Journal of Oceanology and Limnology
Vol. 38 No. 1, P. 195-203, 2020
https://doi.org/10.1007/s00343-019-8323-6

Characteristics of intracellular polyphosphate granules and phosphorus-absorption of a marine polyphosphate-accumulating bacterium, *Halomonas* sp. YSR-3*

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Received Nov. 6, 2018; accepted in principle Dec. 24, 2018; accepted for publication Mar. 8, 2019

Abstract *Halomonas* sp. YSR-3 was isolated from the Yellow Sea and identified as a polyphosphate-accumulating bacterium and the characteristics of its intracellular polyphosphate (polyP) granules and phosphorus absorption were studied. Most YSR-3 cells stored one or two polyP granules in regular appearance and high-density. The diameter of the granules was about 400 nm measuring by a transmission electron microscope (TEM). After stained with 4,6-diamidino-2-phenylindole (DAPI) and visualized by a fluorescence microscope, the cells turned blue and the granules were bright yellow. The composition of granules includes P (major ingredient), Mg, S, K, and Ca as detected by an energy dispersive X-ray spectrometer (EDS). When inorganic phosphorus (PO_4^{3-}) and ferric ion (Fe^{3+}) were added into media, the biomass increased and the cells formed intracellular polyP granules owing to the phosphorus assimilation from media. The YSR-3 obtained higher biomass by adding 0.02 g/L $FePO_4$ than 0.005 g/L and 0.01 g/L $FePO_4$; however, the phosphorus absorption was higher with 0.01 g/L $FePO_4$ than 0.005 g/L and 0.02 g/L $FePO_4$. The optical density at wavelength 480 nm ($OD_{480\,nm}$) was 0.79 and 100% cells could form intracellular polyP granules. These results show that strain YSR-3 is able to acquire higher biomass and absorb more inorganic phosphorus when 0.01 g/L $FePO_4$ is added. The characteristics of absorbing and storing phosphorus as intracellular inorganic polyP granules have a potential for application in high-efficiency phosphorus removal in wastewater treatment.

Keyword: polyphosphate-accumulating bacterium; polyphosphate granule; *Halomonas*; enhanced biological phosphorus removal

1 INTRODUCTION

Eutrophication (extraordinary growth of algae) of water bodies is attracting more and more attention (Saleem et al., 2011). One of its main controlling factors is phosphorus (Lucas et al., 2010; Cai et al., 2011). Phosphorus in wastewater is a major control factor to the eutrophication of rivers, lakes, and seas worldwide. When excessive phosphorus is released into natural water bodies such as lakes and inland seas, a large variety of seriously harmful outcomes may occur. It is necessary to remove phosphorus from wastewater to avoid eutrophication when wastewater is discharged into water bodies (El-Kamah et al., 2011). Using water treatment methods, people have put forward measures to prevent and control phosphorus pollution (Gillor et al., 2010). Activated sludge processes with alternating anaerobic and aerobic conditions have been used successfully for

* Supported by the National Natural Science Foundation of China (Nos. 51308245, 31570061), the Open Project of Jiangsu Provincial Engineering Laboratory for Advanced Materials of Salt Chemical Industry (No. SF201408), and the Open Project of Jiangsu Provincial Key Construction Laboratory of Probiotics Preparation (No. JSYSZJ2017006)
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enhanced biological phosphorus removal (EBPR) from wastewater (Akar et al., 2006).

In the EBPR technology, polyphosphate-accumulating bacteria (PAB) play a crucial role because of their super phosphorus absorption properties. Understanding the general structures of microbial communities of activated sludge and their metabolism process, especially phosphorus metabolism pathway, is important to improve the EBPR technology (Luan et al., 2013). Quite a few of pure cultures of PAB have been isolated in EBPR. However, research on manipulating the properties of EBPR focused on enriched-community cultures but not a pure culture. Without pure culture, understanding the microbiology and biochemistry of EBPR is a challenge (Chaudhry and Nautiyal, 2011). The application of EBPR in wastewater treatment is therefore limited by the acquisition of PAB pure culture and the instability of EBPR (Akar et al., 2006).

Some kinds of PAB have been exploited in EBPR to assimilate and store phosphorus as intracellular polyphosphate (polyP) granules. *Acinetobacter* spp. is a common species in wastewater and can be isolated by EBPR process for treatment plants. Because *Acinetobacter* spp. is isolated from activated sludge and dominant in the communities of EBPR microflora, they are considered the predominant polyphosphate-accumulating organisms for carrying out functions of EBPR process (Ong et al., 2014). Moreover, other similar microbes can accumulate polyP in wastewater treatment plants. For example, *Microlunatus phosphovorus* strain NM-1 is originally isolated from an EBPR process and can accumulate large amounts of polyP (an approximate maximum 48% of its dry weight as phosphate) in a glucose medium (Hirota et al., 2010). It is a gram-positive, coccus-shaped, non-spore-forming bacterium (Hirota et al., 2010). Most PAB can form polyP granules, which is an inorganic molecular and thought to be used as a reservoir of intracellular phosphate (Ong et al., 2014). PolyP is observed in all living organisms including yeast and mammals, and consists of three to many thousands of inorganic phosphate residues. In prokaryotes, polyP regulates a large range of biological functions (Alcántara et al., 2014).

