Copper tolerance of the thermoacidophilic archaeon *Sulfolobus metallicus*: possible role of polyphosphate metabolism

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It has been postulated that inorganic polyphosphate (polyP) and transport of metal–phosphate complexes could participate in heavy metal tolerance in some bacteria. To study if such a system exists in archaea, the presence of polyP was determined by the electron energy loss spectroscopy (EELS) procedure and quantified by using specific enzymic methods in *Sulfolobus acidocaldarius*, *Sulfolobus metallicus* and *Sulfolobus solfataricus*. All three micro-organisms synthesized polyP during growth, but only *S. metallicus* greatly accumulated polyP granules. The differences in the capacity to accumulate polyP between these archaea may reflect adaptive responses to their natural environment. Thus, *S. metallicus* could grow in and tolerate up to 200 mM copper sulfate, with a concomitant decrease in its polyP levels with increasing copper concentrations. On the other hand, *S. solfataricus* could not grow in or tolerate more than 1–5 mM copper sulfate, most likely due to its low levels of polyP. Shifting *S. metallicus* cells to copper sulfate concentrations up to 100 mM led to a rapid increase in their exopolyphosphatase (PPX) activity which was concomitant in time with a decrease in their polyP levels and a stimulation of phosphate efflux. Furthermore, copper in the range of 10 μM greatly stimulated PPX activity in cell-free extracts from *S. metallicus*. The results strongly suggest that a metal tolerance mechanism mediated through polyP is functional in members of the genus *Sulfolobus*. This ability to accumulate and hydrolyse polyP may play an important role not only in the survival of these micro-organisms in sulfidic mineral environments containing high toxic metals concentrations, but also in their applications in biomining.

INTRODUCTION

Polyphosphates (polyP) are present in most organisms including bacteria, archaea and eukaryotes (Kornberg *et al.*, 1999). PolyP is a linear polymer of hundreds of orthophosphate residues linked by phosphoanhydride bonds that has a variety of physiological functions, such as serving as a reservoir of phosphate, substitution for ATP in kinase reactions, serving as a chelator of metals, and adaptation to different stress conditions (Kornberg *et al.*, 1999). The main enzyme involved in the biosynthesis of polyP is polyphosphate kinase (PPK), which catalyses the reversible conversion of the terminal phosphate of ATP into polyP (Akiyama *et al.*, 1992). An exopolyphosphatase (PPX), on the other hand, is known to hydrolyse polyP, liberating inorganic phosphate (P_i) (Akiyama *et al.*, 1993). These enzymes have been purified from *Escherichia coli* and their genes have been identified in several bacteria (Kornberg *et al.*, 1999; Alvarez & Jerez, 2004). One of the proposed mechanisms for metal tolerance is the sequestration of metal cations by polyP (Kornberg *et al.*, 1999). Many heavy metal resistance systems involve either an active efflux or a detoxification of metal ions by different transformations (Silver & Phung, 1996). For copper, these include intracellular complexation, decreased accumulation, extracellular complexation or sequestration in the periplasm (Rouch *et al.*, 1989; Harwood & Gordon, 1994). It has also been proposed that the hydrolysis of polyP detoxifies the metals (Aiking *et al.*, 1984; Keasling, 1997). Van Veen (1997) has shown that the inorganic phosphate transport system (Pit) in *Escherichia coli* and *Acinetobacter johnsonii* can reversibly transport metal phosphates. Keasling & Hupf (1996), using genetically engineered strains of *E. coli*, obtained results indicating that not only a large quantity of intracellular polyP is important for tolerance to heavy metals but also the ability to synthesize and degrade polyP. Based on these results and those mentioned above, Keasling (1997) proposed a model in which the intracellular cation concentration in bacteria would regulate the activity of PPX, which would in turn degrade polyP, and the P_i generated...
accompanies by cation transport would be removed from the cell through the Pit system. We have recently found that _Acidithiobacillus ferrooxidans_, a micro-organism that can tolerate very high concentrations of heavy metals in its normal environment, normally accumulates high amounts of polyP granules and that the levels of intracellular polyP are greatly decreased when the bacterium is grown in or shifted to 100 mM Cu^{2+}. In the presence of this metal, PPX activity and P_i efflux greatly increased, supporting a model for metal tolerance mediated through polyP (Alvarez & Jerez, 2004).

