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Influence of different electron donors on metal reduction by *Desulfovibrio dechloracetivorans* strain SF3

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Abstract: Chlororespiring, sulfate-reducing bacterium *Desulfovibrio dechloracetivorans* strain SF3 is capable of dissimilatively reducing Fe(Ⅲ), Cr(Ⅵ) and Co(Ⅲ). Acetate, ethanol, hydrogen, formate, pyruvate, lactate and citrate can be used as electron donors for soluble Fe(Ⅲ) reduction, whereas poorly crystalline Fe(Ⅲ)-oxide can be reduced only in the presence of pyruvate or H_2. Our experimental results show that the ratio of Fe(Ⅲ)-EDTA reduced to acetate consumed is 7.76±0.35, and no growth companied the Fe(Ⅲ) reduction in the presence of acetate. Reduction of Cr(Ⅵ) and Co(Ⅲ) can be stimulated by hydrogen, pyruvate and lactate, but addition of acetate had no effect. To our knowledge, it is the first report that an SRB (sulfate-reducing bacteria) can utilize acetate as an electron donor for soluble Fe(Ⅲ) reduction but not for insoluble Fe(Ⅲ), and Cr(Ⅵ), Co(Ⅲ) reduction, which indicates that a complex electron transfer system is hired by strain SF3 for metal reduction.

Key words: metal reduction; Fe(Ⅲ); Cr(Ⅵ); Co(Ⅲ); *Desulfovibrio dechloracetivorans* strain SF3

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研究快报

不同电子供体对 *Desulfovibrio dechloracetivorans* strain SF3 金属还原的影响

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摘要:还原脱氯,脱硫细菌 *Desulfovibrio dechloracetivorans* strain SF3 可异化还原三价铁,六价铬和三价钴. 乙酸盐,乙醇,氢气,丙酮酸盐,乳酸盐和柠檬酸盐可作为还原可溶性三价铁的电子供体,而只有丙酮酸盐和氢气做电子供体时 SF3 才能还原不可溶的非晶态三价铁氧化物. 实测定 Fe(Ⅲ)-EDTA 还原对乙酸盐氧化的比例为 7.76±0.35,而且该过程中没有伴随细菌的生长. SF3 可利用氢气,丙酮酸盐或乳酸盐作为电子供体还原六价铬和三价钴,但这一过程不能利用乙酸盐作为电子供体. 据我们所知,这是首次发现一

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种 SRB(硫酸盐还原菌)可利用乙酸盐还原可溶性三价铁,但无法利用其还原不可溶三价铁以及六价铬和三价钴,该发现说明 SF3 在金属还原过程中利用了较复杂的电子传递系统.

关键词:金属还原;铁;铬;钴;*Desulfovibrio dechloracetivorans* strain SF3

Bacterial Fe(Ⅲ) reduction is the first form of microbial respiration in the Earth[1,2], and plays a great role in element cycle and organic compound degradation in today's world[3~5], especially in submerged soils and aquatic sediments.

Besides Fe(Ⅲ), many other high-valence state heavy metals, such as Cr(Ⅵ), Co(Ⅲ) and U(Ⅵ), can be enzymatically reduced by metal-reducing microorganisms with the oxidation of organic substrates or hydrogen. These reduced heavy metals are less toxic, less soluble and tend to form strong complexes with hydroxides or sulfides, thus, they are easy to be precipitated from aquatic environments[6,7].

Considering the environmental significance of dissimilatory metal reduction, metal-reducing microorganisms have been intensively studied over the last two decades. A great feature of these metal-reducing microorganisms is their wide phylogenetic diversity. Sulfate-reducing bacteria (SRB), which are ubiquitously distributed in nature, were found to be capable of reducing heavy metals like Fe(Ⅲ), Cr(Ⅵ), Co(Ⅲ), and U(Ⅵ) enzymatically, in addition to an abiotic process via the production of sulfides[6,8~12]. Due to their resistance to high concentrations of heavy metals, SRBs appear to be a great candidate for bioremediation of toxic metals[13]. Besides practical significances, metal reduction by SRB may greatly stimulate the degradation of organic matters and might be a very important inhibitory factor to sulfate reduction in sediments[8,11], and thus greatly affect the global sulfur and carbon cycles.

