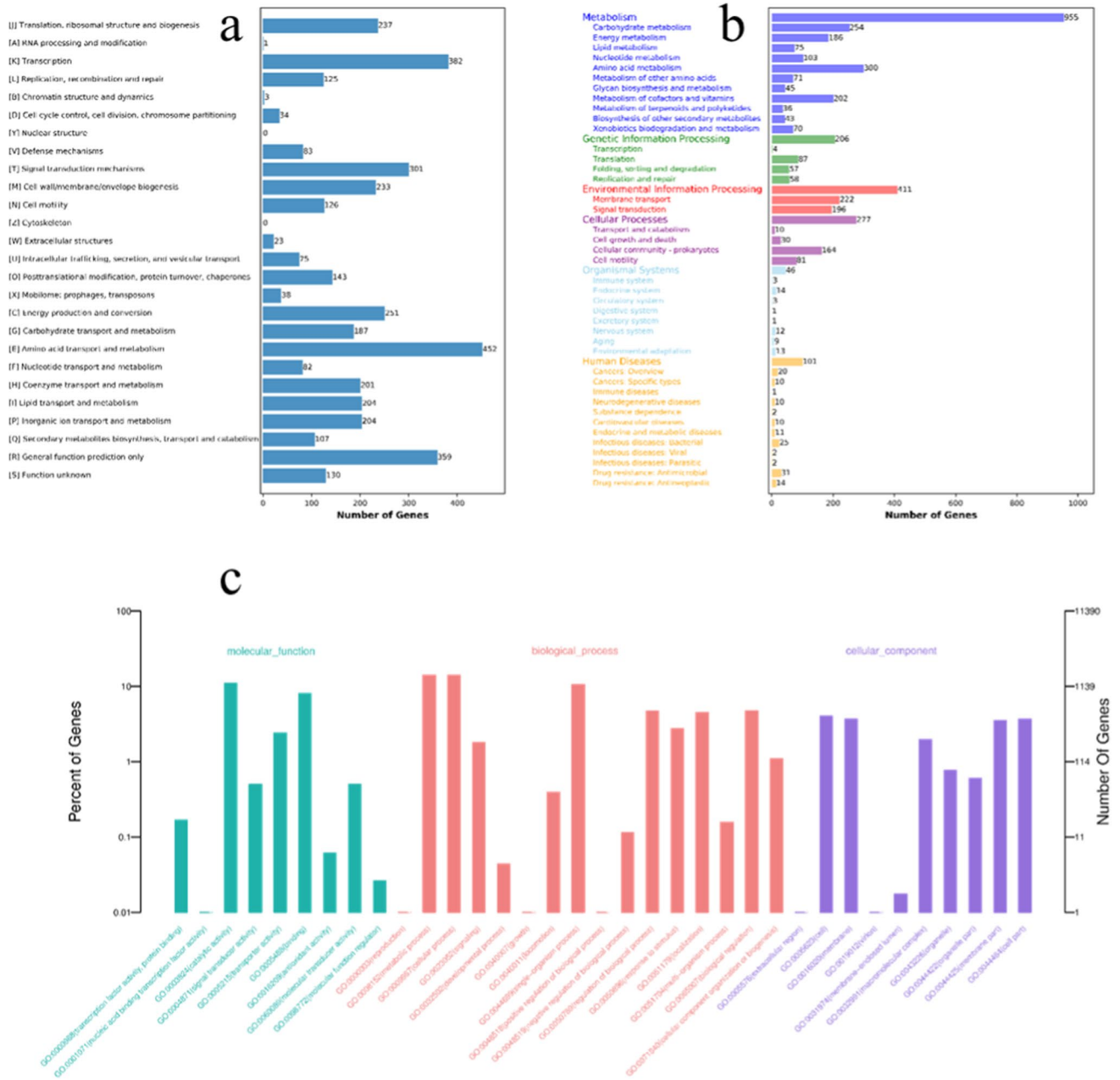

Fig. 4 Percentage, distribution, and number of functionally annotated genes obtained from COG (**a**), KEGG (**b**), and GO (**c**)

Table 3 Predicted antibiotics resistance-related genes in *Pseudomonas* sp. TC952 genome