Strain YSR-3, presented in this paper, was a PAB isolated from the Yellow Sea in our earlier study, and classified as a *Halomonas* sp. based on the sequence of 16S rDNA (Ren et al., 2008). Its cell was rod-shape, 3.5 μm×1 μm, gram-negative and aerobic. It could accumulate polyP and form intracellular metachromatic granules. Cultivated in domestic wastewater, its ratio of phosphorus-removal efficiency was approximate by 72.2%. Although it was previously shown that YSR-3 could absorb phosphate, the information of intracellular polyP appearance, the factors influencing the formation of polyP granules and its characteristics of phosphorus absorption are unknown. In this study, we analyzed the components and characteristics of intracellular polyP granules, the features of growth and inorganic phosphorus absorption with different compounds. These results may help enhance wastewater treatment efficiency and promote future application of strain YSR-3 in the industrial field.

2 MATERIAL AND METHOD

2.1 Bacterial strain and culture conditions

The bacterial strain used in this study is *Halomonas* sp. YSR-3 and the cells are cultivated in 250-mL shake flasks with 100 mL media including (L): 5 g peptone, 1 g yeast extract, 2 mL 0.01 mol/L ferric quinic acid ($FeC_7H_{12}O_6$), seawater to 1 L, adjust pH to 6.5, grow at 180 r/min in 24°C. In order to obtain start seed, culture YSR-3 is inoculated in the above-mentioned fresh media from freshly streaked plates and cultivated for 12 h.

2.2 Cell preparation

Cells of *Halomonas* sp. YSR-3 are harvested by centrifugation (3 200×*g* for 5 min) at the stationary phase of growth (cultivated for 20 h). The samples are washed twice with phosphate-buffered saline (PBS) (0.4 mol/L Na_2HPO_4/NaH_2PO_4, 150 mmol/L NaCl, pH 7.2) and harvested by centrifugation.

2.3 Visualization of polyP granules by transmission electron microscopy (TEM)

Cells originating from the same colony on fresh plate are diluted in sterile seawater and mixed well. The cells are fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at room temperature and then washed in phosphate buffer. This cells solution is directly dropped on the 200-mesh copper-grid and the copper-grid is placed in a desiccator for dehydration overnight, and then examined by TEM (Hitach H8100). Electron micrographs are taken with a slow-scan camera (Gatan model 679). The size of the polyP granules is calculated with software Image-Pro Plus 5.1.

2.4 Cells stained with 4,6-diamidino-2-phenylindole (DAPI)

A 30-μmol/L DAPI stock solution is added into the cell suspension of strain YSR-3 harvested in stationary phase, until its final concentration reach 1 mg/L, mixed well and incubated for at least 8 h. After stainning, the cells is filtered and poured on a 0.22-μm millipore filter by vacuum filtration, and then examined by fluorescence microscope (Olympus BH-2, filter 450/50) (Tian et al., 2013; Weissbrodt et al., 2013).

2.5 Analysis of polyP granules by EDS

The copper-grid holding cells of strain YSR-3 was detected by TEM (JEX 2000FX JEOL, 200 kv), then the eyeshot is located on the cells containing intracellular polyP granules. Subsequently the components of intracellular polyP granule are analyzed by an energy dispersive X-ray spectrometer (Tracor Series II micro-analytical EDS-system), and the non-granule zone of cell and the blank field of the copper-grid are served as controls.

