SCIENCE CHINA
Earth Sciences

• RESEARCH PAPER •

December 2013 Vol.56 No.12: 2135–2140
doi: 10.1007/s11430-013-4704-4

Differences in the gene expressive quantities of carbonic anhydrase and cysteine synthase in the weathering of potassium-bearing minerals by *Aspergillus niger*

SUN LeiLei[1], XIAO LeiLei[1], XIAO Bo[1], WANG WeiYing[1], PAN Chen[1], WANG ShiJie[2] & LIAN Bin[1,2*]

[1] *Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Microbiology, College of Life Science, Nanjing Normal University, Nanjing 210023, China;*
[2] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China*

Received July 24, 2012; accepted March 28, 2013; published online September 17, 2013

We investigated the differences in the gene expression of carbonic anhydrase (CA) and cysteine synthase (CysM) between two weathering conditions, with either soluble potassium or insoluble potassium. We cultured a strain of *A. niger* by adopting a variant Czapek medium (using Na_2HPO_4 as a substitute for K_2HPO_4) in two groups, Group A (containing silicate minerals bearing potassium but without KCl) and Group B (with KCl) . We extracted the mRNAs of CA and CysM from these two groups and performed real-time quantitative polymerase chain reactions (RT-qPCR). We constructed relative standard curves by using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference to confirm a consistent amplification efficiency of the target genes (CA and CysM) and the reference gene and quantified the gene expression of the targets in a relative manner. Our results showed that CA and CysM in Group A were upregulated for 1.7 times and 11.7 times, respectively, compared with those in Group B. Furthermore, we also analyzed some metabolic pathways and functions of the *A. niger*-induced weathering of potassium-bearing minerals, which involved the synthesizing of these two enzymes. Thus our work provides materials for further study of the roles of *A. niger* in the metabolic regulation during the weathering process of potassium-bearing minerals.

***Aspergillus niger*, potassium-bearing minerals, potassium feldspar, carbonic anhydrase, cysteine synthase, RT-qPCR**

Citation: Sun L L, Xiao L L, Xiao B, et al. Differences in the gene expressive quantities of carbonic anhydrase and cysteine synthase in the weathering of potassium-bearing minerals by *Aspergillus niger*. Science China: Earth Sciences, 2013, 56: 2135−2140, doi: 10.1007/s11430-013-4704-4

Potassium is one of the three essential nutrient elements for plant growth and it plays a significant role in agricultural production. In China, soluble potassium is a scarce resource with only a 35% self-supply to the market needs, and thus there is a heavy dependence on import. With the increasing monopoly for production and marketing of potassium fertilizer, China's grain security is threatened. However, insoluble potassium (silicate minerals bearing potassium) is in ubiquitous abundance throughout China (Ma et al., 2010; Liu et al., 2011), but direct absorption of potassium as its feldspathic or other silicate forms is impossible for plants. Production of bio-effective and plant-absorbable potassium from potassium feldspar typically requires energy-intensive, polluting procedures such as heating and acid hydrolysis (Liu et al., 2011; Gu et al., 2011). Therefore, it is worthy to look into a microbial method through which one could produce organic-compound potassium by bio-transforming and extracting it from abundant low-grade potassium feldspar, and it has currently received broad attention (Basak et al.,

*Corresponding author (email: bin2368@vip.163.com)

