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Do Bacterial Secreted Proteins Play a Role in The Weathering of Potassium-Bearing Rock Powder?

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Bacillus mucilaginosus **has already been proved to be capable of degrading silicate minerals, but it is not very clear about the molecular mechanisms of bacterial mineral weathering. To understand the relationship between bacterial weathering of minerals and bacterial secreted proteins, *B. mucilaginosus* was chosen to study the expression of its extracellular proteins in the process of weathering potassium minerals. This article reveals that certain secreted proteins, related to weathering of potassium minerals, can be induced under conditions such as bacterial nutritional deficiency and the existence of K-bearing rock powders. This suggests direct evidence of the metabolic changes of extracellular enzymes in bacteria during the process of weathering of potassium minerals. It was speculated that these secreted proteins, together with extracellular polymers like polysaccharides, may accelerate the weathering of potassium minerals, resulting in the release of K^+ needed for the bacterial growth.**

Keywords microbial weathering, *Bacillus mucilaginosus*, K-release, K-bearing rock, biodegradation, biogeochemical cycling

INTRODUCTION

Microbial weathering is thought to be a prevalent biogeochemical reaction on the earth's surface. In the process of growth and reproduction, microorganisms enhance the dissolution of minerals through their metabolic activity and metabolites, resulting in that some substances were released from rocks or minerals, which affecting the composition and content of the soil (Rogers and Bennett 2004; Rogers et al. 1998).

Although the microbial weathering of minerals is a major driving force in biogeochemical cycling, the effect of microbial activities in migration, transformation and cycling of mineral elements is still poorly understood (Skidmore et al. 2005). As a key, widespread step in the biological and geochemical processes, microbial mineral weathering not only has ecological significance, but also affects the composition of the food chain and human health, and even the architecture and artistic patrimony (Uroz et al. 2009).

The known and potential mechanisms of microbial weathering may include redox reactions through the production of organic acids and chelating molecules for mineral degradation (Lian et al. 2008; Uroz et al. 2007; Uroz et al. 2009). In addition to organic acids, microorganisms (such as bacteria, algae, fungi and protozoa) can use carbonic acid formed from carbon dioxide (CO_2) to attack the mineral surface, promoting the chemical weathering of rocks and minerals (Gadd 2007; Park et al. 2009). Lian et al (2008) reported that there were three major reaction pathways utilized by *Aspergillus fumigatus* to release potassium from potassium minerals.

These pathways include: acid hydrolysis, through the secretion of soluble small molecules; a chelating reaction of mineral elements, via the secretion of insoluble macromolecules and polymers bound in the cell membrane; and direct bio-physical forces which can fracture mineral grains to decrease particle sizes and generate fresh and more reactive surfaces.

K is an essential nutrient of life on Earth, it plays a key role in many physiological and biochemical processes.

Some kinds of aluminosilicate minerals such as feldspars and micas constitute the biggest potassium pool in soils, however potassium contained in this pool is mostly not available to plants (Barré et al. 2007). *B. mucilaginosus* has great K-releasing capability and can promote the release of potassium through weathering of silicate minerals (Hu et al. 2006; Lian 1998; Zhao et al. 2006, 2008). This observation is confirmed by XRD (X-ray

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[powder] diffraction) analysis of mineral residues and chemical analysis of the K^+ concentration, which is increased in the supernatant after the bacteria react on K-bearing silicate minerals (Lian et al. 1998, 2005). *B. mucilaginosus* could increase 67.8–82.6% potassium-release rates from K-feldspar, muscovite and illite compared with the control group (Sheng and Huang 2002).

The bacteria also could promote K-release from a variety of potassium-bearing silicate minerals more effectively to increase yields of wheat, maize, tomato, mung bean, celery et al. (Lin et al. 2002; Nishanth and Biswas 2008; Xie et al. 2010; Yan et al. 2009), and significantly improve K intake rates of sudan grass (*Sorghum vulgare* Pers.) (Basak and Biswas 2009). Transgenic methods have been applied in *B. mucilaginosus* research, which was used as a microbial fertilizer for plant growth (Li et al. 2005, 2007).

The *B. mucilaginosus* K02 strain, used in this study, also demonstrates an enhancement in silicate mineral weathering (Du et al. 2008; Hu et al. 2011; Mo and Lian 2011; Zhou et al. 2010).

