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Geochip-based analysis of microbial functional genes diversity in rutile bio-desilication reactor

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Abstract: Biological desilication process is an effective way to remove silicate from rutile so that high purity rutile could be obtained. However, little is known about the molecular mechanism of this process. In this work, a newly developed rutile bio-desilication reactor was applied to enrich rutile from rough rutile concentrate obtained from Nanzhao rutile mine and a comprehensive high through-put functional gene array (GeoChip 4.0) was used to analyze the functional gene diversity, structure and metabolic potential of microbial communities in the biological desilication reactor. The results show that TiO_2 grade of the rutile concentrate could increase from 78.21% to above 90% and the recovery rate could reach to 96% or more in 8−12 d. The results also show that almost all the key functional genes involved in the geochemical cycling process, totally 4324 and 4983 functional microorganism genes, are detected in the liquid and ore surface, respectively. There are totally 712 and 831 functional genes involved in nitrogen cycling for liquid and ore surface samples, respectively. The relative abundance of functional genes involved in the phosphorus and sulfur cycling is higher in the ore surface than liquid. These results indicate that nitrogen, phosphorus and sulfur cycling are also present in the desiliconization process of rutile. Acetogenesis genes are detected in the liquid and ore surface, which indicates that the desiliconizing process mainly depends on the function of acetic acid and other organic acids. Four silicon transporting genes are also detected in the sample, which proves that the bacteria have the potential to transfer silicon in the molecule level. It is shown that bio-desilication is an effective and environmental-friendly way for enrichment of rough rutile concentrate and presents an overview of functional diversity and structure of desilication microbial communities, which also provides insights into our understanding of metabolic potential in biological desilication reactor ecosystems.

Key words: rutile; functional gene diversity; silicate bacteria; bioleaching

1 Introduction

Biohydrometallurgy, so-called bioleaching, is a subfield within hydrometallurgy and is an efficient, economical and environmental-friendly technology to recover valuable metals from complex, barren ores and secondary resources. It has been widely used to recover copper from low grade sulphide ores [1−4], and to pre-biooxidatedly treat gold concentration to increase the recovery of finely disseminated gold from refractory ores containing pyrite and arsenopyrite [5−7]. Besides copper and gold, there are several studies focused on bio-desilication of bauxite, and impurities such as silica, calcium, and iron could be removed by silicate bacteria [8−13]. Although biohydrometallurgy is widely applied to those aspects, the removal of silica from rutile by using silicate bacteria has not yet been reported. The traditional rutile enrichment process mainly includes gravity separation, magnetic separation, electric separation, flotation and combined process. However, the TiO_2 grades of rough concentrates recovered by these processes are generally around 80% [14−19]. Many impurities, such as silicate minerals and carbonate minerals, exist in the edge and fissure of rutile particles. To remove these impure minerals, acid leaching process is often used for the enrichment of rutile concentrate. However, acid leaching process needs acid resistance devices, and by-products containing heavy metal and acid are harmful to environment.

The objectives of this work are to enrich rutile from rough rutile concentrate by bio-desilication process with silicate bacterial and to understand the bacterial functional genes diversity, structure and metabolic

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potential in the bio-desilication process. A newly developed rutile bio-desilication reactor was applied to enrich rutile from rough rutile concentrate and a comprehensive Geochip 4.0 was used for analyzing the diversity of microbial functional genes in this process.

2 Materials and methods

2.1 Primary rutile concentrate

The primary rutile concentrate used in this work was obtained from Nanzhao rutile mine in Henan, China. The chemical and mineralogical compositions of the sample are listed in Tables 1 and 2.