2009; Ma et al., 2010; Liu et al., 2011). *A. niger*, a type of saprophytic fungus widely existing in nature, is an important fermentative microbe for industrial use in the production of enzymes, heterogeneous proteins, organic acids, secondary metabolites, etc. To be specific, it is used in producing amylase, acid protease, cellulose, pectinase, glucose-oxidase, citric acid, gluconic acid, and gallic acid, and fulfils other functions such as steroid transformation and traditional fermentation (Guo et al., 2010). As an effective ore-leaching fungal strain, it has been proved as an effective agent in the weathering of potassium feldspar (Hu et al., 2011a) and apatite (Chen et al., 2009) Knowledge of the mechanism by which *A. niger* weathers potassium silicate has progressed rapidly (Hu et al., 2011b). However, little light has been shed on the subject of the differences in gene expression during this weathering process, or indeed the control functions of associated proteins. This research suggests that carbonic anhydrase (CA) may exert a significant influence on the process of the fungal weathering of potassium silicate (Xiao et al., 2012a). Olsson-Francis et al. (2010) discovered that the gene expression quantities of cysteine synthase (CysM) in *Cupriavidus metallidurans* rises notably during the weathering of siderite, indicating that these two genes could play important roles in the process of microbial weathering of minerals. This research uses real-time quantitative polymerase chain reaction (RT-qPCR) techniques to investigate the differences in gene expression during the *A. niger*-induced weathering of potassium mineral powder into soluble potassium and sheds light upon the possible reasons. This research aims to further elucidate the mechanisms governing the *A. niger*-induced weathering of potassium-bearing minerals under experimental conditions.

1 Material and methods

1.1 Materials

The strain of *A. niger* used was provided by China's General Microbiological Culture Collection Centre (CGMCC) where it is recorded under strain preservation number 3.3928. A basic medium was prepared in accordance with the composition of Czapek's medium with modification by removing elements containing potassium incorporated (Ma et al., 2008). The compositions of basic medium were: $NaNO_3$ 0.45 g, $Na_2HPO_4 \cdot 12H_2O$ 0.3257 g, $MgSO_4$ 0.037 g, $FeSO_4 \cdot 4H_2O$ 0.0015 g, sucrose 4.5 g, ultra-pure water 150 ml. The pH was adjusted to 6.8.

The potassium powder used in these experiments was obtained from Daoping Town of Fuquan City, Guizhou Province. Analysis of compositions by X-ray diffraction (XRD) showed that potassium feldspar accounted for 76% by mass; analysis using X-ray fluorescence showed that K_2O, Al_2O_3, and SiO_2 accounted for 9.67%, 18.06%, and 57.75% by mass respectively. The mineral powder was crushed and passed through a 200 mesh sieve.

The RNA extraction kit, reverse transcription kit, and fluorescence quantification PCR kit were sourced from RNAprep pure Plant Kit (TIANGEN Ltd., China); the BD SMART PCR cDNA Synthesis Kit was sourced from Clontech (Japan), and the SYBR Green master mix from Applied Biosystems (America). All primers used were synthesised by Nanjing Genescript Biotechnology Ltd. (China).

1.2 Fungi culture

Three types of culture mediums were prepared: Group A with potassium mineral powder, Group B with KCl instead of the mineral powder, and Group C with potassium mineral powder as an experimental control. Among them, 1 g of potassium mineral powder was added to each of Groups A and C's medium per 150 mL of basic medium, whereas 0.075 g KCl was similarly added to medium in Group B. Additionally, 1 mL spore suspension of *A. niger* was inoculated to both Group A and B (a ring of *A. niger* spore was injected into 2 mL of ultra-pure, continually stirred water); no spores were added to Group C for asepsis control. All three groups were cultured at 28°C and agitated at 120 rpm for for 2–3 days.

1.3 The potassium-releasing function of *A. niger*

Supernatant liquid was collected after the logarithmic phase (the first two to three days), and filtered by needle strainer. The concentration of potassium ions in the liquid was tested by employing inductively coupled plasma atomic emission spectrometry (ICP-AES). The mycelial pellets of *A. niger* were collected and placed in 150 mL of 1 mol/L ammonium acetate solution and treated by ultrasonic wave for 30 min. The potassium ions concentration in the supernatant was then measured by ICP-AES as mentioned (Hu et al., 2011b).

1.4 Extraction and reverse transcription of total RNA

Total RNA from the fermented *A. niger* mycelial pellet was extracted using RNAprep pure Plant Kit: after its optical density (OD) value had been confirmed, the integrity and the degradation level of total RNA were detected by 1.5% agarose gel electrophoresis, and then the reverse transcription kit was used for reverse transcription.