The mechanism of K release from silicate minerals by *B. mucilaginosus* is a complicated process. To investigate *B. mucilaginosus* weathering of potassium feldspar and illite, Lian et al. (2002) proposed a phased model that illustrates the bacterial K releasing mechanism. Bacterial extracellular polysaccharides promote the formation of a bacteria-mineral complex. At the bacteria-mineral complex interface, organic acids and low pH enhance the dissolution of mineral crystals and the release of soluble potassium.

Another noteworthy phenomenon is that extracellular polysaccharide from *B. mucilaginosus* has a strong tendency to adsorb organic acids (Lian 1998), thus, micro-regions consisting of high organic acid concentration form, leading to further dissolution of the rock surface. In addition, polysaccharides also adsorb SiO_2 and K^+, which drives the stasis between minerals and the liquid phase to move toward dissolving reaction that increase K^+ release and solvation, and promote mineral weathering (Lian et al. 2002; Liu et al. 2006).

K-selected microbes have the capability of constitutively degrading potassium-bearing minerals under potassium deficient conditions, by secreting large amounts of extracellular enzymes with a lower probable return (Ekschmitt et al. 2005). This mechanism has been retained during evolution, perhaps because their secreted proteins could be used to acquire essential mineral nutrients.

The studies of Seneviratne and Indrasena (2006) and Puente et al. (2009) showed that nitrogen-fixing bacteria on mineral surfaces can accelerate mineral decomposition. The link between nitrogen fixation and the acceleration of mineral decomposition may involve changes in various protein expression levels. By SDS-PAGE, Konhauser et al. (2008) found that *Anabaena* sp. would adjust its expression of various specific proteins while weathering silicate minerals. Some induced genes in the microbe-mineral interaction have also been reported (Olsson-Francis et al. 2010).

This study aims to understand the relationship between bacterial secreted proteins and K-bearing mineral weathering by studying the effects of *B. mucilaginosus* secreted proteins in the K-releasing process, so that we can decipher if bacterial secreted proteins are related to the bacterial weathering of K-bearing rock powders. To the best of our knowledge, there have been no reports on differential expression of secreted proteins in the process of bacterial decomposition of potassium minerals, and there is a lack of direct evidence for metabolic mechanisms of bacterial release of potassium from minerals.

MATERIALS AND METHODS

Bacteria

The Gram-negative, *B. mucilaginosus* K02 strain (GenBank database accession number: HM579819) (Mo and Lian 2011), which was used in the study, was stored at the Environmental Biological Science and Technology Research Center, Institute of Geochemistry, Chinese Academy of Sciences.

Isolation and purification of the bacterium were conducted using a nitrogen free medium (sucrose 5.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCO_3$ 0.1 g, Na_2HPO_4 2.0 g, $FeCl_3$ 0.005 g, illite powder 1.0 g, double distilled water 1.0 L, pH 7.0–7.2).

Minerals

The K-bearing mineral (Table 1) used in this study was collected from Fuquan in Guizhou Province. The rock sample was crushed and sieved to collect grains <100 mesh (approximately 150 μm). Mineral powders were ultrasonically cleaned with ddH_2O (double distilled water) (2 min, 3 times at 100 V) to remove the electrostatically charged micro-particles that adsorbed to the surface of the mineral powder.

Bacterial Growth and its Effect on the K-releasing

Nitrogen-containing medium was defined as: sucrose 10.0 g, $(NH_4)_2SO_4$ 1.0 g, $CaCO_3$ 1.0 g, $MgSO_4$ 0.5122 g, KCl 0.1 g, $Na_2HPO_4 \cdot 12H_2O$ 2.507 g, K-feldspar 10.0 g, ddH_2O 1.0 L, pH 7.0–7.2.

Bacteria were grown in four controlled media (Table 2), which were as follows: (A) KCl with no mineral powder added, +KCl/−mineral; (B) lack of KCl but supplied with mineral powder, −KCl/+mineral; (C) KCl supplied with mineral powder, +KCl/+mineral; (D) no KCl and no mineral powder, −KCl/-mineral. The flasks containing 150 ml medium were incubated at 30°C with rotation at 130 rpm. This method was used to study the differential expression of secreted proteins related to the K releasing process. Growth of bacteria was monitored spectrophotometrically (measurement of the optical density at 600 nm).