Related to archael copper resistance mechanisms, some metal efflux pumps have been identified in the genomes of archaea (Pedone et al., 2004). The P-type CPX-ATPases are responsible for the transport of heavy metal ions in all kinds of organisms. One of the two CPX-ATPases of _Sulfolobus solfataricus_ has recently been isolated and characterized (Deigweiher et al., 2004). In Ferroplasma acidarmanus the FerI copper resistance (cop) loci (Ettema et al., 2003), which include genes encoding a putative transcriptional regulator (copY), a putative metal-binding chaperone (copZ) and a putative copper-transporting P-type ATPase (copB) have been described (Baker-Austin et al., 2005). By transcription analyses the authors demonstrated that copZ and copB are co-transcribed, and that the transcript levels increase significantly in response to exposure to high levels of Cu^{2+} ions, suggesting that the transport system is operating for copper efflux.

In archaea, polyP has been reported only in _Methanosarcinae_ (Scherer & Bochem, 1983) and in _Sulfolobus acidocaldarius_ (Skórko et al., 1989). However, nothing is known about the possible role of polyP in archaea. A partially purified PPK was reported in _S. acidocaldarius_ (Skórko et al., 1989). However, we demonstrated later that this protein was rather a glycogen synthase (Cardona et al., 2001). Despite the fact that no bacterial-type PPKs have been found in the majority of the available archael genomic sequences, we recently reported the isolation of a PPX enzyme from _Sulfolobus solfataricus_ (Cardona et al., 2002). In the present work, we have studied the effect of different metals in several _Sulfolobus_ species and have found that the presence of these cations decreased polyP levels and increased both PPX activity and phosphate efflux, clearly supporting the proposed metal tolerance model.

**METHODS**

**Strains and growth conditions.** _S. metallicus_ DSM 6482 was grown at 65°C in medium 88 (Deutsche Sammlung von Mikroorganismen und Zellkulturen) with 0-05% (w/v) elemental sulfur and 0-02% (w/v) yeast extract. _S. acidocaldarius_ DSM 639 was grown at 70°C in the same medium but with 0-1% (w/v) yeast extract and 0-2% (w/v) sucrose, according to Skórko et al. (1989). _S. solfataricus_ DSM 1617 was grown at 70°C in medium 182 (Deutsche Sammlung von Mikroorganismen und Zellkulturen) with 0-1% (w/v) yeast extract and 0-1% (w/v) Casamino acids. To study copper or cadmium tolerance of _S. metallicus_ and _S. solfataricus_, the micro-organisms were grown in their respective media, except that different concentrations of CuSO_4_ (5–200 mM) or CdSO_4_ (0-01–3 mM) were present initially, as indicated in the corresponding experiments. For shift experiments, _S. metallicus_ cells were grown in the absence of copper to the early stationary phase and after removing the medium from the cells by centrifugation, they were then shifted to a new medium containing 10, 20 or 100 mM CuSO_4_. As a control, a shift of cells to 100 mM (NH_4)_2SO_4_ was used. Aliquots were taken at different times and polyphosphate levels and PPX activity were determined. Growth was monitored by measuring unstained cell numbers by means of a Petroff-Hauser chamber under a phase-contrast microscope.

**Electron microscopy.** Unstained cells from _S. metallicus_ or _S. solfataricus_ were routinely examined for the presence of electron-dense bodies by transmission electron microscopy (Gonzalez & Jensen, 1998). Early stationary-phase cells from different growth conditions were suspended in distilled water and then placed onto carbon-coated nickel grids. After allowing the micro-organisms to settle on the grid, the excess liquid was drained off with filter paper, and the preparations were air-dried. For analysis, a transmission electron microscope (Philips Tecnai 12) operating at 80 kV was used.

**Electron energy loss spectroscopy (EELS).** EELS analysis (Lünsdorf et al., 2000) was performed with a Zeiss CEM 902 integrated energy-filtered transmission electron microscope. The microscope was operated in the electron spectroscopic imaging (ESI) mode for element mapping, and parallel EELS performed for spectrum registration with the aid of ESI-Vision software (Soft Imagining System). Aperture settings described by Lünsdorf et al. (2000) were used. Commercial hydroxyapatite was used as the internal phosphate standard (Chávez et al., 2004).

**PolyP quantification.** Purified recombinant His6-PPK was prepared by using _E. coli_ strain NR 100 as described previously (Ahn & Kornberg, 1990; Kumble et al., 1996), and this preparation was used in the polyP assay described below. The protein concentration was determined by the method of Bradford (Coomassie Plus protein assay reagent, Pierce).