1.5 Real-time fluorescence quantification PCR

When using the SYBR Green master mix kit for RT-qPCR, the reaction system comprised: 2 × Master Mix 12.5 μL, foword and reverse primers (10 μmol/L) of each of 0.75 μL, cDNA 0.5 μL, and 10.5 μL DEPC H_2O: details of the primers used are summarized in Table 1.

The following reaction condition for RT-qPCR was applied: 94°C for 5 min, 94°C for 10 s, 60°C for 15 s, and 72°C for 20 s, a total of 40 cycles. A dissociation curve

Table 1 Primers and their series for RT-qPCR detection

Primers	Series 5′–3′
Carbonic anhydrase-F	TAACAGCACCATCACCCTCT
Carbonic anhydrase-R	TTCCATCCTCATCCGAAAAC
Cysteine synthase-F	ATGCTGGAGAAACGCAAGAG
Cysteine synthase-R	TCAGGAGGTTGGATGAAAAG
GAPDH-F	ACAAGGACTGGCGTGGTG
GAPDH-R	ACCGTTCAGGTCGGAGGAG

analysis: temperature between 55 and 99°C, a heating rate of 12°C per minute, and one plate-reading per second were used. The gene standard curve was determined by using different dilution concentration (10-fold dilution successively) cDNA as a template and the same gene primers. Glyceraldehyde-3-phosphate dehydrogenase, GAPDH, the endogenous housekeeping gene, was used for internal reference (Perez et al., 2007). Amplified samples of this template were replaced with pure water for negative control. The real-time fluorescence quantification PCR instrument cabin of software 7500 system SDS was used for analysing the Ct value(s) of the target genes and the initial copy number, and to quantify the target gene relatively.

1.6 Statistical analysis

A Q-Test, assuming that $P<0.05$ indicated statistical significance, was used for inter-group comparisons. Statistical analysis was performed using SPSS 13.0.

2 Results

2.1 Growth of *A. niger* and its utilisation of potassium-bearing minerals

In Group A (with mineral, without KCl), the growing hypha of *A. niger* wrapped up the mineral powder to form mycelial pellets, or fugus-mineral aggregates (Hu et al., 2011a, 2011b; Chen et al., 2009). The size of mycelial pellet was relatively small (diameter approximately 1 to 2 mm) due to the culture time being controlled in the logarithmic phase. In Group B (without mineral but with KCl), growth of *A. niger* was better with a pure white colour observed. The mycelial pellet in Group B was slightly larger than that of Group A, which appeared darker.

In the presence of different potassium resources as the following conditions: the concentration of K^+ ions in the supernatant of Group A was 2.43 ppm and that in the mycelial pellet was 21.16 ppm. For Group B, the concentration of K^+ ions was 207.79 ppm whereas it was 95.52 ppm in the mycelial pellet. Group C was the asepsis control with a concentration of 2.94 in its supernatant. Compared to control Group C, differences in concentration inside and outside the mycelial pellets were significant: 8.7 times higher than that found outside, in Group A, indicating that the fungi can release potassium from the mineral powder and that most of the potassium released was wrapped up in the mycelial pellet (a portion was adsorbed on its surface) while a small portion was released in the culture. It can be concluded that soluble potassium was obtained in Group A during the process of *A. niger* mycelial pellet weathering of insoluble potassium.

In Group B, the potassium concentration in the culture was far greater than that in the mycelial pellet, indicating a regulation function exerted by the mycelial pellet, formed with fungal growth, reducing the osmotic pressure inside the mycelial pellet.

2.2 Determination of standard curve of fluorescence quantification PCR and relative expression of mRNA

Through continuing optimisation of primers and reaction conditions, the amplification efficiency of CA and CysM on the target gene and GAPDH of the internal reference gene were at a similar level, hence the relative standard curve method could be applied for the analysis of the relative quantification of the target gene. The relative standard curve was determined by amplifying both target and internal reference gene after 10-fold sample dilution of cDNA. RT-qPCR technique was used for testing the effect of genes expression of *A. niger* CA and CysM on different potassium resources; mRNA relative expression quantities of CA and CysM are shown in Figures 1 and 2.