Though the phenomenon of metal reduction of SRB has been intensively investigated[3~5,8,11], few experiments have focused on the effects of different electron donors on dissimilatory metal reduction. *Desulfovibrio dechloracetivorans* strain SF3, a marine SRB, is noted for its ability to degrade 2-chlorophenol (2-CP)[14]. One of its unique features is that it hires different electron donors for the reduction of different electron acceptors: Acetate can be utilized for reductive dechlorination but not for sulfate and nitrate reduction. And, hydrogen and formate, two common electron donors for *Desulfovibrio*, can not be used for reductive dechlorination[14]. Considering this characteristic, we designed a set of experiments to investigate how strain SF3 responds to different electron donors during metal reduction. Electron accepters we used include Fe(Ⅲ)-EDTA, Fe(Ⅲ)-NTA, poorly crystalline Fe(Ⅲ)-oxide, K_2CrO_4 and Co(Ⅲ)-EDTA.

Influence of different electron donors on dissimilatory Cr(Ⅵ), Co(Ⅲ) reduction In all experiments, standard anaerobic techniques were employed. *D. dechloracetivorans* strain SF3 (ATCC 700912) was routinely grown in 1 L glass serum bottles with 600 mL of modified seawater media[14]. To avoid chemical reduction of the metals, sulfate is replaced by chloride in the basal medium. For metal reduction experiments, log-phase bacteria grown in media containing 10 mmol/L sodium pyruvate, 30 mmol/L sodium sulfate and 0.1% yeast extract were harvested by centrifugation at 10 000 g for 30 min, washed twice by anaerobic bicarbonate buffer ($NaHCO_3$, 30 mmol/L; NaCl, 427 mmol/L; $MgCl_2$, 14 mmol/L; $CaCl_2$, 0.9 mmol/L; pH 7.4), and then resuspended in the same buffer or anaerobic basal media to a final density of ca. 5×10^6 cells/mL (in buffer) or 3×10^6 cells/mL (in medium). 250~500 μmol/L K_2CrO_4 or 3 mmol/L Co(Ⅲ)-EDTA was added from sterile anaerobic solutions by syringe needles. Electron donors tested in our experiments included acetate, pyruvate, lactate at 5 mmol/L each or H_2 at 22 mmol/L. Co(Ⅲ)-EDTA was prepared using hydrogen peroxide method[15], and

in the experiments with Co(Ⅲ)-EDTA, resazurin was omitted from the medium to avoid the disturbance of the spectrophotometrical absorbance of Co(Ⅲ) at 535 nm. Reduction of Cr(Ⅵ) was monitored by the standard 1, 5-diphenyl carbazide method[7] at 540 nm. Co(Ⅲ) concentration was directly measured for maximum absorbance at 535 nm[16].

In our experiments, both cultures and cell suspensions of strain SF3 directly reduced 250～500 μmol/L Cr(Ⅵ) or 3 mmol/L Co(Ⅲ) without any extra electron donors added, and similar results have been obtained in previous investigations[10]. A probable reason for this phenomenon is the utilization of endogenous electron donors[17]. However, addition of 5 mmol/L pyruvate, lactate or 22 mmol/L hydrogen led to a significant acceleration in reduction velocity, whereas 5 mmol/L acetate had no such effect (Fig. 1). For Cr(Ⅵ) reduction, pyruvate was the most favourable electron donor among the four species of potential electron donor tested, and hydrogen was as favorable as lactate (Fig. 1(a)). In the case of Co(Ⅲ) reduction, hydrogen and pyruvate were equally effective and lactate induced a lower reduction activity (Fig. 1(b)).

Influence of different electron donors on dissimilatory soluble Fe(Ⅲ) reduction Cells of strain SF3 were collected and resuspended as described above. Fe(Ⅲ)-EDTA or Fe(Ⅲ)-NTA at 10 mmol/L each were added as electron acceptors. Electron donors, including acetate, pyruvate, lactate, ethanol, citrate, formate at 10 mmol/L each or H_2 at 44 mmol/L, were also added by syringe needles. Fe(Ⅲ)-NTA was synthesized using Roden and Lovley's method[18]. Fe(Ⅱ) concentration was spectrophotometrically measured by a modified acid extraction-ferrozine method[19] at 562 nm.