Table 1 Chemical mineralogical composition of primary rutile concentrate (mass fraction, %)

TiO_2	SiO_2	K_2O	MgO	CaO
78.21	12.84	1.66	0.21	0.67
Al_2O_3	Fe_2O_3	Others	Total	
2.92	1.91	1.58	100	

Table 2 Mineralogical composition of primary rutile concentrate (mass fraction, %)

Rutile	Quartz	Feldspar	Hornblende
78	10	5	2
Mica	Limonite	Others	Total
2	2	1	100

2.2 Strain and culture medium

The original silicate strains were isolated from the mine water of a silicate mine in Henan Province, China. Strain YJ-6 was obtained by the process of UV mutagenesis and domestication culture. Strain YJ-6 showed good desilication performance and was selected in this work.

The culture medium used for the bio-desilication consisted of the following ingredients (g/L): Glucose 5.0, Na_2HPO_4 0.2, $MgSO_4$ 0.2, $CaCO_3$ 0.1, $FeCl_3$ 0.005, and NH_4NO_3 1.0.

2.3 Bio-desilication and sample preparation

Bio-desilication experiments were carried out in a 10 L stainless steel bioreactor with a mineral concentration of 10% (w/v) at 30 °C with initial pH of 7.0. The initial cell density was about 1×10^8 cells/mL. The negative control with abiotic condition was also performed.

Liquid and rutile sediment samples were separated from 10 L bio-desilication reactor by filtration after running for 8 d. Cells in liquid were collected by centrifugation at 8000*g* for 10 min and prepared for DNA extraction. The rutile sediment was directly used for DNA extraction.

2.4 DNA extraction and purification

Total community DNA was extracted using a protocol described by ZHOU et al [20]. The crude DNA was further purified using the Promega Wizard DNA Clean-Up System (Madison, WI, USA) according to the manufacturer's instruction. DNA quality was evaluated by the gel electrophoresis with 1% agarose gel stained with 0.5 μg/mL ethidium bromide. The DNA absorbance ratios at $A_{260/280}$ and $A_{260/230}$ were detected by using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). Purified DNA was stored at −80 °C until it was used.

2.5 Whole genome amplification, labeling, hybridization and gene chip scanning

Total 100 ng DNA was used as template for the whole genome amplification by using the Templiphi kit (GE Healthcare, Piscataway, NJ) with some improvements. 0.1 μmol/L spermidine and 260 ng/μL single stranded DNA binding protein (Single Stranded DNA Binding Protein, SSB) were added to enhance the amplification efficiency and representativeness [21]. The details are as follows. To avid contamination, the whole process was conducted in the UV sterilized fuming cupboard, and positive and negative control was a must for operation. Firstly, 5 μL total DNA template was added into 10 μL sample buffer and the buffer was kept at room temperature for 10 min; next, 10 μL reaction buffer, 0.6 μL enzyme, 1.25 μL SSB and 1 μL spermidine were added in sequence; the sample and the reagents were well mixed with the pipette and later placed in the PCR meter and bathed at 37 °C for 6 h and 95 °C for 3 min and finally the temperature was kept at 4 °C. 1 μL amplified DNA was selected for quantitative determination with gel electrophoresis method and labeled directly after the successful amplification. About 2.5 μg amplified whole genome was used for Cy5 (GE Healthcare) for fluorescence labeling (random primer) [21−22]. The details are as follows. 20 μL random primer (2.5 X Random Primer, 750 μg/mL, Invitrogen, USA) was added into about 25 μL amplified DNA and the two were well mixed with pipette head and later heated at 99.9 °C for 5 min and then cooled down rapidly on ice. 2.5 μL dNTP mixture (5 mmol/L dA/G/CTP and 2.5 mmol/L dTTP), 2 μL Klenow (40 U/μL) and 1 μL Cy5 Dye (25 mmol/L) were added in sequence after the reaction system was cooled down thoroughly. Then, the reaction system was mixed well by the pipette and placed into the polymerase chain reaction (PCR) meter and bathed at 37 °C for 3 h and 95 °C for 3 min and finally kept at 4 °C for further use. Note that the whole experiment should be conducted in darkroom since Cy5