PolyP was quantified by using a two-step conversion of polyP into ATP by PPK and quantification of ATP by using luciferase to generate light (Ault-Riche et al., 1998). First, polyP was extracted by using Glassmilk, from the different cells grown in the absence or in the presence of copper once they reached the early stationary phase. The polyP extracted was assayed by using the reverse reaction of _E. coli_ PPK in ADP excess. Finally, the ATP generated was determined by using the luciferase (Boehringer Mannheim) reaction, and the luminescence was measured with a luminometer (BioScan Lumi/96). Concentration of polyP was expressed in terms of _P_i_ residues.

**In vitro preparation of [32P]polyP_750_ as a substrate for PPX.** Radioactively labelled polyP with a chain length of 750 residues was prepared as described by Ault-Riche et al. (1998), with the modifications of Cardona et al. (2001) and Alvarez & Jerez (2004). The identity and purity of [32P]polyP_750_ were determined by its susceptibility to hydrolysis by ScPPX1 as described previously (Ault-Riche et al., 1998; Cardona et al., 2001).

**Preparation of cell-free extracts from _S. metallicus_.** Cells from 800 ml of a control culture grown to 10^8 cells ml^{-1}, or cultures shifted to different CuSO_4_ concentrations or to 100 mM (NH_4)_2SO_4_, were harvested by centrifugation (7700 g for 15 min). The pellets were washed with M88 medium to remove the sulfur. Then the cells were resuspended in 50 mM Tris/acetate (pH 7-0)-10% sucrose buffer (20 ml μg wet weight), frozen, and sonicated eight times for 30 s each time. The lysate was centrifuged (4300 g for 5 min) to eliminate cellular debris, and the supernatant was used to measure PPX activity.
Assay for PPX activity and TLC analysis of the reaction products. PPX activity was determined as described by Cardona et al. (2002), with the following modifications. The 50 µl reaction mixture contained 50 mM Tris/acetate (pH 7.0), 1 mM MnCl₂, 100 mM KCl, 50 µg extract protein and 250 µM [³²P]polyP750. After incubation of the mixture for 60 min at 65°C, the reactions were stopped; 4 µl was taken from each reaction mixture and loaded on polyethyleneimine-cellulose plates (Merck). For TLC, samples of 4 µl were developed in 0-75 M KH₂PO₄ (pH 3-5). Radioactive spots were visualized and quantified by using a Phosphorimager (Molecular Imager FX Systems, Bio-Rad). One unit of enzyme was defined as the amount releasing 1 pmol phosphate from polyP min⁻¹.

In vivo labelling of S. metallicus with ³²P. Cells were grown in medium 88 supplemented with 0-05% (w/v) sulfur and 0-02% (w/v) yeast extract to mid-exponential phase in Pi-sufficient conditions (2 mM Pi). Cells were collected by centrifugation and resuspended at a higher cell density (10¹⁰ cells ml⁻¹) in a medium with a lower Pi concentration (0-2 mM Pi). To label the cells, [³²P]KH₂PO₄ [100 µCi ml⁻¹ (3-7 MBq ml⁻¹)] was added and the microorganisms were further incubated for 20 h, after which the radioactively labelled cells were harvested by centrifugation.

Pi efflux measurements. The ³²P-labelled cells were exhaustively washed (five times) by resuspension and centrifugation with fresh medium 88 containing sufficient Pi (2 mM) to eliminate the non-incorporated radioactive label and finally were resuspended in the same medium to a cell density of 10⁹ cells ml⁻¹, in the presence or absence of CuSO₄. To determine the amount of ³²P released to the medium, samples (1-5 ml) were taken periodically and the supernatants obtained by their centrifugation at 12 000 g for 10 min were measured by scintillation counting and analysed by TLC on polyethyleneimine-cellulose with ³⁵P as a standard (Keasling et al., 1993).