Both cell suspensions and cultures of strain SF3 effectively coupled the reduction of Fe(Ⅲ)-EDTA and Fe(Ⅲ)-NTA to the oxidation of various electron donors including acetate, formate,

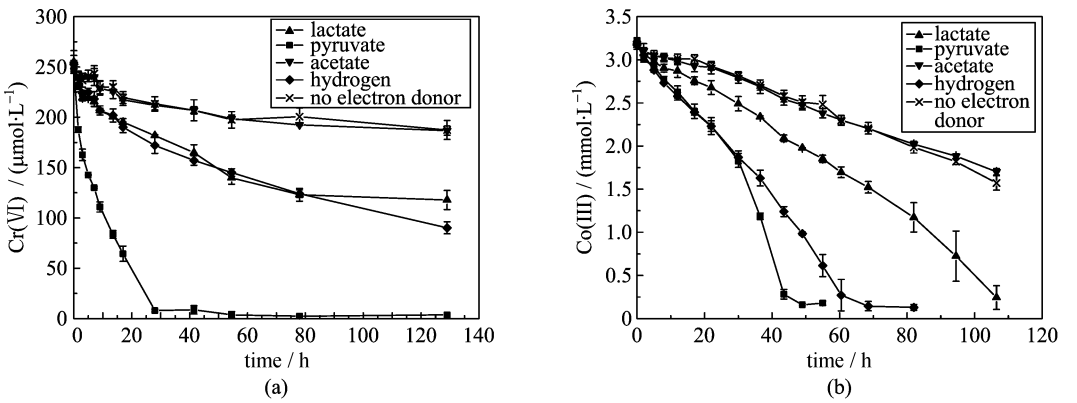

Fig. 1 250 μmol/L Cr(Ⅵ) (a) and 3 mmol/L Co(Ⅲ) (b) reduction by strain SF3 in anaerobic bicarbonate buffer

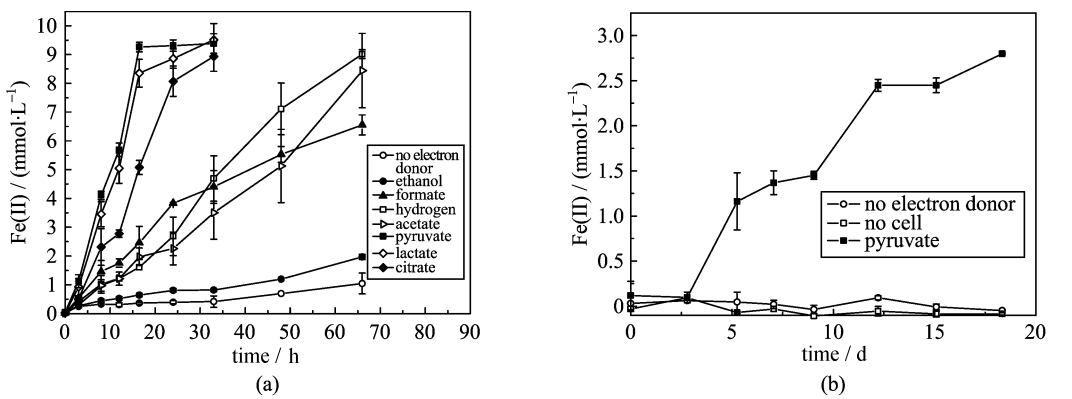

Fig. 2 Soluble and insoluble Fe(Ⅲ) reduction by strain SF3

hydrogen, ethanol, pyruvate, lactate and citrate (Fig. 2(a)). Pyruvate, lactate and citrate were favourable electron donors for Fe(Ⅲ)-EDTA and Fe(Ⅲ)-NTA reduction. When pyruvate was the electron donor, a higher Fe(Ⅲ)-reducing activity was measured when Fe(Ⅲ)-EDTA was the electron acceptor, and lower (ca. 29%) activity was detected in the case of Fe(Ⅲ)-NTA (data not shown).