Potassium ion concentrations in the growth media were analyzed by AAS (atom absorption spectrometry, Perkin-Elmer PE-5100). Take 1 ml samples from three flasks of culture B (−KCl, +mineral) and C (+KCl, +mineral) for each day. The samples were mixed with 4-times volume ddH_2O, standed at

TABLE 1
Mineralogical and chemical compositions of the mineral

Mineralogical composition (%) by XRD[a]							
K-feldspar	Mica	Chlorite	Montmorillonite	Hornblende	Iron ore		
78.39	12.18	3.25	4.24	0.54	1.40		
Chemical composition (%) by XRF[b]							
K_2O	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	LOI
9.47	54.55	17.52	4.59	3.99	3.44	0.15	5.43

[a]X-ray diffractometry, Rigaku D/Max-2200, Cu$K\alpha$ at 40 kV and 30 mA, and 3°/min scan rate.
[b]X-ray fluorescence spectrometry, Panalytical Axios PW4400.

40°C for 1 hour and then centrifuged at 8000 rpm for 10 minutes to collect the supernatant. The supernatants were filtered by 0.45 μm filter paper before AAS analysis. The average values of the three samples were multiply by 5 as the K^+ concentrations of the mediums. Mineral grains collected after 10 days' experiments were air-dried and analyzed by SEM (scanning electron microscopy, KYKY-AMRAY 1000B).

Preparation of Extracellular Proteins

Bacterial cells growed in the four controlled media (30°C, 130 rpm, 48 h) were removed by two successive centrifugations at 8,000 × g for 20 minutes at 4°C. The supernatant was precipitated with 85% saturated $(NH_4)_2SO_4$ solution and the raw protein precipitates were cleaned by the trichloroacetic acid (TCA)/acetone cleaning method (Gómez-Vidal et al. 2008). The obtained proteins were dissolved in rehydration buffer (7 M urea, 2M Thiourea, 4% w/v 3-[(3-Cholamido propyl)dimethylammonio] propanesulfonate (CHAPS), 40 mM Dithiothreitol (DTT), 0.8% v/v Bio-lytes pH 5–8 (BioRad) and 0.002% w/v bromophenol blue, all chemicals were analytical or biochemical grade), which can be used immediately or stored at −70°C. The concentration of the proteins was determined using the Bradford assay (Bradford 1976). Three repeats of each sample were prepared.

Two-dimensional Electrophoresis (2-DE)

This study targeted only the extracellular proteins of bacteria, because they may directly act on minerals (Fredrickson and Zachara 2008). Compared with the intracellular proteins, the high abundance proteins within the extracellular proteome may exhibit higher concentrations. Isoelectric focusing of extracellular proteins revealed that highly abundant proteins impact the results, leading to the appearance of a dark protein smear in the 2-DE protein image. Limiting the amount of the protein loaded onto the gel resulted in the absence of signal for low molecular weight proteins.

Bacterial secreted protein (80 μg) was diluted to a final volume of 200 μl with rehydration buffer. IPG strips (length 11 cm) in the linear pH range of 5–8 (Immobiline; BioRad) were used because preliminary experiments revealed few proteins below pH 5 and none above pH 8. The IPG strips were rehydrated for 14 hours with the rehydration solution at 20°C. The electrophoresis was performed using Ettan IPGphor 3 (GE healthcare) with the running conditions as a gradient to 200 V for 30 minutes; a gradient to 500 V for 30 minutes; a gradient to 1000 V for 1 hour; a gradient to 8000 V for 1 h; 8000 V for 4 hours; total ∼ 40 kV h.

After completion of the first isoelectric focusing electrophoresis, the IPG strips were treated by reduction and alkylation (Fanous et al. 2008), followed by second dimensional SDS-PAGE with the SE 600 Ruby system (Amersham Biosciences), using a vertical slab gel (10% T), running at 10 mA/gel for 15 min, and 20 mA/gel for 6 h. After electrophoresis, the gel was stained with silver nitrate (Nebrich et al. 2007), and protein images were captured by ImageScanner III (GE healthcare) using Imagemaster 2D software (version 6.0) for identification and quantitative intensity analysis of protein spots on the image. These protein spots were manually checked once more for their accuracy. Only protein spots showing significant up- or down-regulation (2-fold change in signal intensity at least) were