RESULTS AND DISCUSSION

Accumulation of polyphosphate in members of the genus Sulfolobus

We have previously described the presence of abundant polyP granules in the acidophilic A. ferrooxidans, which may be related to metal tolerance in this biominning bacterium (Alvarez & Jerez, 2004). To detect the presence of these granules in members of the genus Sulfolobus, we analysed unstained cells of the micro-organisms by electron microscopy (Fig. 1a, b). When S. solfataricus (Fig. 1a) or S. acidocaldarius (not shown) was examined, very faint and small granules, if any, were present. In contrast, two or three large electron-dense granules per cell were seen in S. metallicus (Fig. 1b). To obtain detailed information about the polyphosphate-like inclusions, ultrastructural determinations were made by using scanning electron microscopy coupled to EELS analysis with an integrated energy-filtered transmission electron microscope. Fig. 1(c) shows the electron energy loss spectra obtained from cell areas containing granules in S. metallicus and in S. acidocaldarius compared with that for hydroxyapatite as a phosphate reference after background subtraction and spectrum filtering. The ionization energy onset of the P₁₂,₃ edge is at 135 eV for all three spectra, followed by characteristic energy-loss near edge structures (ELNES) features in the 50 eV higher energy loss region (indicated by the bar in Fig. 1c). All three main maxima, i.e. I, II and III, were present in the spectra obtained, although the intensities in S. acidocaldarius were very small due to its low content of polyP granules, which was very similar to that of S. solfataricus (not shown). Thus, by using electron microscopic microanalyses we demonstrated that the electron-dense granules accumulated by S. metallicus were mainly composed of phosphate and most likely corresponded to polyP.

Enzymic determinations of polyP

To determine whether the electron-dense granules composed of phosphate were indeed due to a higher accumulation of polyP, we quantified polyP in S. metallicus, S. acidocaldarius and S. solfataricus during their growth phases (Fig. 2). Using cell-free extracts obtained for each growth time point, polyP was enzymically quantified. All three micro-organisms synthesized increasing amounts of polyP as growth proceeded, levelling off when the stationary phase was reached.

Accumulations of polyP in S. metallicus were remarkable for being so high and sustained in time compared to those in E.
coli, which does not form visible polyP granules under common growth conditions (Kornberg et al., 1999). The levels of 180 nmol polyP (mg protein)$^{-1}$ (230 mM) reached at the stationary phase of growth (Fig. 2a) are roughly 10-fold greater than those seen in S. solfataricus [20 nmol polyP (mg protein)$^{-1}$ or 1 mM] (Fig. 2b) and S. acidocaldarius [10 nmol polyP (mg protein)$^{-1}$ or 0.5 mM] (Fig. 2c). The amounts of polyP accumulated by S. metallicus are even higher than those reported (150 nmol mg$^{-1}$) for another micro-organism accumulating polyP granules – Vibrio cholerae during the exponential phase of growth with a rich medium (Ogawa et al., 2000) – and are closer to the 400 nmol (mg protein)$^{-1}$ accumulated by A. ferrooxidans (Alvarez & Jerez, 2004). It is possible that the polyP accumulations observed in these crenarchaea contribute not only to their energy supply but also to their enhanced survival and stress resistance in the presence of metals, as suggested for bacteria (Keasling, 1997; Alvarez & Jerez, 2004).

**Effect of CuSO$_4$ and CdSO$_4$ in the growth of S. metallicus and S. solfataricus**

The S. metallicus cells utilized in these experiments were previously adapted to grow at different concentrations of metals. There was a small decrease in the growth rates and the curves reached the plateaus at slightly lower cell densities when the micro-organisms were grown in the presence of increasing Cu$^{2+}$ concentrations compared with the control culture in the absence of added Cu$^{2+}$ (Fig. 3a). However, at 200 mM Cu$^{2+}$, there was a more severe effect of copper on growth, with an approximately 30% decrease in cell numbers. On the other hand, S. solfataricus was not able to grow in any of the copper concentrations tested (Fig. 3b). These results are in agreement with previous reports in which S. solfataricus was able to grow only in the presence of 0.1–1.0 mM copper, being completely inhibited by growth in the presence of 10 mM copper (Miller et al., 1992). This behaviour was similar to that seen for S. acidocaldarius, which also did not grow in the presence of 10 mM Cu$^{2+}$ (Miller et al., 1992). These results clearly contrast with the very high tolerance of S. metallicus to concentrations of copper sulphate as high as 100 and 200 mM (Fig. 3a). S. metallicus could grow in the presence of CdSO$_4$ concentrations up to 1 mM without a noticeable effect in the cell numbers obtained at the stationary phase. At higher concentrations (2–3 mM) there was an extended lag period and the culture reached significantly lower cell numbers (results

![Fig. 2. PolyP levels during growth of different species of Sulfolobus. S. metallicus (a), S. solfataricus (b) and S. acidocaldarius (c) were grown in their respective media and the cell numbers (○) and polyP levels (■) were determined at the indicated times of growth.](image)

![Fig. 3. Growth of Sulfolobus species in the presence of CuSO$_4$. S. metallicus (a) and S. solfataricus (b) cells were inoculated in their respective growth media in the absence of added CuSO$_4$ (●), or were supplemented with 5 mM (○), 10 mM (■), 50 mM (△), 100 mM (◇) or 200 mM (×) CuSO$_4$, and cells were counted daily.](image)
not shown). On the other hand, S. solfataricus growth was seriously affected by CdSO₄ concentrations as low as 10 μM (results not shown).