2.6 The growth and phosphorus absorption of YSR-3 with different substrates

To detect the influence of Na_3PO_4 on the growth and phosphorus absorption, YSR-3 is cultivated in medium 1 containing (L): 5-g peptone, 1-g yeast extract, 2-mL 0.01 mol/L $FeC_7H_{12}O_6$, and 30-g NaCl, added 0.82-g Na_3PO_4 as control. The medium 2 containing (L): 5-g peptone, 1-g yeast extract, and 30-g NaCl, respectively added with 2-mL 0.01 mol/L $FeC_7H_{12}O_6$, 0.016-g FeCHO (ferric citric acid), 0.012-g $FePO_4$ (ferric phosphate), and 0.011-g $FeCl_3$ (ferric trichloride), until the final concentration of Fe^{3+} is 0.066 mmol/L, was used to observe the influence of different iron compounds, no Fe^{3+} adding as blank control. Medium 3 containing (L): 5-g peptone, 1-g yeast extract, and 30-g NaCl, respectively added with 0.005-g $FePO_4$ (0.033 mmol/L Fe^{3+}), 0.01-g $FePO_4$ (0.066 mmol/L Fe^{3+}), and 0.02-g $FePO_4$ (0.132 mmol/L Fe^{3+}) is used to examine the influence of $FePO_4$ concentration. The medium is replenished to 1 L with distilled water, adjusted pH to 6.5 with NaOH solution, and sterilized at 113°C for 20 min. The biomass of strain YSR-3 was measured by a spectrophotometer (Unic 7200) at $OD_{480\,nm}$. The amount of PO_4^{3-} in the supernatant is indicated by $OD_{882\,nm}$ via the ammonium molybdate spectrophotometric method (GB 11893-1989). The average value is deduced from triplicate experiments.

2.7 The formation of polyP granules by strain YSR-3

A single colony is picked up and diluted in 2-mL sterilized water to prepare high-concentration cell-solution. A 1-mL solution of medium 4 containing (L): 1-g $C_4H_4Na_2O_4$ (succinic acid disodium), 0.25-g $NaNO_3$, 0.2-g CH_3COONa, 0.075-g $C_2H_3NaO_2S$ (sodium thioglycolate), 10-mL Wolfe's vitamin solution, 10-mL Wolfe's mineral solution (Wolin et al., 1963), 0.01-g $FePO_4$ (0.066 mmol/L Fe^{3+}), and 30-g NaCl is inoculated and incubated at 24°C at 180 r/min. Both the biomass and the amounts of PO_4^{3-} in supernatant are measured by a spectrophotometer (Unic 7200) per 12 h, and then respectively marked as $OD_{480\,nm}$ and $OD_{882\,nm}$. After DAPI staining and fluorescence microscopy visualization, the numbers of total cells and the cells forming intracellular polyP granules are calculated. The ratio is calculated by the equation: (the number of cells containing polyP granules / the number of total cells) × 100%. The average value is deduced from triplicate experiments.

3 RESULT

3.1 Morphology of intracellular polyP granules

The TEM photo of strain YSR-3 show that there are one or two granules in the polar position of cells, and the granules were round with regular appearance and high-density. Its diameter is about 400 nm (Fig.1a). After stained with DAPI, the color of the cytoplasm is blue and the intracellular polyP granules are bright yellow. Similar to the TEM image, the yellow granules are located at one or two ends of cells (Fig.1b).

3.2 Phosphorus stored as polyP granules by YSR-3

The components of granules are P, Mg, S, K, and Ca as determined by EDS (Fig.2). Comparing the cytoplasm zone with blank field (only copper-grid), the major ingredient of granules is phosphorus. Combining the result of DAPI staining, we conclude that these intracellular granules contain polyP.

3.3 Na_3PO_4 improved growth and phosphorus absorption of strain YSR-3

When 0.82 g/L Na_3PO_4 was added into the medium 1, the $OD_{480\,nm}$ was up to 0.85 at 24 h and lowered slightly in subsequent cultivation. After the strain was cultivated for 48 h, the biomass became stable (Fig.3a). While strain YSR-3 grew and absorbed

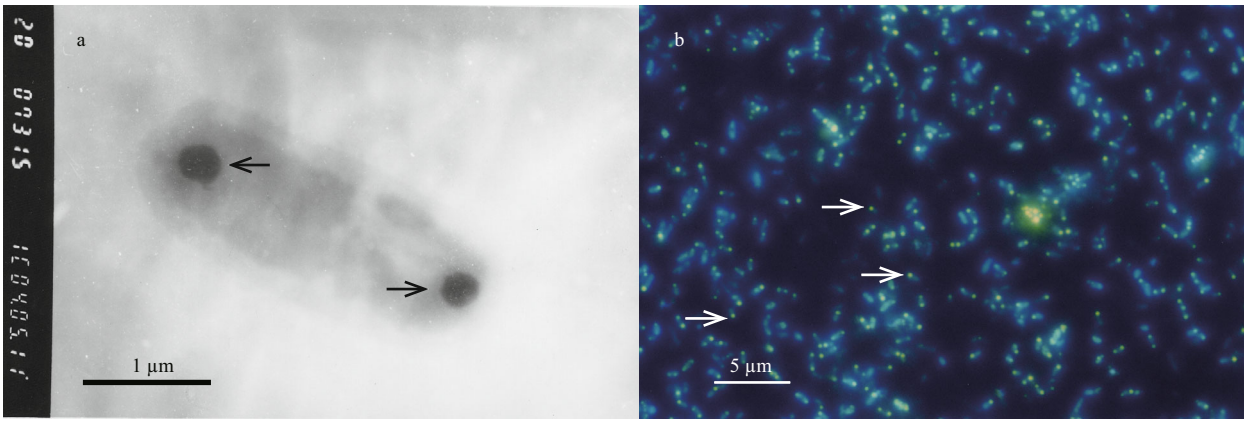

Fig.1 PolyP photo of strain YSR-3

a. photo taken by TEM (×10 000). PolyP granules pointed by arrow showed deep color and about 400 nm; b. photo taken by fluorescence microscope after staining with DAPI (×100). Cells showed blue. PolyP granules pointed by arrow was about 400 nm and showed bright yellow.