To our knowledge, strain SF3 is the only member of *desulfovibrio* that can reduce both metals and chlorinated compounds. A great feature of strain SF3 is that it can oxidize various kinds of electron donors for soluble Fe(Ⅲ)-reduction. Among these electron donors, acetate is used most for Fe(Ⅲ) reduction in many sedimentary environments[4,9,20]. However, most members of *Desulfovibrio* can not utilize acetate for the reduction of any electron acceptor, which may lead to a competitive disadvantage over acetate oxidizers[3,21,22]. To date, strain SF3 and *Desulfovibrio profundus* are the only two members of *Desulfovibrio* capable of acetate oxidation[23]. This feature may provide the two species with greater competitiveness in anaerobic sediments. Another interesting feature of strain SF3 is that acetate can be utilized for reductive dechlorination but not for sulfate and nitrate reduction. And, hydrogen and formate, which are two common electron donors for *Desulfovibrio*, can not be used for reductive dechlorination[14]. However, all of these substrates supported soluble Fe(Ⅲ)-reduction by strain SF3 in our investigations, suggesting a much more complex electron transport system in the bacterium. The fact that acetate cannot be used in Cr(Ⅵ) and Co(Ⅲ) reduction is also supportive of this observation.

Influence of different electron donors on dissimilatory insoluble Fe(Ⅲ) reduction　The ability of strain SF3 to utilize different electron donors for insoluble Fe(Ⅲ) reduction was investigated using 10 mmol/L poorly crystalline Fe(Ⅲ)-oxide as the electron acceptor. Poorly crystalline Fe(Ⅲ)-oxide was synthesized by neutralizing a ferric chloride solution with NaOH as previously described[19]. Strain SF3 reduced poorly crystalline Fe(Ⅲ)-oxide in the presence of pyruvate (Fig. 2(b)). And a limited poorly crystalline Fe(Ⅲ)-oxide reducing ability was shown in the presence of H_2: after 24 days of incubation in anaerobic medium, only about 0.7 mmol/L Fe(Ⅱ) was synthesized by strain SF3 (data not shown). Whereas other electron donors tested had no effect on insoluble Fe(Ⅲ) reduction. The phenomenon that strain SF3 partially reduced poorly crystalline Fe(Ⅲ)-oxide only in the presence of pyruvate and H_2 was observed in both buffer and medium experiments, eliminating the possibility that it's due to the higher cell concentration by fermentation when pyruvate was the electron donor. The color change of the Fe(Ⅲ)- oxide precipitate and the incomplete reduction of total Fe(Ⅲ) (ca. 30% and ca. 7% total Fe(Ⅲ) reduction when pyruvate and H_2 are used as electron donors, respectively) suggest that the reduction of insoluble Fe(Ⅲ) was processed locally on the surface of poorly crystalline Fe(Ⅲ)-oxide and magnetite and siderite were probably the end products of poorly crystalline Fe(Ⅲ)-oxide reduction by strain SF3. Previous studies proposed that high concentration of organic acids may artificially stimulate Fe(Ⅲ)-oxide reduction by chemically chelate Fe(Ⅲ)[4]. However, the inability of strain SF3 to reduce poorly crystalline Fe(Ⅲ)-oxide by oxidation of 10 mmol/L lactate suggested that the coupling of Fe(Ⅲ)-oxide reduction and pyruvate oxidation was due to a direct enzymatic process.

A distinct characteristic of strain SF3 is that it showed a different electron donor preference in soluble Fe(Ⅲ) reduction compared with the reduction of poorly crystalline Fe(Ⅲ)-oxide. This feature is consistent with the recent finding that different electron transport pathways are employed in soluble versus insoluble Fe(Ⅲ) reduction by

both *Geobacter sulfurreducens* and *Shewanella oneidensis*[24~27]. In previous investigations about genus *Geobacter and Shewanella*, all the terminal insoluble Fe(Ⅲ) reductase that had been identified to function *in vivo* are located on the outer membrane[25,28~30]. However, more and more recent evidence suggests that, though many specific metal reductase has already been identified in genus *Geobacter* and *Shewanella*, some soluble metal ions like chelated Fe(Ⅲ), Cr(Ⅵ), Co(Ⅲ) and U(Ⅵ) can penetrate through the outer membrane and thus be nonspecifically reduced by low potential proteins, for example, some *c*-type cytochromes, present in the periplasm, inner membrane or cytoplast of these bacteria[24,31~33]. This hypothesis can explain the phenomenon that strain SF3 can oxidize far more various kinds of electron donors for soluble than insoluble Fe(Ⅲ)-reduction.