is very sensitive to light. Labeled DNA was later purified by using the QIAquick purification kit (Qiagen) according to the instruction. Cy5 concentration was determined with NanoDrop after purification. The labeling was successful if the fluorescence labeling gross went beyond 50 pmol and then the next procedures could be conducted. Successfully labeled DNA was dried in vacuum drier (SpeedVac, ThermoSavant, USA) at 45 °C for 35−45 min. The dried DNA could be stored below −20 °C for no longer than 2 mon or used for hybridization directly. The labeled DNA after drying was re-dissolved into 130 μL hybridization buffer (50% formamide, 3×SSC, 0.3% SDS, 0.7 g/μL Herring sperm DNA and 0.85 mmol/L DTT) and denatured at 98 °C for 3 min after being thoroughly dissolved and then kept at 65 °C until the spot hybridization. All the hybridization reactions here were set with 3 replicates and the arrays were later scanned by the ScanArray 500 array scanner (PerkinElmer, Boston, MA, USA). Laser with 90% 633 nm wavelength and 75% photomultiplier tube (photomultiplier tube, PMT) were used to obtain signals. Images obtained from scanning were analyzed with ImaGene (version 6.1, BioDiscovery, El Segundo, CA, USA) to convert the images into digital information.

2.6 Array data preprocessing

Original data obtained by ImaGene was uploaded to the website of Institute for Environmental Genomics (IEG, http://ieg.ou.edu/microarray/) for preprocessing. The main processing contents include: 1) remove the points defined with low mass (defined as 1 or 3) after analysis by ImaGene 6.1; 2) remove the points with signal to noise ratio (SNR, R_{cn}) smaller than 2, and $R_{sn}=(I_s-I_b)/D_i$ (where I_s is the signal intensity at the sample point, I_b is the background signal intensity) and D_i is the standard deviation of background signal intensity; 3) remove the sample points whose balance between the signal value of three replicates and the mean signal value was greater than twice the standard deviation; finally, only those sample points witnessing at least 2 of 3 replicates could be used as real sample points for further analysis.

3 Results

3.1 Bio-desilication of rutile

To test the effect of developed bio-desilication reactor, totally 1 kg rutile concentrate was put into the reactor. The SiO_2 grades of rutile concentrate decrease very fast from 12.85% to 8.34% and the purity of rutile reaches to 92% after 8 d (Fig. 1). The purity of rutile increases from 92% to 93% from 8th to 22nd day. These results indicate that bio-desilication reactor could effectively remove silicate from rutile and therefore purify rutile. Considering the energy consumption and the purity of rutile, 8−12 d is believed the optimal time for bio-desilication.

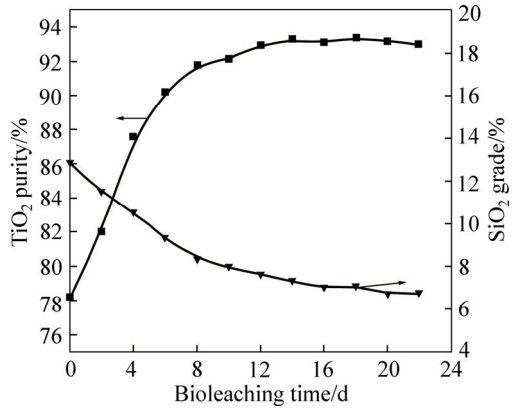

Fig. 1 Changes of rutile purity and SiO_2 grades in bio-desilication reactor

3.2 Overview of functional gene of bio-desilication reactor

The examined microbial communities show little differences among the liquid and rutile surface measured by the number of detected genes and signal intensity. There are totally 4324 and 4983 functional genes detected in the liquid and on the rutile surface, respectively. These genes include carbon degradation (1191 and 2175), carbon fixation (79 and 85), methane oxidation (19 and 22), methanol production (26 and 31), nitrogen cycling (712 and 831), metal resistance (486 and 584), sulfate reductase (337 and 388), sulfur oxidation (90 and 107) and phosphorous utilization (372 and 433), as seen in Table 3 and Fig. 2. Besides these genes, functional genes for acetic acid generation and silicon transporting are also detected in the liquid and on rutile surface samples, which indicates that the rutile desilication microorganism might have the ability to generate acetic acid and transfer silicate from solid to liquid phase.