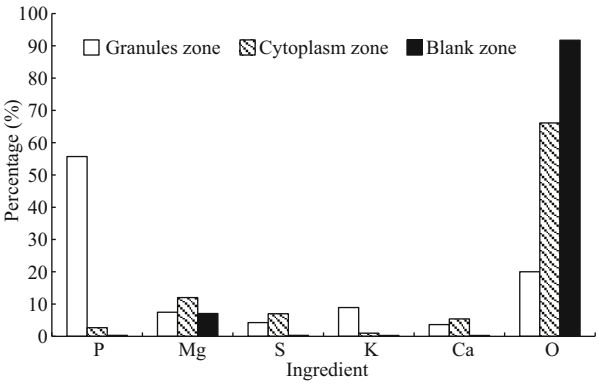

Fig.2 The polyP ingredients of strain YSR-3 by EDS

The main composition of the granules is phosphorus.

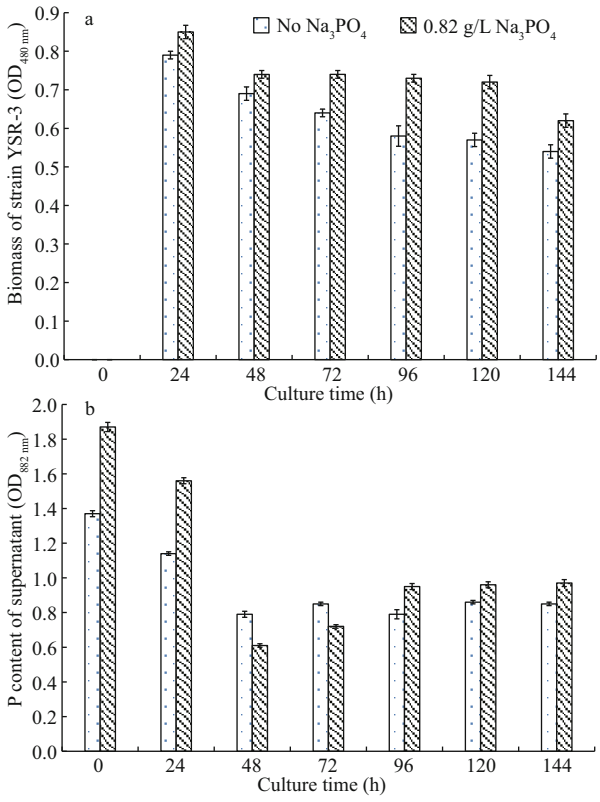

Fig.3 Effect of Na_3PO_4 on strain YSR-3

a. growth was improved by adding 0.82 g/L Na_3PO_4; b. after adding 0.82 g/L Na_3PO_4, phosphorus content of supernatant was decreased meaning phosphorus was absorbed by YSR-3. Strain was cultivated in media without Na_3PO_4 as control.

phosphorus, the amount of phosphorus in supernatant reduced and the value of $OD_{882\,nm}$ lowered. The $OD_{882\,nm}$ gradually decreased during culture process and was down to 0.61 at 48 h, which was at the lowest point. After 48 h, the $OD_{882\,nm}$ slightly increased, and then kept stable from 96 h to 144 h (Fig.3b). These results show that phosphorus is an important factor to the growth of strain YSR-3 and can simultaneously improve the cells' capacity of phosphorus absorption. Therefore, strain YSR-3 can absorb the phosphorus in media and accommodate them as intracellular polyP granules that are bound stably in the cells and cannot be released easily.