**Effect of copper ions on the levels of polyP in S. metallicus**

PolyP levels showed a gradual decrease with increasing concentration of copper in the growth medium of S. metallicus until a clear drop (more than 90%) was seen when the Cu²⁺ concentration was raised to 200 mM (Fig. 4a). This behaviour correlated exactly with the disappearance of the polyP granules observed when cells were subjected to those high copper levels (Fig. 4b, c, d). In the absence of copper, 96% of the cells contained two or three large polyP granules per cell (Fig. 4b), whereas at 200 mM copper sulphate the granules were very small, with only 46% of the cells containing only one of these aggregates (Fig. 4d). These results strongly indicate a possible relationship between the polyP levels and the adaptation of S. metallicus to grow in the presence of Cu²⁺.

**PPX activity in cell-free extracts after addition of different concentrations of metals**

A possible mechanism to explain the observed decrease in polyP levels when cells were exposed to copper ions was an increase of the PPX activity. Therefore, the in vitro effect of copper and other metals on the PPX activity present in cell-free extracts of non-adapted S. metallicus was determined (Fig. 5). Copper greatly stimulated PPX activity at very low concentrations. The same maximal PPX activity was reached with both copper and manganese. However, the metal concentration requirement for this was 10 μM for copper (Fig. 5) and 500 μM for manganese (results not shown). At concentrations of copper higher than 10 μM there was an inhibition of the activity, whereas manganese continued to stimulate PPX. Other metals such as cadmium and zinc also stimulated PPX activity in the micromolar range although to a lower extent (results not shown). These results suggest a stimulatory effect of copper and other metal ions on PPX activity and polyP hydrolysis.

**Fig. 4.** Reduction in polyP content during exposure of S. metallicus to copper ions. (a) Cells grown in the absence or presence of the CuSO₄ concentrations indicated were harvested in the early stationary phase (6–7 days of growth) and polyP was quantified by the non-radioactive enzymic method. Three independent determinations were performed. The error bars represent the standard deviations. (b–d) Cells harvested from the same cultures as shown in Fig. 3(a) in the absence of CuSO₄ (b), or in the presence of 100 mM (c) or 200 mM (d) CuSO₄ were analysed by electron microscopy without staining. Arrows indicate some of the polyP granules.

**Fig. 5.** PPX response to CuSO₄ in vitro. PPX activity was determined using the standard assay with cell-free extracts from S. metallicus grown in the absence of copper. The indicated amounts of MnSO₄ (○) or CuSO₄ (●) were added to the assay reaction mixtures.
To further investigate the effect of copper ions on PPX activity and polyP levels, non-adapted cells were shifted to the presence of different CuSO₄ concentrations. Cells were collected at different time intervals and the PPX activity and polyP levels were measured in the cell-free extracts. A rapid increase in the PPX activity was seen when cells were exposed to copper, with the severest effect at the 100 mM concentration (Fig. 6a). No significant effect on PPX activity or polyP levels was seen when the cells were shifted to 100 mM (NH₄)₂SO₄ (results not shown). The increase in PPX activity corresponded in time with the decrease in polyP levels seen in Fig. 6(b), strongly suggesting that the decrease in polyP levels observed when cells were shifted to the presence of copper was due to an increase of this PPX activity.

Effect of copper ions on the efflux of P_i from S. metallicus cells

The decrease in polyP levels due to increased PPX activity in S. metallicus cells subjected to Cu²⁺ ions should generate free P_i. To determine whether this P_i was transported out of the cells, S. metallicus was grown to early stationary phase and labelled in vivo with ³²P_i as indicated in Methods. After exhaustively washing the radioactively labelled cells to eliminate the non-incorporated label, they were resuspended in sulfur medium with or without Cu²⁺ ions and the P_i efflux was determined (Fig. 7).