Fe(Ⅲ) reduction coupled to acetate oxidation

When acetate was the electron donor, the ratio of Fe(Ⅲ) reduced to acetate consumed has been measured. Cell suspensions of SF3 were added into 100 mL medium containing 5 mmol/L sodium acetate and 10 mmol/L Fe(Ⅲ)-EDTA to a density of 3.05×10^6 cells/mL. Control experiments were also conducted with the absence of either acetate or Fe(Ⅲ)-EDTA, respectively. Cell concentrations were determined by plate-counting method and protein concentrations were tested by Bradford method[34] after alkaline cell lysis[10]. Acetate was measured by gas chromatography (Agilent GC 6890N). The result (Fig. 3) shows that the ratio of Fe(Ⅲ) reduced to acetate consumed is 7.76 ± 0.35, which is very close to the constant of 8 when acetate was completely oxidized to CO_2 coupled to the reduction of Fe(Ⅲ) according to the formula:

$$CH_3COO^- + 8Fe(Ⅲ) + 2H_2O \rightarrow 2CO_2 + 8Fe(Ⅱ) + 7H^+$$

No significant acetate consumption or Fe(Ⅱ) accumulation occurred in no electron donor or no electron acceptor controls. Although strain SF3 reduced almost 10 mmol/L Fe(Ⅲ) over an incubation period of 12 days in the presence of 5 mmol/L acetate, neither plate counting nor Bradford protein assay detectable growth occurred (data not shown). However, the reduction of Fe(Ⅲ)-EDTA was accelerated during the incubation in anaerobic medium when acetate was the electron donor (Fig. 3), and this higher reduction activity can be retained when these bacteria were inoculated into fresh acetate-Fe(Ⅲ)-EDTA medium (data not shown), suggesting that the acetate-dependent soluble Fe(Ⅲ) reduction activity is inducible, while other electron donors didn't show this effect. Except soluble Fe(Ⅲ), acetate can only be oxidized when 2-CP is used as the electron acceptor, and this dechlorination process is also inducible[14]. Thus possibly the inducing of acetate dependent soluble Fe(Ⅲ) reduction activity is due to the synthesis of acetate dehydrogenases after the stimulation of Fe(Ⅲ) ions. Alternatively, due to the inability for strain SF3 to oxidize acetate for Cr(Ⅵ) and Co(Ⅲ) reduction, it is also possible that it is due to a inducible specific acetate-dependent Fe(Ⅲ)-reductase. No matter what the reason is, these phenomena indicated a much more complex electron transport system in the bacterium.

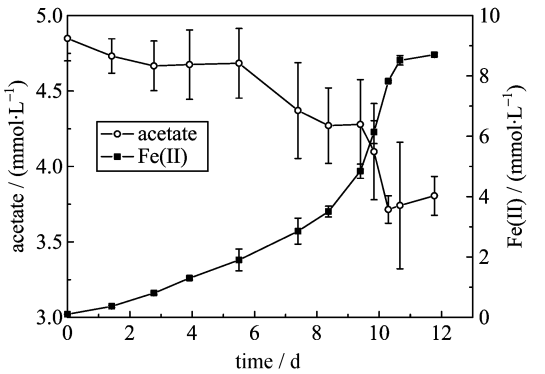

Fig. 3 Stoichiometric investigation on acetate oxidation coupled to Fe(Ⅲ) reduction

The broad range of electron donors for Fe(Ⅲ)-reduction and the inability of growth by dissimilatory metal reduction in strain SF3 indicate that reduction of Fe(Ⅲ), as well as other metal ions, is probably a protective by-pass reaction.

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Possibly, unlike other metal reducers, such as *Geobacter and Shewanella*, the physiological function of these enzymatic processes is not to conserve energy for growth but is to protect themselves from inhibition of sulfate reduction by toxic metal ions and competition of electron donors by bacteria using these alternative electron acceptors. This hypothesis can also explain the phenomenon that strain SF3 can not reduce poorly crystalline Fe(Ⅲ)-oxide unless pyruvate or H_2 exists because insoluble Fe(Ⅲ) has far less influence on the inside of bacterial cells than soluble metal ions. However, there are still many unsolved questions, such as why strain SF3 cannot utilize acetate for Cr(Ⅵ) and Co(Ⅲ) reduction? Why poorly crystalline Fe(Ⅲ)-oxide can be reduced in the presence of pyruvate or H_2? And how the ability to utilize acetate is induced? To answer these questions, more intensive and sophisticated experiments are needed.

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