3.4 $FePO_4$ enhanced growth and phosphorus absorption of strain YSR-3

For some strains belonging to genus *Halomonas*, Fe^{3+} influences their growth and phenotypic resistance (Church et al., 2000; Harrison et al., 2015). When four kinds of iron compounds, $FeC_7H_{12}O_6$, FeCHO, $FePO_4$ and $FeCl_3$ were respectively added into the medium 2, strain YSR-3 grew well. In the media containing 0.012 g/L $FePO_4$, the $OD_{480\,nm}$ was 0.86 at 24 h, which is higher than that in other media (Fig.4a). In subsequent growth phase, the biomass gradually decreased. Because the initial amount of phosphorus is not equal, the capacity of phosphorus absorption is indicated by $\Delta OD_{882\,nm}$ ($\Delta OD_{882\,nm}=OD_{882\,nm}i-$

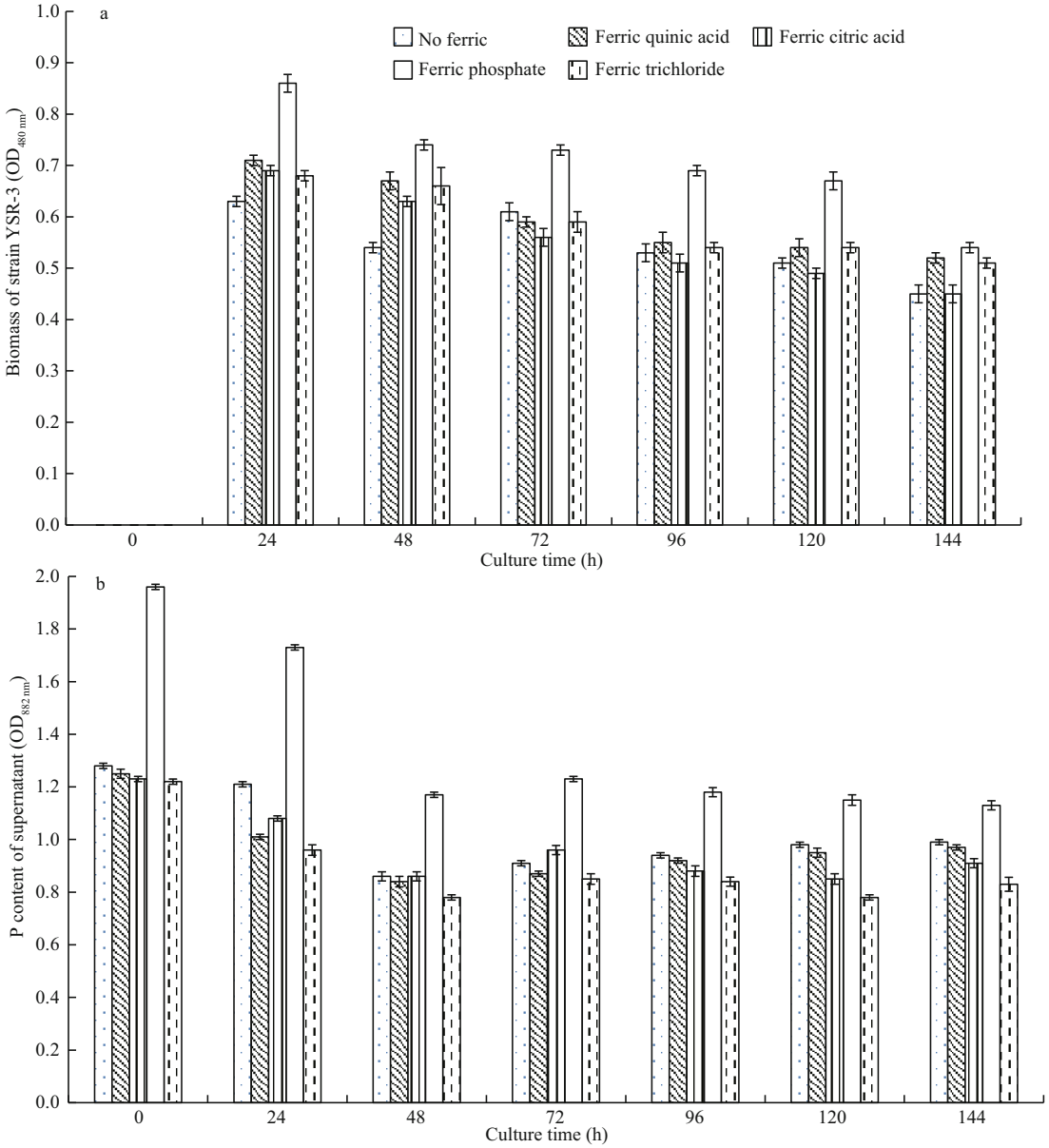

Fig.4 Effect of different iron compounds on strain YSR-3

a. growth of YSR-3 was better with $FePO_4$; b. decline of phosphorus content in supernatant was largest with $FePO_4$. $FePO_4$ was propitious to growth and phosphorus absorption. Final concentration of Fe^{3+} was 0.066 mmol/L.

$OD_{882\ nm}0$, $OD_{882\ nm}i$=$OD_{882\ nm}$ at i h, $OD_{882\ nm}0$=$OD_{882\ nm}$ at 0 h). The higher the $\Delta OD_{882\ nm}$ is, the stronger the ability of phosphorus absorption would be. When adding 0.012 g/L $FePO_4$, the $\Delta OD_{882\ nm}$ was 0.7 at 48 h, which is higher than that with other iron compounds. These results indicate that $FePO_4$ could enhance the growth and phosphorus absorption capacity of strain YSR-3 (Fig.4b).