LI et al [23] believed that hydroxylation reaction might take place between SiO_2 and organic acid molecules directly. In the hydroxylation reaction, SiO_2 is hydrolyzed and ionized in the water first, and then, H^+ ions provided by the hydroxyl will spread to the diffusion layer of the SiO_2 surface, H^+ ions react with the electron pairs and transfer the electrons to Si atoms, and lead to the enhanced electron cloud density around the Si atoms, and finally generate Si—OH bonds.

3.3 Functional genes involved in carbon cycling

Carbon cycling plays an important role in the microbial ecosystem. Genes related to the generation of acetic acid, carbon fixation, carbon degradation, methanol generation and methanol oxidation have all

Table 3 Signal intensity and numbers of functional genes detected

Functional gene category	Subcategory	Number of functional gene	
		Liquid	Ore surface
Carbon cycling	Acetogenesis	83	109
Carbon cycling	Carbon degradataion	1918	2174
Carbon cycling	Carbon fixation	79	85
Carbon cycling	Methane oxidation	19	22
Carbon cycling	Methane production	26	31
Metal Resistance	Metal Resistance	486	584
Nitrogen	Ammonification	152	177
Nitrogen	Anammox	2	2
Nitrogen	Assimilatory N reduction	41	44
Nitrogen	Dissimilatory N reduction	496	585
Nitrogen	Nitrification	74	87
Nitrogen	Nitrogen fixation	145	151
Phosphorus	Phosphorus utilization	372	433
Silicon	Silicon_transporter	4	4
Sulphur	Sulfate reductase	51	64
Sulphur	Sulfite reductase	286	324
Sulphur	Sulfur oxidation	90	107
Total		4324	4983

been detected in the liquid and ores (Figs. 3 and 4).

A key enzyme, *D*-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), involved in carbon fixation via the Calvin cycle, is detected in both liquid and the ore. There are 64 and 69 RuBisCO genes detected in the liquid and ore, respectively. Furthermore,

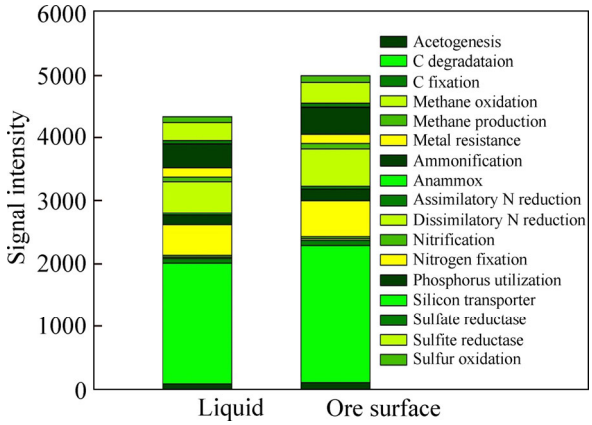

Fig. 2 Relative abundance of all functional gene groups detected

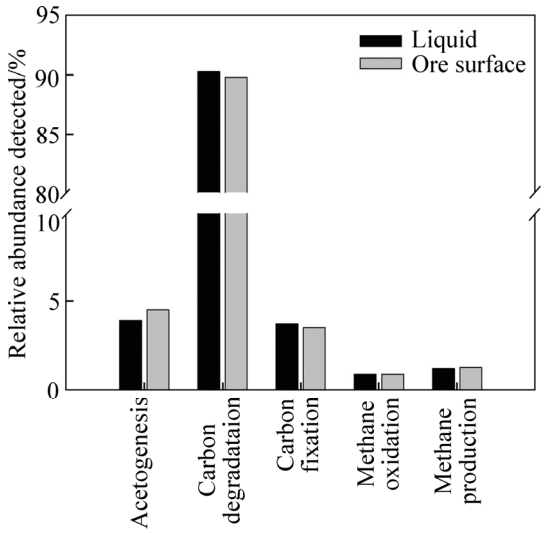

Fig. 3 Relative abundance of detected gene groups involved in carbon cycling in liquid and ore surface of microbial communities