TABLE 2
The composition of the four media used in this study

	Sucrose	$(NH_4)_2SO_4$	$CaCO_3$	$MgSO_4$	KCl	$Na_2HPO_4{\cdot}12H_2O$	Mineral powder
A	10. 0g/l	1.0 g/l	1.0 g/l	0.51 g/l	0.1 g/l	2.51 g/l	None
B	10. 0 g/l	1.0 g/l	1.0 g/l	0.51 g/l	None	2.51 g/l	10.0 g/l
C	10. 0 g/l	1.0 g/l	1.0 g/l	0.51 g/l	0.1 g/l	2.51 g/l	10.0 g/l
D	10. 0 g/l	1.0 g/l	1.0 g/l	0.51 g/l	None	2.51 g/l	None

Note: Medium D contains no KCl and no mineral powder, thus has no K^+ source required by bacteria for growth and reproduction. Therefore, more bacterial cells were inoculated into the flask to get the secreted proteins. It is possible to bring some K^+ into the medium during inoculation.

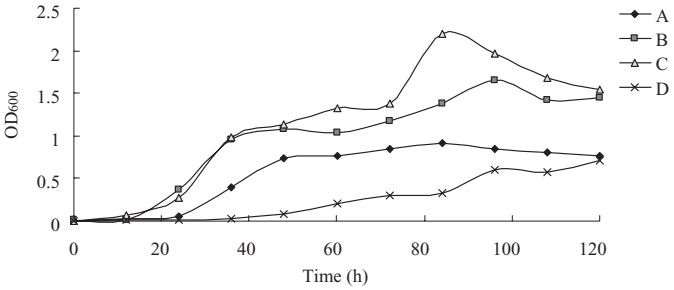

FIG. 1. The growth curve of *B. mucilaginosus* measured by OD600. A: the medium containing KCl but without the mineral powder, +KCl/−mineral; B: the medium containing the mineral powder but without KCl, −KCl/+mineral; C: the medium containing the mineral powder and KCl, +KCl/+mineral; D: the medium without the mineral powder and KCl, −KCl/−mineral.

considered as differentially expressed. The experiment for 2-DE electrophoresis of each sample was repeated at least three times.

Protein Identification by Tandem Mass Spectrometry (MS/MS)

Some protein spots associated with the K-releasing process were excised from the polyacrylamide gel, which was stained by using Colloidal Coomassie (Candiano et al. 2004), and then analysed by tandem mass spectrometry (MS/MS) (Molina et al. 2007; Nesvizhskii et al. 2007) on the 4800 plus MALDI-TOF-TOF Analyzer (Applied Biosystems Ltd.) performed by Shanghai BoYuan Biotechnology Co., Ltd.

RESULTS

Bacterial Growth Curves

The growth curves of the bacteria in the four different media (Table 2), under the conditions of 30°C with a shaking speed of 130 rpm are shown in Figure 1. The bacteria incubated for 48 hours were still in the exponential phase. The OD600 (optical density at 600 nm) values of samples B and C were higher than that of sample A and reached an inflection point due to the disturbance of the mineral powder. Due to a lack of potassium nutrition, the bacteria grew very slowly in medium D. To obtain the secreted proteins, bacterial inoculates of sample D were increased to three loops of bacterial culture to obtain a better yield. The measurements of bacterial growth were replicated twice. Perhaps because all media contained calcium carbonate, the acidity of each sample ranged from a pH of 6.36 to 7.57.

The complexity of metabolism at stationary phase has a negative impact on the differential expression of secreted K-releasing related proteins. The bacteria in the stationary phase might need less K ion than in the growth phase. So, the time for extraction of the secreted proteins for 2-DE was final chosen as in the middle phase of the exponential growth (48h, see Fig. 1).

K-release by the Bacteria

In a 5-day incubation period, the potassium ion concentration of culture B (−KCl, +mineral) slowly rose from 5.3 to 10.4 ppm, and then maintained at 8.2–8.5 ppm. But the control group of culture B (no inoculation) remained about 5 ppm all the time in 5 days. The K^+ concentration of culture C (+KCl, +mineral) slowly declined from 104.1 to 96.4 ppm in the first day. Then it rapidly decreased to 56.9 and 51.1 ppm at the second and third day, respectively, during the rapid growth of bacteria. After that, it maintained at 45.2–46.6 ppm. The results show that the bacteria use K nutrition from the minerals in the culture B, but use KCl in culture C.