At different concentrations of $FePO_4$ in medium 3 as 0.005 g/L (0.033 mmol/L Fe^{3+}), 0.01 g/L (0.066 mmol/L Fe^{3+}), and 0.02 g/L (0.132 mmol/L Fe^{3+}), the cells grew well. When the concentration of $FePO_4$ increased, the biomass increased. In the media with 0.02 g/L $FePO_4$, the $OD_{480\ nm}$ was 0.86 and higher than that in other two concentrations at 24 h (Fig.5a). In subsequent growth phase, the $OD_{480\ nm}$ slowly decreased. At 48 h, the $\Delta OD_{882\ nm}$ was up to 0.87 in the media with 0.01 g/L $FePO_4$ and higher than that in other two concentrations. The $\Delta OD_{882\ nm}$ slightly decreased from 48 h to 144 h. These results show that higher phosphorus and iron concentrations improved cells growth but did not increase phosphorus absorption ability of strain YSR-3.

3.5 100% cells formed intracellular polyP granules

Because 0.02 g/L $FePO_4$ was beneficial to the

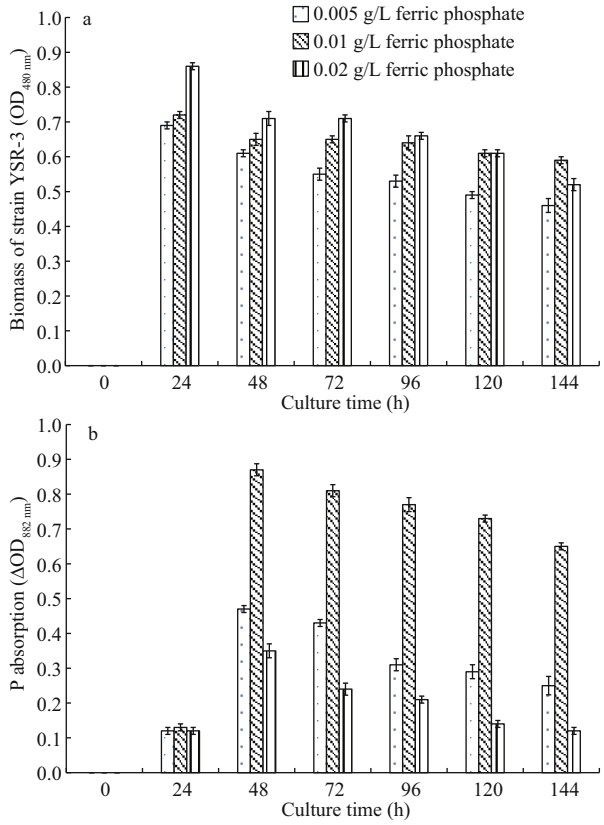

Fig.5 Effect of $FePO_4$ concentrations on strain YSR-3

a. YSR-3 got higher biomass at 0.02 g/L $FePO_4$; b. phosphorus absorption was stronger at 0.01 g/L $FePO_4$.

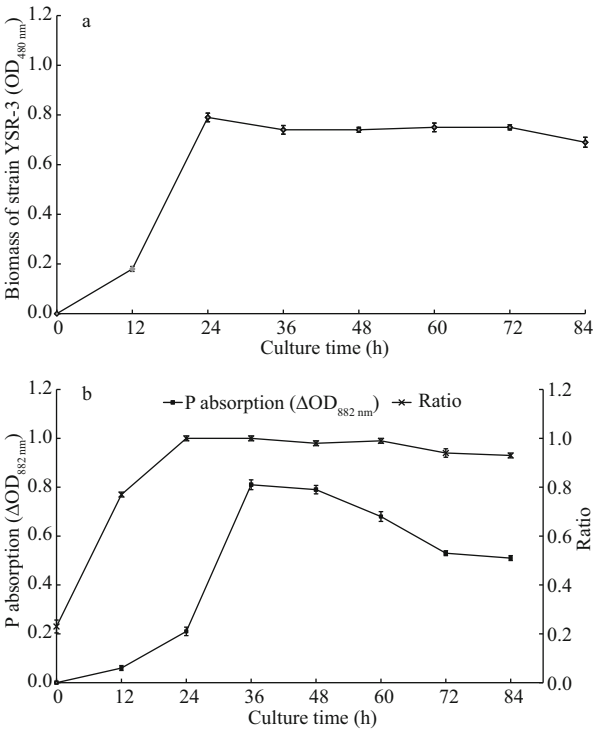

Fig.6 Correlation of growth, phosphorus absorption and polyP granules formation of YSR-3

a. growth of strain YSR-3; b. 100% cells formed intracellular polyP granules when cells assimilated phosphate from media. YSR-3 was cultivated in media with 0.01 g/L $FePO_4$.