The results in Figures 1 and 2 indicated that there were significant differences in enzyme gene expression of CA and CysM. During the *A. niger*-induced weathering of potassium minerals, the enzyme gene expressions of CA and CsyM increased 1.7 and 11.7 times respectively. The results implied that there may be a causal connection linking the expression of these two genes and the functional process of their potassium utilisation from potassium mineral powders.

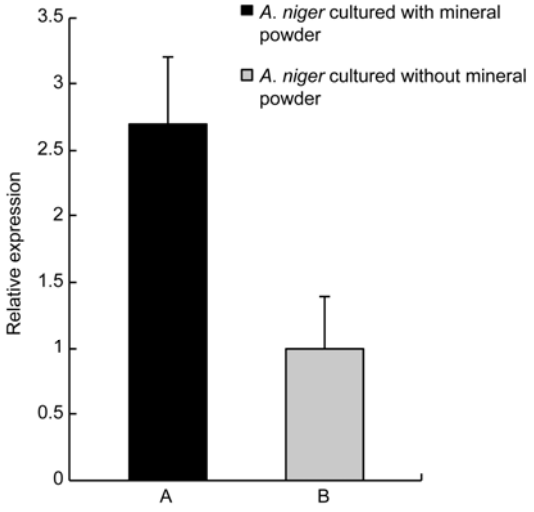

Figure 1 RT-qPCR analysis of the expression of CA genes in *A. niger* cultured with or without mineral powder, using GAPDH as an internal reference gene. Note: statistical significance at $P<0.01$.

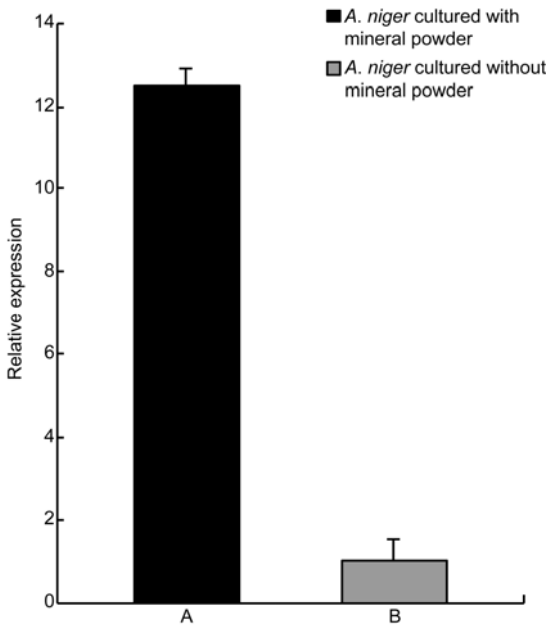

Figure 2 RT-qPCR analysis of the expression of CysM genes in *A. niger* cultured with or without mineral powder, using GAPDH as an internal reference gene. Statistical significance at $P<0.01$.

3 Discussion

Our experimental results showed CA gene expression of *A. niger* increased with the induction of potassium-bearing minerals. CA, one of the enzymes known to have amongst the highest catalytic rate (Deng et al., 2010), catalyzed the inter-transformation of carbon dioxide and bicarbonate. For example, in marine eco-systems, some marine diatoms may form CA under low-carbon conditions and then use CA for catalysing this inter-transformation of CO_2 and HCO_3^- to collect carbon resource for photosynthesis. In animal bodies, CA is essential to forming both exo- and endo-skeletons in both invertebrates and vertebrates (Richier et al., 2009). CA exists in nearly all organisms revealing the involvement of both atmospheric and lithospheric bio-activity in evolutionary geochemical processes innate to life's origins and evolution. Soil microorganisms may accelerate the weathering process of silicate mineral bearing potassium by CA (Xiao et al., 2012a), and may utilise carbonic acid formed by the dissolution of CO_2 for dissolving minerals and obtaining inorganic nutrients (Xiao et al., 2012a; Du et al., 2008). This is essential not only for the formation and evolution of soil, but also for the fixation of CO_2 and concomitant relief of the greenhouse effect (Chen et al., 2001; Lian et al., 2011). This research provided direct evidence of increased mRNA gene expression of CA in the process of *A. niger*-induced weathering of potassium silicate. During the process, increased CA gene expression catalysed the hydration of CO_2, and ultimately accelerated the weathering.