The SEM observations on bacterial mineral degradation in culture B and C are shown in Figure 2. The surface of mineral grain in sample B is covered with more organic matters and the bacteria may hide in biofilm. The mineral grain in sample C has more distinct edges and less surrounding organic matters. This

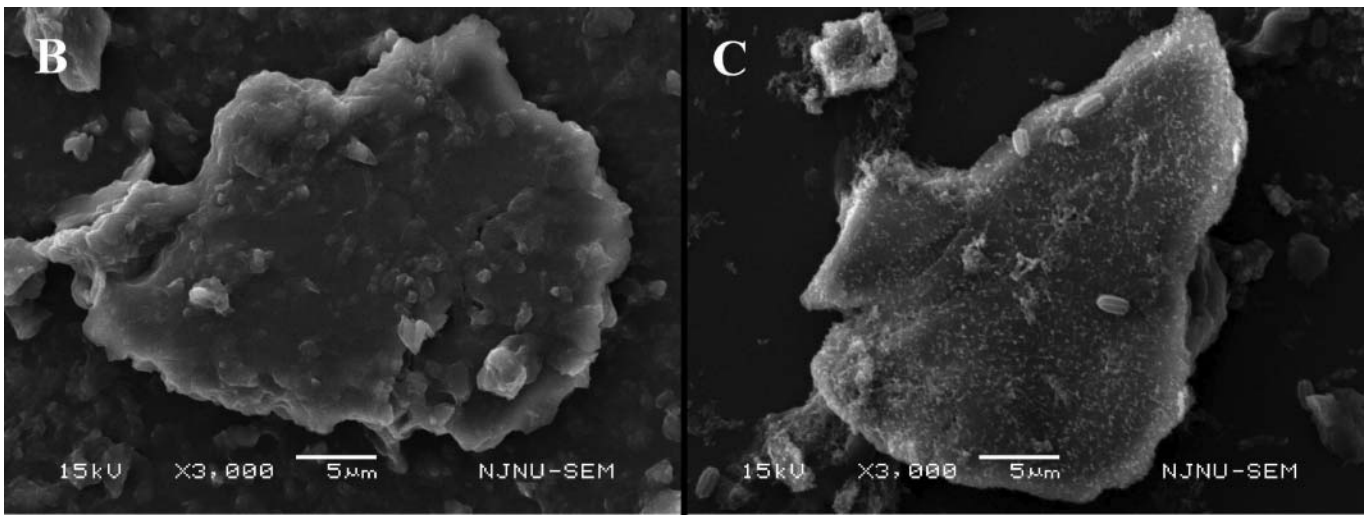

FIG. 2. The SEM observations of mineral grains in media B and C after 10 days experiments (Fig. 2B and 2C correspond to media B and C).