growth but not to the phosphorus absorption, 0.01 g/L $FePO_4$ was added into the media. Cultivating strain YSR-3 in medium 4, the $OD_{480\ nm}$ was 0.79 at 24 h, which reached the highest value, and then slightly decreased. The amount of phosphorus assimilated by YSR-3 largely increased, because the $\Delta OD_{882\ nm}$ was up to 0.81 at 48 h. In subsequent culture, the $\Delta OD_{882\ nm}$ was getting smaller. After stained with DAPI and viewed by fluorescence microscope, the ratio of cells containing polyP granules to total cells was calculated. The ratio was 1 at 24 h, indicating that 100% cells formed intracellular polyP granules (Fig.6). The ratio slightly decreased in subsequent cultivation. In the early growth phase (0–24 h), the cells absorbed phosphorus for fundamental metabolism. At the 24 h, the biomass peaked and 100% cells formed intracellular polyP granules but the phosphorus absorption capacity of YSR-3 was not the strongest. Until the 48 h, the rate of growth decreased, then the cells accumulated the largest amount of phosphorus. The time delay between the largest biomass and the strongest phosphorus absorption might result in that the elongation of polyP polymers was mainly processed in the latter growth phase. Eventually, the phosphorus was accumulated and stored as intracellular polyP granules. The content of phosphorus in supernatant started to increase when the cells entered death phase, which is corresponding to the lower ratio of cells containing granules to total cells.

4 DISCUSSION

The microbial community structure could significantly affect the removal efficiency of contaminants in EBPR process. PAB are very important in this process owing to their phosphorus-absorbing capability, which removes the phosphorus from wastewater and accumulates as intracellular polyP. The polyP is a high-energy compound and its hydrolysis could supply energy to various biochemical reactions in the cell. Therefore, it is urgent to study the formation process and biochemistry of intracellular polyP in the application field of EBPR and theoretical research.

The polyP has high-energy bonds (analogous to those in ATP), so it is employed as a microbial phosphagen in many biochemical reactions and shows properties of a polyanion. It also serves as a reservoir

of phosphorus in the acidocalcisomes and a buffer against alkalis in the algae *Dunaliella Salina* (Seufferheld et al., 2008). Additionally, polyP appears as a multifunctional metal-chelating agent for the coalescent of Ca^{2+} affecting bacterial transformation, and can be accumulated in many bacteria when grows under environmental stresses (Bru et al., 2016). Furthermore, we confirmed the importance of polyP in many other bio-processes, such as antioxidative protection, signaling and regulation, cell viability and proliferation pathogen virulence, structural component and chemical chaperoning, production of poly-3-hydroxybutyrate, and modulation of the microbial stress response (Bru et al., 2016). In *Caulobacter crescentus*, the biogenesis and localization of polyP is controlled as a function of the cell cycle, ensuring regular partitioning of polyP granules between mother and daughter cells (Henry and Crosson, 2013). These functions indicate that polyP is an important molecule in the prokaryotic world.

This paper presents some interesting results in these aspects of strain YSR-3, which is a PAB isolated from the Yellow Sea, and classified as *Halomonas* sp. It accumulates phosphorus and forms intracellular metachromatic granules. The highest phosphorus-removal efficiency of strain YSR-3 in domestic wastewater is up to 72.2% (Ren et al., 2008). In some kind of bacteria such as *Lactobacillus* strains forming intracellular polyP granules, the positions occupied by polyP granules appears as holes in EDS analysis (Van Dien and Keasling, 1999). The strain YSR-3 is unlike *Lactobacillus* strains. The polyP granules of strain YSR-3 show black zone that are not easy to split under treatment of EDS owing to its high compactness. In addition, the polyP granules have a narrow size range (about 400 nm) and regular arrangement within cells (one or two ends, Fig.1a and 1b), which is similar to magnetosome. Magnetosome is synthesized by magnetotactic bacteria and comprised by crystals of magnetic iron minerals. It is a special intracellular organelle, nanometer-scaled and membrane-embraced. Due to the unique magnetosome, magnetotactic bacteria can orient and migrate along geomagnetic field lines. Nowadays it becomes a typical model of biomineralization owing to its fine structure and bioformation (Du et al., 2017). These similar characteristics indicate that the synthesis of polyP granules might be precisely controlled by genetic manipulation and involved in biomineralization. The strain YSR-3 might provide suitable materials for biomineralization research.