Increased gene expression of CA indicated that it may have had a significant impact upon the promotion of rock weathering. Existing research proves that adding CA to Karst systems may catalyse the formation of carbonic acid from CO_2, resulting in accelerated dissolution (Yuan et al., 2000). Although past research has reached the consensus that microorganisms play an essential role in carbonate rock formation and the existence of massive carbonate minerals may be the result of the reaction of CO_2 with silicates, in the absence of understanding of the catalytic function of CA in weathering, the rates of organism function in weathering, and the sedimentation of CO_2, were underestimated (Liu, 2001).

CA, besides its function as the direct catalyst for the inter-transformation of CO_2 and HCO_3^-, is involved in the formation of malonyl-CoA by the catalysis of the Acetyl-CoA carboxylase with bicarbonate as substrate. Moreover, it works in lipid metabolism and ties closely with physiological activity on the surface of membranes and films (Milce et al., 2007). In an environment lacking potassium, *A. niger* needs to actively seek a potassium resource; its metabolic properties as well as its membrane film flow-capabilities are needed to adjust to such potassium-scarce environments, or to those containing insoluble, feldspathic, potassium.

Cysteine groups in microorganism secretion are considered to perform a function in oxidoreduction (Uroz et al., 2009). The determinants of the chemical properties of minerals' surfaces were chemical composition, atomic structure, and micro-texture. Chemical reaction of organisms upon the mineral surface was restricted to depths of several nanometres. In most cases, the chemical properties of the mineral surface rarely showed the entirety of the mineral as the unsaturated nature of the surface exposed to the external environment may have caused self-initiative structural adjustment. While with adsorbed molecular processes, structural readjustment may manifest itself in different forms (Lu, 2005). Cell pigment proteins of microorganism membranes can transport electrons through direct, and proximate, contact with mineral oxides (Fredrickson et al., 2008). The mineral crystal structure may be destroyed by oxidation-reduction reactions of compounds on, and in, the mineral surface, thereby reducing its stability, with dissolution being initiated at crystalline defects. CysM, a 60–70 kda homo-dimer, widely exists in organisms and plant cells: it can catalyse O-acetyleserine OAS and Cys of which gene expression increased significantly during *A. niger*-induced weathering of minerals, indicating that cysteine may have served a specific function in the context of fungal weathering of this mineral (Olsson et al., 2010). Free nercaptan groups of cysteine contributed to the formation of disulfide bonds and were critical with regard to maintaining the stability of some proteins: they also acted as catalysis, and oxidation-reduction, centres for a variety of enzymes, accessory factors, and regulatory proteins. Moreover, cysteine is a restrictive nutrient for glutathione—an important oxidation-reduction buffer—and plays a role in cell detoxification

(Kaur et al., 2007). Cysteine, as a glycopeptide synthesis connector in the body of an organism, is involved in the modification of translating galactofuranos (Dancho et al., 2011), and confers benefits upon the formation process of relative glycoproteins in the process of *A. niger*-induced weathering of potassium silicates.