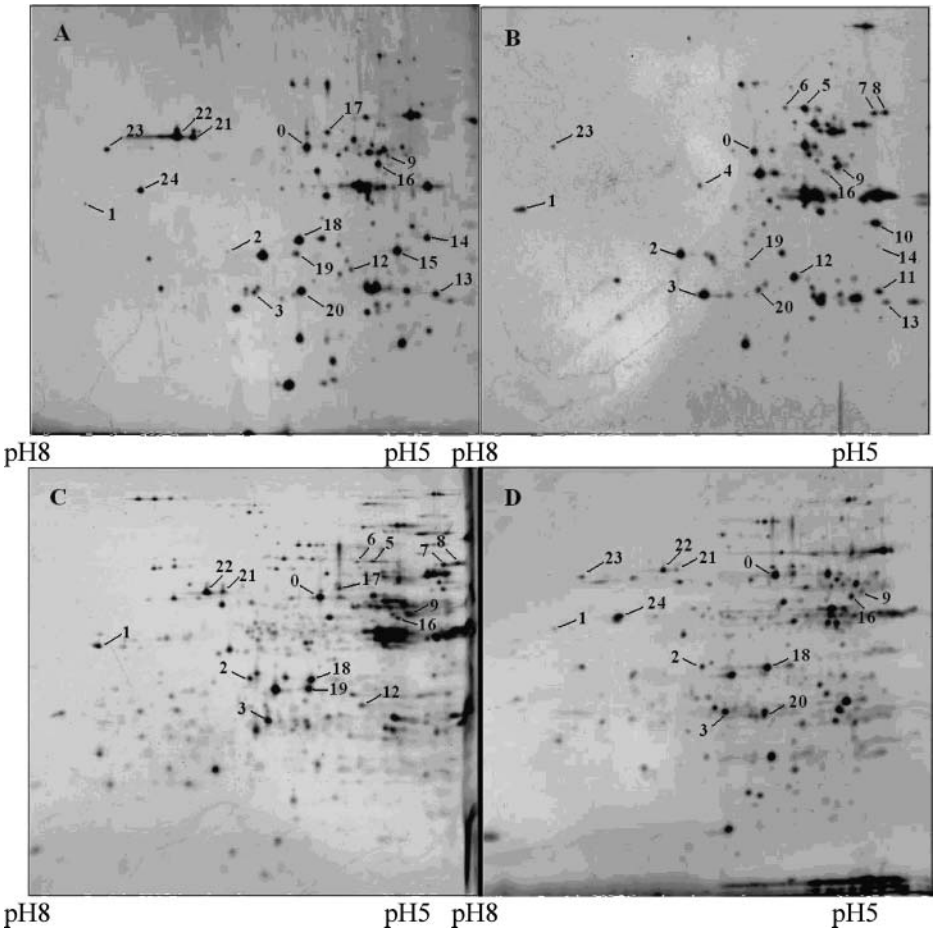

FIG. 3. Silver staining image of two-dimensional electrophoresis of *B. mucilaginosus* secreted proteins (Fig. 3A–3D correspond to sample A–D). A: +KCl/−mineral; B: −KCl/+mineral; C: +KCl/+mineral; D: −KCl/−mineral. Spot 0 is a reference protein spot, which is a housekeeping protein.

phenomenon means bio-weathering to the minerals occuring because of the bacteria growth at K^+-lacking medium.

Comparison of 2-DE Gels

The 2-DE analyses of *B. mucilaginosus* proteins secreted under the four different media conditions are shown in Figure 3. By comparison of Figure 3A to Figure 3B, there were 12 up-regulated protein spots (spot 1–12) and 12 down-regulated protein spots (spot 13–24) under bacterial potassium weathering conditions.

At the same time, the representative spots 1–12 and 13–24 shown for the gel of sample C (+KCl/−mineral) and those shown in the gel of the sample D (−KCl/−mineral) were compared with sample A(+KCl/−mineral) and B (−KCl/+mineral). Figure 3C shows the protein profiles for sample C, from which some K-releasing associated proteins were up-regulated (spot 1–3, 5–9, 12), but the increase of the expression was less than those from sample B (Fig. 3B); besides, the expression levels of some down-regulated proteins (spot 16–19, 21, 22) associated with potassium metabolism were not severely reduced. Figure 3D shows the protein profiles for sample D, from which not only protein spots 1, 2,3 and 9 were not up-regulated, but spots 16, 18 and 20–24 were not severely down-regulated as well, indicating that with a lack of potassium and mineral powder, bacterial protein profiles were closer to those produced in whole nutrient medium (Fig. 3A).

Protein Identification

At first, 1 to 12 spots excised from the silver nitrate staining gel were sent for analysis. Only spots 1 and 8 had a low homology hit, the other 10 spots did not reveal any matches. Secondly, the Colloidal Coomassie method was used to get protein spots 1, 2, 3 and 5, of which spots 1, 2, 3 were matched with three protein sequences in the NCBInr database. Tandem mass spectrometry database matching results are listed in Table 3. The analysis reveals three related proteins: glycine hydroxymethyltransferase, phosphoserine aminotransferase, and ketol-acid reductoisomerase.

DISCUSSION

With proteomics, gene expression of proteins related to microbial physiological activities and function under different

TABLE 3
List of identified *B. mucilaginosus* secreted proteins whose expressions were stimulated by K-feldspar when incubated without K^+

Protein spot	Protein identified	Accession number	species	datebase	Matched peptides in database	Mascot MOWSE score	Mass (kDa)
1	Glycine hydroxymethyltransferase	gi|251799794	*Paenibacillus* sp. JDR-2	ncbinr	KPWAAAIDK, NAIPFDPT-SPFVTSGIR	161	44.72
2	phosphoserine aminotransferase	gi|28868952	*Pseudomonas syringae* pv. tomato str. DC3000	ncbinr	FGMIYAGAQK	84	39.74
3	ketol-acid reductoisomerase	gi|226311207	*Brevibacillus brevis* NBRC 100599	ncbinr	TIAIIGYGSQG–HAQAQNLR	122	37.74
5	ABC transporter, ATP-binding protein	gi|254786291	*Teredinibacter turnerae* T7901	ncbinr	AAELLEKLGLAK	51 (invalid)	invalid
8	hypothetical protein BDI_3520	gi|150010100	*Parabacteroides distasonis* ATCC 8503	ncbinr	IQTMLILR	49 (invalid)	Invalid