PolyP is an inorganic polymer of phosphate residues and accumulated in some microorganisms. In their life circle, cells constantly assimilate orthophosphate from the water to synthesize phospholipids, polyP, and nucleotides. The polyP plays significant biochemical or/and physiological roles such as participating in the response to nutritional stringencies and environmental stresses (Bru et al., 2016). When Na_3PO_4 was added into the media, strain YSR-3 grew well and absorbed more phosphorus to synthesize intracellular polyP granules (Fig.3). The capacity of phosphorus absorption was improved when more phosphorus was added into the media. In different environmental stresses, cell might adopt different phosphorus uptake mechanisms to survive (Martin et al., 2014; Muszyński and Miłobędzka, 2015). In addition, the strain YSR-3 could regulate phosphorus uptake ability to survive and accommodate to outer variable circumstances. The research interest is gradually turned into the application of microorganisms as heavy metal detoxification agents (Monachese et al., 2012). For some bacteria, such as PAB, the synthesis of intracellular polyP granules may counteract with heavy metals toxicity because intracellular polyP granules could be used as a chelating agent. The polyP could combine heavy metals and was potentially responsible for detoxification (Alcántara et al., 2014). Strain YSR-3 could grow and absorb phosphorus in the media added with different iron compounds (Fig.4). The $FePO_4$ was the best for the growth and phosphorus absorption of strain YSR-3 as $FePO_4$ might provide phosphorus for YSR-3 growth and stimulate its potential to synthesize intracellular polyP granules. The growth was concentration-dependent and increased with concentration augment of $FePO_4$ (Fig.5). To some degrees, strain YSR-3 could assimilate and detain iron. However, higher $FePO_4$ content (up to 0.02 g/L) could not enhance the phosphorus absorption of strain YSR-3. We speculated that iron-rich conditions inhibited the phosphorus uptake of strain YSR-3 owing to the toxicity of higher iron concentration. *Halomonas hydrothermalis* was micro-aerobically cultivated under iron starvation condition, and then the growth rates decreased by as much as 83% comparing to those of cells with abundant iron (Bru et al., 2016). In addition, strain YSR-3 belongs to genus *Halomonas*. These results show that iron could affect the growth of strains belonging to genus *Halomonas*. Normally, adaptability of microorganism to external

habitats is often influenced by complicated interactions among several physicochemical and biological factors rather than one factor alone (Harrison et al., 2015). Therefore, the iron (Fe^{3+}) is necessary for the growth of *Halomonas* sp. YSR-3, but iron-rich conditions could inhibit its capability of phosphorus absorption. Strain YSR-3 had a better tolerance to 5% NaCl (w/v), which is the same to other strains belonging to genus *Halomonas* (Ren et al., 2008). Strain YSR-3 has a specific tolerance to high salinity, which is very important in the research of wastewater treatment, especially in the high-salinity wastewater treatment and the phosphorus resource recycling in the ocean.

In order to examine the relationship among cell growth, phosphorus absorption, and polyP granule formation, it is necessary to comprehensively detect and identify strain YSR-3. When the cells have accomplished phosphorus absorption, the intracellular polyP granules would be stored and sealed in the media even after cultivating for 84 h. In other published cases, PAB stored polyP granules under aerobic incubation conditions; however, it decomposed polyP granules and released phosphorus into media under anaerobic incubation condition (Nakamura et al., 1995; Akar et al., 2006). In the early growth phase of strain YSR-3, phosphorus provided raw materials for cell growth and no polyP granules were formed in most of the cells. In the latter stationary phase, all cells stored phosphorus as intracellular polyP granules. Corresponding to other reported PAB, strain YSR-3 could sustain polyP granules within cells under continuous aerobic incubation conditions. Owing to these specific properties, strain YSR-3 may be used to improve the efficiency of wastewater treatment and phosphorus recycling because the cells containing polyP granules could be conveniently removed from the water body by filtration or centrifugation.

5 CONCLUSION

In order to provide strains or techniques for improving wastewater treatment efficiency, we analyzed the components and characteristics of intracellular polyP granules of strain YSR-3. The growth and capability of absorbing inorganic phosphorus, which existed in the media, were measured. The strain YSR-3 had some specific characteristics, which is very important for its application. In future study, the whole genomic sequence of YSR-3 shall be determined in high-throughput sequencing technique. The genes responsible for polyP synthesis and degradation, polyP transport, and polyP retention, will be analyzed by bioinformatics methods. The similarities and differences of intracellular polyP formation between marine PAB and freshwater PAB will be explored. Studying the formation process of intracellular polyP granules in strain YSR-3 provides a supporting technology for resolving eutrophication problem by bioremediation in situ. The capacity to recover phosphorus and recycle phosphorus from wastewater is potential to benefit both the environment and the economy.

6 DATA AVAILABILITY STATEMENT

All data used for this project are publicly available and accessible online. We have annotated the entire data building process and empirical techniques presented in the paper. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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