ARO name	Gene ID	Position	Resistance mechanism	Drug class
adeF	NPGAP_04810	1,072,565.0.1075717	Antibiotics efflux	Fluoroquinolone antibiotic, tetracycline antibiotic
adeF	NPGAP_11395	2,504,371.0.2507550	Antibiotics efflux	Fluoroquinolone antibiotic, tetracycline antibiotic
adeF	NPGAP_12490	2,757,904.0.2761026	Antibiotics efflux	Fluoroquinolone antibiotic, tetracycline antibiotic
tet(A)	NPGAP_19265	4,185,144.0.4186343	Antibiotics efflux	Tetracycline antibiotic

Table 4 Predicted phosphorus solubilizing related genes in *Pseudomonas* sp. TC952 genome

Gene name	Gene ID	Position	Functional description
pqqD	NPGAP_23630	5,124,973…5,125,248	A small, redox-active molecule and a cofactor for glucose dehydrogenase, responsible for dehydrogenase activity
gcd	NPGAP_05105	1,139,431…1,141,842	Catalyzes the conversion of glucose to gluconic acid
gcd	NPGAP_13480	2,979,683…2,982,100	Catalyzes the conversion of glucose to gluconic acid
eno	NPGAP_19720	4,272,969…4,274,258	Enolase gene, phosphopyruvate hydratase, responsible for phosphorus solubilization
fepC	NPGAP_10435	2,289,313…2,290,101	ABC-type cobalamin/Fe^{3+}-siderophores transport system
cirA	NPGAP_02790	626,011…628,068	Iron complex outer membrane receptor protein; TonB-dependent receptor
cirA	NPGAP_03955	886,354…888,744	Siderophore transmembrane transporter activity; TonB-dependent siderophore receptor
cirA	NPGAP_05115	1,143,461…1,145,503	Iron complex outer membrane receptor protein; TonB-dependent receptor plug domain
cirA	NPGAP_08840	1,969,069…1,971,504	TonB-dependent siderophore receptor
cirA	NPGAP_14780	3,255,380…3257,500	Iron complex outer membrane receptor protein; TonB-dependent receptor
cirA	NPGAP_15715	3,431,565…3,433,625	Iron complex outer membrane receptor protein; TonB-dependent receptor
cirA	NPGAP_16655	3,590,427…3,592,574	Iron complex outer membrane receptor protein; TonB-dependent receptor
cirA	NPGAP_20475	4,444,686…44,47,271	TonB-dependent heme/hemoglobin receptor family protein
cirA	NPGAP_23760	5,153,590…5,156,395	Siderophore transmembrane transporter activity; TonB-dependent siderophore receptor
fepD	NPGAP_03485	786,528…787,565	ABC-type Fe^{3+}-siderophore transport system; permease component
fepD	NPGAP_10440	2,290,098…2,291,114	ABC-type Fe^{3+}-siderophore transport system; permease component

Conclusion

This study deepens our understanding of the influence of the phosphate-solubilizing process on TC removal by the bacterial strain *Pseudomonas* sp. TC952. Strain TC952 was capable of solubilizing inorganic phosphate, and the soluble phosphate concentration increased to 400 mg/L in 2 days without/with TC stress. TC stress didn't influence the phosphate solubilizing of strain TC952 in a short time, but promoted in a long incubation time. The removal of TC in inorganic phosphate medium was due to adsorption. During the phosphate-solubilizing process of strain TC952, the solution was acidified into pH 4.3, and Ca^{2+} and PO_4^{3-} were released into the solution. The acidified solution and PO_4^{3-} promoted TC hydrolysis significantly. Four tetracycline antibiotic resistance genes and 16 inorganic phosphate-solubilizing-related genes were identified in the genome of strain TC952. The genome analysis revealed that strain TC952 can secrete organic acid and siderophore to acidification and chelation which were responsible for inorganic phosphate solubilizing.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Zewen Tan and Xiuyue Yang. Investigation and sample testing were performed by Zewen Tan and Jianpeng Gao. Supervision, writing—reviewing and editing, and funding acquisition were performed by Yongtao Li and Beini Gong. The first draft of the manuscript was written by Zewen Tan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Not applicable.

Declarations

Ethical approval All authors of the article have accepted and observed the ethical issues.

Consent to participate and publish All authors have expressed their consent to participate in the writing and publication of the article.

Competing interests The authors declare no competing interests.

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