environmental conditions can be elucidated (Fanous et al. 2008). In general, the biodegradation capacity of microorganisms was achieved through the secretion of metabolic intermediates or extracellular enzymes (De Graef et al. 2005). Song et al. (2007) suggested that bacteria can selectively use minerals, and were inclined to attach to the mineral containing necessary elements. In nitrogen-containing medium, the *B. mucilaginosus* K02 strain produces extracellular polymers with high protein content and improves K-release compared to nitrogen-free medium (Du et al. 2008).

By comparing the 2-DE analyses of bacteria secreted protein profiles in sample B (−KCl/+mineral) to that of sample A (+KCl/−mineral), we found a series of up-regulated or down-regulated protein spots, indicating that mineral powder can induce bacteria to express mineral weathering associated proteins. The comparison of bacterial secreted protein profiles among sample A, B and C (culture C: +KCl/+mineral) suggesting that bacteria may be more inclined to use soluble K^+ for the energe saving in media containing $K^+$and K-bearing mineral, and may not be sensitive to mineral powder.

On the other hand, the comparison among sample A, B and D (culture D: −KCl/−mineral) suggests that just K-nutrition deficiency (without adding K-bearing minerals) is not severe enough to induce a sufficient number of secreted proteins needed for mineral weathering. The difference between sample A and D should result from potassium nutrient deficiency. These observations indicates that the bacterial mineral weathering related proteins are more greatly expressed under conditions of K^+ deprivation but with K-bearing minerals. Bacteria are conditioned to use soluble K^+; when inorganic nutrients are plentiful, the minerals have limited impact on the induction of secreted proteins expression (those proteins associated with mineral dissolution). Nutritional deficiencies signal an urgent need to increase metabolism in bacteria; however, when minerals were not present, even nutritional deficiencies were not sufficient to induce the expression of a large number of proteins associated with mineral weathering.

When bacteria incubated in medium B (−KCl, +mineral), the concentration of water-soluble potassium ion except those utilized and adsorbed by the bacteria could rise to 10.4 ppm, which shows the bacteria can release potassium from minerals for its growth. But the concentration of K^+ in medium C (+KCl, +mineral) declined with the growth of bacteria, which means the K^+ was utilized and adsorbed without enough compensation from minerals contrast to that in medium B. Associated with the secreted protein profiles (Fig. 3), in which the spots 1∼12 were all significantly up-regulated in sample B but is different in sample C, those extracellular proteins have positive correlation with bacterial K-release. The mineral grains in medium B (Fig. 2B) also might be corroded more severely than it in medium C (Fig. 2C) observed by SEM.

The related secreted proteins may accelerate bacterial or microbial degradation of potassium minerals. In this text, we propose the following two opinions on the possible roles that *B. mucilaginosus* secreted K-releasing related proteins play in the process of K-release and mineral degradation.

(1) Mineral weathering promoted by redox reactions
Under conditions where specific nutrient elements are absent, bacteria (through more redox processes), transfer electrons between metal ions or groups on mineral surfaces resulting in the destruction of mineral structure; finally, nutrient release is promoted.

Some bacteria are more inclined to produce redox reactions. Research shows that multiheme cytochromes are located in bacterial outer membrane surface and these structures allow proteins to transfer electrons easily through direct and close contact with the surface of the oxide (Fredrickson and Zachara 2008). Theoretically, for the mosaic structure of the compound in a complex subject, redox processes will result in the instability of the mineral crystals, allowing them to be dissolved more readily (Uroz et al. 2009).

(2) The efficiency of mineral weathering is enhanced by the combined action of bacterial secreted proteins and exopolysaccharides (EPS)
Previous studies have shown that extracellular polysaccharides have a consistent absorption of free potassium and phosphorus, which are released into solution (Du et al. 2008; Yi and Huang 2008); together with bacterial secreted proteins, they drive the homeostatic balance of insoluble mineral dissolution towards the forward dissolving reaction, thereby enhancing the efficiency of mineral weathering.