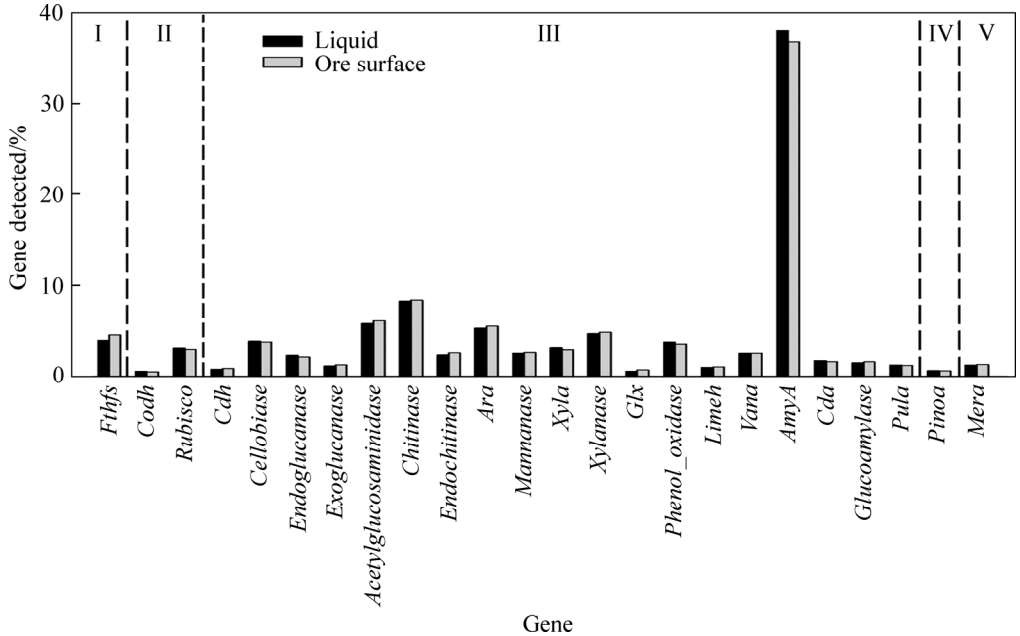

Fig. 4 Signal intensity of functional genes involved in carbon cycling

83 and 109 acetogenesis functional genes *fthfs* are detected in the liquid and ore, respectively. Other carbon degradation genes, including the cellulose, the chitin and lignin degradation genes, are also detected.

The signal intensity of methanol oxidation genes (*mmoX* and *pmoA*) is weaker than that of the methanol production genes (Fig. 4) and most *mcr* genes come from uncultured archaea, which indicates that archaea plays a relatively important role in the methanol cycling process, especially the production process.

3.4 Fuctional genes involved in nitrogen cycling

Almost all the functional genes involved in nitrogen cycling are detected in the liquid and ore surface (Figs. 5 and 6). Among all the nitrogen cycling processes, nitrogen fixation genes (*nifH*), ammonization genes (*ureC*) and denitrification genes (*narG*) enjoy relatively high abundance (Figs. 5 and 6). Totally 145 and 151 *nifH* genes, 101 and 125 *ureC* genes, 182 and 218 *narG* genes are detected in the liquid and ore surface, respectively. All these results indicate that most of the functional genes involved in the nitrogen cycle are present in the desiliconization process of rutile. The function of these nitrogen cycling might provide nitrogen sources for desiliconizing microorganisms. Therefore, it is recommended to add nitrogen, ammonium salt and nitrate during the desiliconization process to enrich the nitrogen source and finally strengthen the desiliconization process.