Potassium-bearing minerals arising from *A. niger*-induced weathering in this research indicated that the content of CysM in the group with mineral powder was increased by 11.7 times. The activity and content of those enzymes in living bodies were strictly controlled by a series of related genes that ensured that the adjustment of enzyme activity was foremost followed by content solely with unsatisfied physiological needs. On the other hand, increased CysM in the group with mineral powder was remarkably high, indicating that in the environment containing potassium-bearing minerals but lacking soluble potassium, *A. niger* was inclined to synthesise cysteine massively to become further involved in the elimination of free radicals, the relief of ions or organic intoxication status (e.g., chloride, benzene, phenol, etc.), which may jeopardise cell growth. Active sulfhydryl may have been involved in electron transportation on the mineral surface and the underlying crystalline structure may have been destroyed by the oxidation-reduction reaction of compounds on, and in, the mineral surface, leading to a reduction in its stability. On the other hand, free mercaptan groups in cysteine contributed to formation of disulfide bonds and were deemed critical for the continued stability of some proteins, as well as acting as catalysis and oxidation-reduction centres for a variety of enzymes, accessory factors, and regulatory proteins. Under the experimental conditions herein, a lack of potassium may have caused accelerated weathering in a quest for inorganic nutrients. During the weathering process, a series of changes in *A. niger* occurred, including obtaining more carbon resources, significantly involving the transfer of a one-carbon unit. Transfer of this one-carbon unit did not only relate to amino acid metabolism, but was also involved in the biosyntheses of purine and pyrimidine as well as S-adenosylmethionine, which are the major resources underpinning compound methylation in micro-organic bodies (Qiao, et al., 2004).

In the process of *A. niger*-induced weathering of potassium silicates, the first step was to enrich carbon dioxide by utilising carbonic anhydrase, which then increased local regional acidity on the ore surface, accelerated lipid metabolism on the cell membrane, and ultimately accelerated the weathering. Meanwhile, cysteine may have functioned in transgenic methyl and sulphur-based biochemical processes, for the antioxidation and elimination of free radicals, as well as having some involvement in the translating modification of glycoprotein, inside the cell membrane.

The aforementioned activities were apparently conducive to *A. niger* weathering and utilisation of potassium-bearing minerals. The process was considered complex: the organism could actively weather a series of potassium-bearing minerals by overcoming the difficulty of a lack of soluble potassium and self-adjusting its enzymatic activity and contents. Microbial weathering could destroy the crystal structure and release potassium, which is an essential nutrient for organic growth (Xiao et al., 2012a, 2012b).

4 Conclusions

This research studied two enzymes in the *A. niger* metabolic process using a RT-qPCR method. It was found that the gene expression of both enzymes upregulated in the microbial-induced weathering process. These results provided solid evidence for further discussion of CA and CysM functionality in the weathering process of potassium-bearing minerals by *A. niger*. Meanwhile, the research laid a molecular microbial foundation for further research analysing the changes that occurred in *A. niger* when it is performed in potassium-scarce environments.

This work was jointly supported by the National Natural Science Foundation of China (Grant No. 41173091), National Key Basic Research Program of China (Grant No. 2013CB956702), and the Priority Academic Program Development of Jiangsu Higher Education Institutions. We thank the two anonymous reviewers for their insightful comments and suggestions.

Basak B B, Biswas D R. 2009. Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. Plant Soil, 317: 235–255

Chen J, Yang J D, Li C L. 2001. The continental weathering and the global climatic change (in Chinese). Adv Earth Sci, 16: 399–405

Chen S, Liu C Q, Lian B. 2009. Effect of a strain of *Aspergillus niger* on weathering of phosphorite (in Chinese). Acta Minal Sin, 29: 103–107

Costa M, Borges C L, Bailao A M, et al. 2007. Transcriptome profiling of *Paracoccidioides brasiliensis* yeast-phase cells recovered from infected mice brings new insights into fungal response upon host interaction. Microbiology, 153: 4194–4207

Dancho D, Laurent L G, Richard D, et al. 2011. Direct access to new D-galactofuranoconjugates: Application to the synthesis of galactofuranosyl-L-cysteine and L-serine. Tetrahedron Lett, 52: 1121–1123