Among the secreted proteins of *B. mucilaginosus*, some K-releasing related proteins have a unique domain, which can trap potassium ions. These proteins may belong to the ion-transporter family. Eckhardt et al. (2001) constructed a tomato (*Lycopersicon esculentum*) root (under iron deficiency stress) cDNA library and isolated two Fe^{2+} uptake-related transport proteins, which were finally identified as zinc transport proteins. It can be speculated that cation transport proteins may share a similar domain. Extracellular protein binding to K^+ is similar to the biomineralization process, in which a K^+-rich region is formed and tiny mineral crystals are further produced.

This process results in the K^+ concentration of other regions falling below the saturation point, which promotes the further dissolution of minerals. Similar results were reported on *B. subtilis*: a highly concentrated metal ion region formed on the cell surface (Konhauser et al. 2008). In yeast undergoing a biomineralization process, protein molecules can be mineralized crystal nuclei (He et al. 2009). In bacteria, the capacity of Fe storage is mediated by ferritin (Wallner et al. 2009).

Based on comprehensive effects of the potassium releasing mechanism (Lian et al. 2002), the role of bacterial secreted proteins is another pathway that explains the mechanisms of how bacteria receive K-limited nutrition from silicate mineral

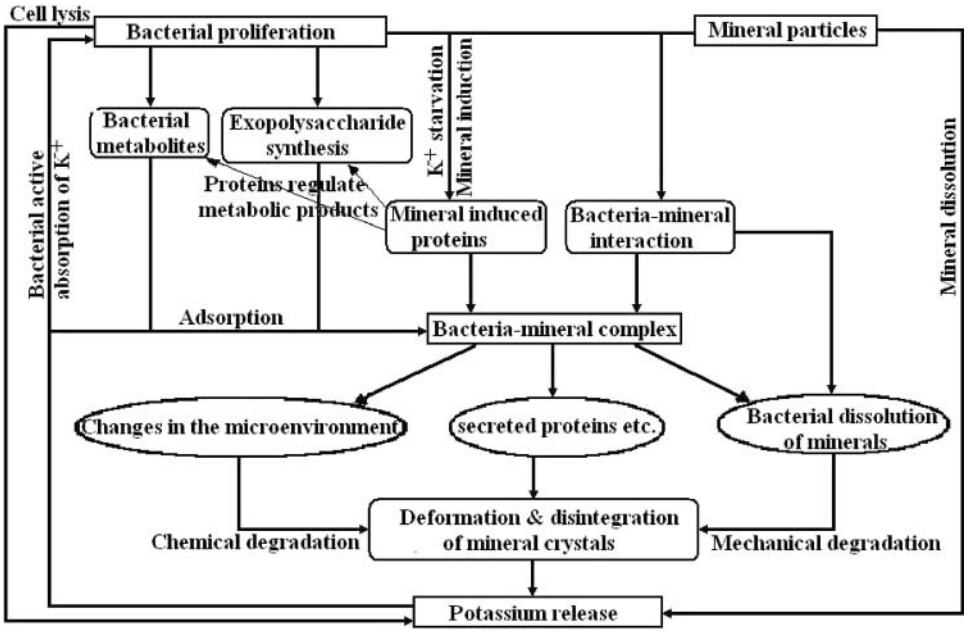

FIG. 4. The schematic diagram of potassium releasing process by *B. mucilaginosus* from potassium- bearing silicate minerals.

weathering. The related functional genomics of microorganisms can regulate protein expression to control the weathering of potassium minerals (Fig. 4); intracellular proteins regulate metabolites and secrete extracellular proteins.

In this study, a total of 12 protein spots (spots 1–12) were up-regulated in the K-releasing process. This data is not strong enough for illustration of the bacterial mineral weathering system. Thus, the metabolic pathway and the mechanism of K^+ release and mineral decomposition by *B. mucilaginosus* requires further investigation.

CONCLUSIONS

In this article, we found that there was a relationship between the expression of secreted proteins and the bacterial capability of dissolving minerals. Two-dimensional electrophoresis and tandem mass spectrometry technology were applied to provide direct evidence that suggests that differential expression of secreted proteins is associated with K-release efficiency in *B. mucilaginosus*. This association suggests that minerals can induce the secretion of various proteins. Such induction was strengthened when microbial need for respective mineral elements occurred, which could explain the regulatory mechanism of microbial weathering. Those bacterial secreted proteins associated with K-bearing mineral weathering may impact the mineral crystal by means of electron transferring. In addition, these secreted proteins, together with polysaccharide or other extracellular polymers, absorb mineral elements in solution to achieve dissolution and weathering of potassium minerals and to acquire inorganic nutrients.

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