3.5 Metal resistance genes

Compared to AMD and other environment, the rutile desiliconization system doesn't contain too many metal ions. As a result, the detected metal resistance genes are relatively little. Totally 486 and 584 metal resistance genes are detected in the liquid and ore surface, respectively (Fig. 7).

3.6 Functional genes involved in sulfur, phosphorus and silicon metabolism

Totally 372 and 433 phosphorous cycling genes, 376 and 431 sulfur cycling genes, 4 and 4 silicon transporting genes are detected in the liquid and ore surface, respectively (Fig. 8). Among the detected phosphorus cycling genes, there are 42 and 49 *phytase* genes, 78 and 90 *ppk* genes, 215 and 254 *ppx* genes, 37 and 40 *pgk* genes, respectively; among the detected sulfur cycling genes, there are 19 and 20 *sqr* genes, 125 and 141 *dsrA* genes, 112 and 125 *dsrB* genes, 30 and 38 *Sir* genes, 6 and 8 *SoxA* genes, 17 and 19 *SoxB* genes, 16 and 22 *SoxC* genes, 4 and 5 *SoxV* genes, 47 and 53 *Soxy* genes, respectively. Four silicon transporting genes are

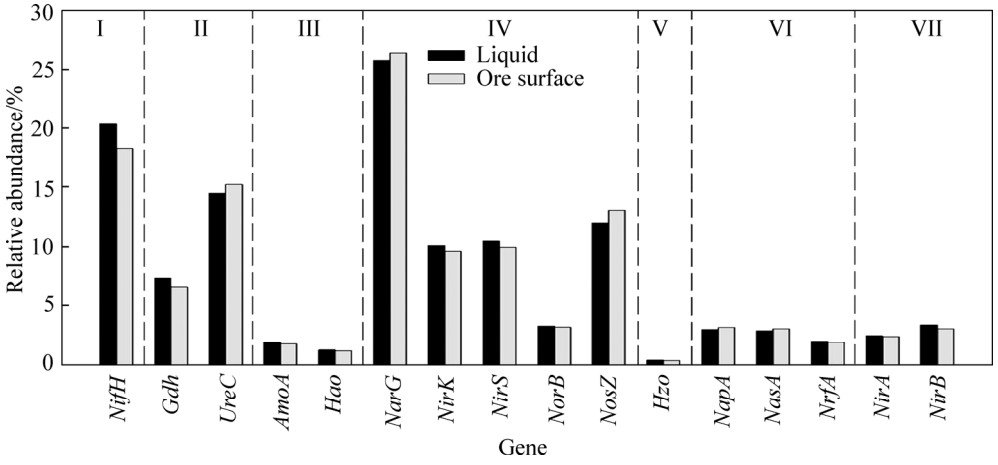

Fig. 5 Total signal intensity of functional genes involved in nitrogen cycling

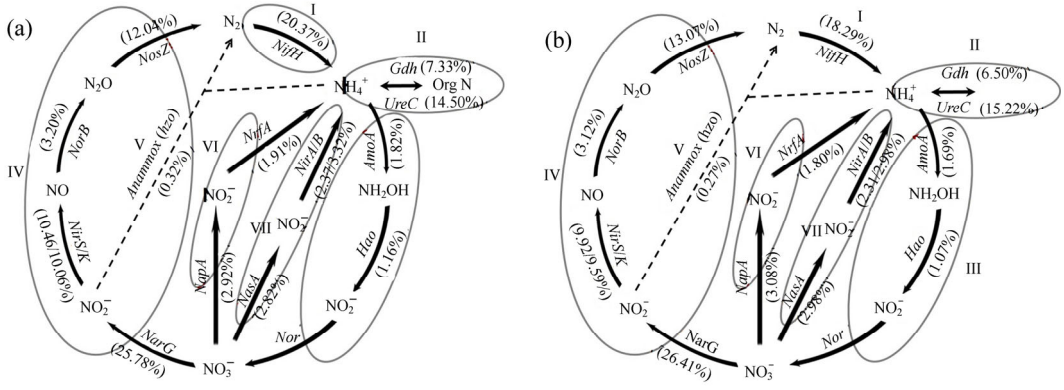

Fig. 6 Functional genes involved in nitrogen cycling: (a) Liquid; (b) Ore surface