Deng Q H, Li M T, Xiang F, et al. 2010. Structure prediction of β carbonic anhydrase from *Brassica napus* (in Chinese). Biotech Bull, 1: 111–117

Du Y, Zhou X Y, Lian B. 2008. The extracellular secretion of *Bacillus mucilaginosus* and its capability of releasing potassium from potassium-bearing minerals (in Chinese). Earth Sci Front, 15: 107–111

Fredrickson J K, Zachara J M. 2008. Electron transfer at the microbe-mineral interface: A grand challenge in biogeochemistry. Geobiology, 6: 245–253

Gu H N, Wang N, Yang Y Q, et al. 2011. Research progress of preparing potash fertilizer with potassium-bearing minerals (in Chinese). Chem Ind Eng Pro (China), 30: 2450–2455

Guo Y M, Zheng P, Sun J B. 2010. *Aspergillus niger* as a potential cellular factory: Prior knowledge and key technology (in Chinese). Chin J Biotech, 26: 1410–1418

Hu J, Lian B, Yu J P, et al. 2011a. Formation of *Aspergillus niger*-mineral aggregation and characterization of polysaccharide from aggregation (in Chinese). Acta Microbiol Sin, 51: 756–763

Hu J, Yu J P, Lian B. 2011b. Capability and mechanism of potassium releasing from potassium-bearing minerals by *Aspergillus niger* (in

Chinese). Bull Mineral Petrol Geochem, 30: 277–285
Kaur J, Bachhawat A K. 2007. Yct1p, a novel, high-affinity, cysteine specific transporter from the yeast *Saccharomyces cerevisiae*. Genetics, 176: 877–890
Lian B, Yuan D X, Liu Z H. 2011. Effect of microbes on karstification in karst ecosystems. Chin Sci Bull, 56: 3743–3747
Liu D F, Lian B, Wang B. 2011. Degradation of potassium rock by earthworms and responses of bacterial communities in its gut and surrounding substrates after being fed with mineral. PLoS ONE, 6: e28803
Liu Z H. 2001. The role of carbonic anhydrase as an activator in carbonate rock dissolution and its significance in atmospheric CO_2 precipitation (in Chinese). Geosici Sin, 22: 477–480
Lu A H. 2005. New advances in the study of environmental mineralogical materials: Pollution treatment by inorganic minerals—the fourth category of pollution treatment methods (in Chinese). Earth Sci Front, 12: 195–205
Ma F, Zhang S, Shan D. 2008. Optimization of the medium composition for culturing of mycelia pellet of *Aspergillus niger* Y3 (in Chinese). China J Envir Sci, 28: 989–933
Ma H W, Su S Q, Liu H, et al. 2010. Potassium resource and sustainable development of potash salt industry in China (in Chinese). Earth Sci Front, 17: 294–310
Olsson F K, Van H R, Mergeay M, et al. 2010. Microarray analysis of a microbe-mineral interaction. Geobiology, 8: 446–456
Perez S, Royo L, Astudillo A, et al. 2007. Identifying the most suitable endogenous control for determining gene expression in hearts from organ donors. BMC Mol Biol, 8: 114
Qiao Q A, Cai Z T, Feng D C. 2004. Progress in the folate coenzyme participated reaction of one-carbon unit transfer (in Chinese). Prog Chem, 16: 785–790
Richier S, Kerros M E, Vargas C, et al. 2009. Light-dependent transcriptional regulation of genes of biogeochemical interest in the diploid and haploid life cycle stages of *Emiliania huxleyi*. Appl Eenviron Microb, 75: 3366–3369
Xiao B, Lian B, Sun L L, et al. 2012a. Gene transcription response to weathering of K-bearing minerals by *Aspergillus fumigatus*. Chem Geol, 306–307: 1–9
Xiao B, Lian B, Shao W L. 2012b. Do bacterial secreted proteins play a role in the weathering of potassium-bearing rock powder? Geomicrobiol J, 29: 497–505