There was a continuous basal release of label from the control cells. However, an increase in the P_i efflux over this basal level was observed when cells were exposed to Cu²⁺ ions. Differences between the kinetics and extent of polyP degraded (Fig. 6) and the P_i released (Fig. 7) were observed. A similar situation has been found in Acinetobacter johnsonii subjected to conditions (anaerobiosis) in which 65% of the polyP is degraded, since intracellular P_i accumulated up to 150 mM in the course of polyP degradation, while the external P_i concentration remained below 11 mM. The amount of ³²P_i released to the medium was the highest in cells exposed to 200 mM Cu²⁺. By analysing the radioactivity released to the medium by TLC on polyethyleneimine-cellulose we found that it consisted mainly of P_i (not shown). No other radioactively labelled cellular metabolites appeared on the TLC, indicating that the label released to the medium was not the product of cellular lysis during the treatment with copper. These results strongly suggest that at least part of the P_i generated from polyP hydrolysis in the presence of Cu²⁺ ions was transported out of the cells. Formation and transport of metal–phosphate complexes have been demonstrated in cells or isolated membrane vesicles from bacteria by determination of changes in the membrane potentials (van Veen et al., 1994). This type of system is not currently available in S. metallicus. Our attempts to label S. metallicus cells with ⁶⁴Cu to determine

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**Fig. 6.** PPX activity and polyP content of cells exposed to copper ions. S. metallicus cells were grown in the absence of copper to the early stationary phase. Cells were then shifted to a fresh medium containing no CuSO₄ (●), 10 mM (○), 20 mM (■) or 100 mM (▲) copper or to 100 mM (NH₄)₂SO₄ as a control (not shown). Aliquots were taken at the indicated times, and PPX activity (a) and polyP (b) were determined. Means ± SE (n=4) are plotted.

**Fig. 7.** Effect of copper ions on the efflux of P_i from S. metallicus cells. Cells were labelled in vivo with H₃³²PO₄ [100 mCi ml⁻¹ (3.7 MBq ml⁻¹)] for 20 h in the presence of 0.2 mM P_i. The cells were then exhaustively washed with cold standard medium and shifted to the same fresh medium with or without copper added (●), or containing 100 mM (▲) or 200 mM (■) CuSO₄. At the times indicated, the cells were removed by centrifugation and the radioactive P_i released to the medium supernatants was determined. Means ± SE (n=4) are plotted.
the formation of metal-phosphate complexes were unsuccessful, probably due to competing protons in the acidic environment of this acidophile. Therefore, we could not determine the efflux of the metal from the cells. Until the formation of copper–phosphate complexes is demonstrated in *S. metallicus*, we cannot rule out that copper may be exerting an indirect effect that alters polyP levels.

It is possible that the proposed model for polyP-mediated metal detoxification also operates in *S. acidocaldarius* and *S. solfataricus*. However, their very low levels of polyP synthesized compared to those in *S. metallicus* make this system less relevant in the former micro-organisms, since they are very sensitive to copper (Dopson *et al.*, 2003), as we have also seen here. The low levels of polyP in *S. acidocaldarius* and *S. solfataricus* are not due to lack of phosphate, because in their natural environments, such as Octopus Spring at Yellowstone National Park or the thermal waters from Pozzo Vasca at Vulcano in the Aeolian Islands, southern Italy, phosphate has been found in different ionic forms depending on the pH of the medium (Amend & Shock, 2001).

The proposed model for metal ion detoxification based on the hydrolysis of polyP involves the transport of metal–phosphate complexes out of the cell. It has been proposed that the inorganic phosphate transport (Pit) system could be a possible candidate for this purpose, because it can reversibly transport metal–phosphate complexes (Keasling, 1997; van Veen, 1997). A Pit-like phosphate transport system was searched for in the available genomes of *S. solfataricus*, *Sulfolobus tokodaii* and *S. acidocaldarius*. A Pit-like transporter was not found in these archaea, but instead we found an open reading frame encoding a protein similar to the porter was not found in these archaea, but instead we found *Sulfolobus tokodaii* and *S. solfataricus* (van Veen, 1997). A Pit-like phosphate transport system was searched for in the available genomes of *S. solfataricus*, *Sulfolobus tokodaii* and *S. acidocaldarius*. A Pit-like transporter was not found in these archaea, but instead we found an open reading frame encoding a protein similar to the porter was not found in these archaea, but instead we found

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