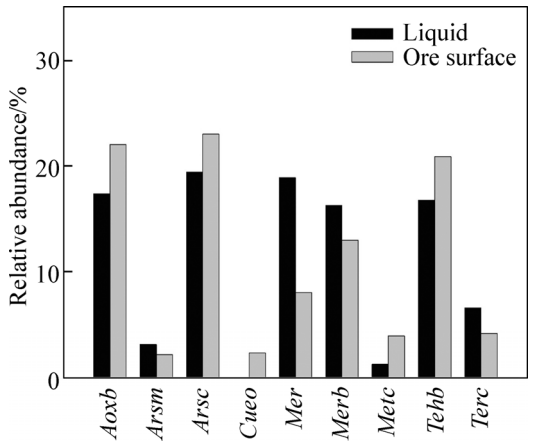

Fig. 7 Detected genes involved in metal resistance in liquid and ore surface

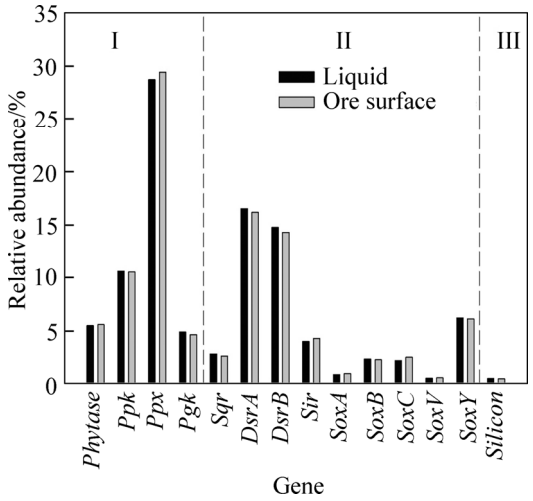

Fig. 8 Detected functional genes involved in sulfur, phosphorus and silicon metabolism

detected too. In a word, the quantity of functional genes involved in phosphorus and sulfur cycling detected in the ore surface is greater than that detected in the liquid, which indicates that the phosphorus and sulfur cycling also plays an important role in the deciliconization process of rutile. Silicon transporting genes could hardly be detected in other samples but here four are detected in the sample, which indicates that the bacteria have the potential to transfer silicon in the molecule level.

4 Conclusions

1) Bio-desilication by using silicate bacterial is an efficient way to remove the silicate in rutile and therefore increase the grade of rutile. The efficiency of rutile bio-desilication reactor is acceptable. TiO_2 grade of the concentrate could increase from 78.21% to 92% and the recovery rate could reach to above 96% in 8−12 d.

2) The functional gene array has detected almost all the key functional genes involved in the geochemical cycling process. Totally 4324 and 4983 functional genes are detected in the liquid and on the ore surface, respectively.

3) There are totally 712 and 831 functional genes involved in nitrogen cycling for liquid and ore surface samples, respectively. The relative abundance of functional genes involved in the phosphorus and sulfur cycling is higher in the ore surface than liquid. These results indicate that nitrogen, phosphorus and sulfur cycling are also present in the desiliconization process of rutile.

4) Acetogenesis genes and silicon transporting genes are detected in the liquid and ore surface, which indicates that the desiliconizing process mainly depends on the function of acetic acid and silicon transportation protein.

5) In the future, electrochemical mechanism on bio-desilication should be studied in depth. At the same time, more silicate bacteria strains should be screened and cultivated for the biological desilication research. The process conditions of continuous bio-leaching should also be further tested so that this technology can be applicated in the large-scale industrial production.

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