

This article was downloaded by: [University of Minnesota Libraries, Twin Cities]

On: 14 September 2014, At: 05:24

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Geomicrobiology Journal

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/ugmb20>

Metabolic Flexibility and Substrate Preference by the Fe(II)-Oxidizing Purple Non-Sulphur Bacterium *Rhodopseudomonas palustris* Strain TIE-1

E. D. Melton^a, C. Schmidt^a, S. Behrens^a, B. Schink^b & A. Kappler^a

^a Department of Geomicrobiology, Center for Applied Geosciences, University of Tübingen, Tübingen, Germany

^b Department of Biology, University of Konstanz, Constance, Germany

Accepted author version posted online: 01 Apr 2014. Published online: 12 Sep 2014.

To cite this article: E. D. Melton, C. Schmidt, S. Behrens, B. Schink & A. Kappler (2014) Metabolic Flexibility and Substrate Preference by the Fe(II)-Oxidizing Purple Non-Sulphur Bacterium *Rhodopseudomonas palustris* Strain TIE-1, *Geomicrobiology Journal*, 31:9, 835-843, DOI: [10.1080/01490451.2014.901439](https://doi.org/10.1080/01490451.2014.901439)

To link to this article: <http://dx.doi.org/10.1080/01490451.2014.901439>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

Metabolic Flexibility and Substrate Preference by the Fe(II)-Oxidizing Purple Non-Sulphur Bacterium *Rhodopseudomonas palustris* Strain TIE-1

E. D. MELTON¹, C. SCHMIDT¹, S. BEHRENS¹, B. SCHINK², and A. KAPPLER^{1*}

¹Department of Geomicrobiology, Center for Applied Geosciences, University of Tübingen, Tübingen, Germany

²Department of Biology, University of Konstanz, Constance, Germany

Received October 2013, Accepted February 2014

It is unknown to which extent phototrophic Fe(II) oxidation takes place in the simultaneous presence of organic electron donors (e.g., acetate/lactate). Therefore, the photoferrotrophic strain *Rhodopseudomonas palustris* TIE-1 was inoculated with various combinations of Fe(II), acetate and lactate to understand metabolic substrate preference. When acetate was provided together with Fe(II), TIE-1 consumed acetate first before Fe(II). When provided lactate plus Fe(II), TIE-1 consumed both substrates in parallel. When all three substrates were provided in one culture, TIE-1 used all substrates simultaneously. Our study suggests that the availability of alternative electron donors in addition to ferrous iron limits phototrophic iron oxidation.

Keywords: carbon flow, iron redox cycling, phototrophic Fe(II) oxidation

Introduction

Iron is an important redox-active transition metal for all living organisms, acting as an essential nutrient and able to serve as an electron source for Fe-metabolizing microorganisms (Kappler and Straub 2005; Konhauser et al. 2011; Weber et al. 2006). At neutral pH, the two environmentally most common iron redox species are ferric (Fe(III)) and ferrous iron (Fe(II)). Ferrous iron is swiftly oxidized abiotically to ferric iron by molecular oxygen at neutral pH (Davison and Seed 1983). Under microoxic and anoxic conditions, microbial Fe(II) oxidation processes become dominant over abiotic Fe(II) oxidation by oxygen (Druschel et al. 2008). Two distinct metabolic groups are capable of Fe(II) oxidation and Fe(III) mineral formation under anoxic conditions; nitrate-reducing Fe(II)-oxidizers (Straub et al. 1996) and photoferrotrophs (Ehrenreich and Widdel 1994; Widdel et al. 1993).

Photoferrotrophy requires light energy, bicarbonate as electron acceptor and carbon source and Fe(II) as electron donor: $\text{HCO}_3^- + 4 \text{Fe}^{2+} + 10\text{H}_2\text{O} \xrightarrow{h\nu} \text{CH}_2\text{O} + 4 \text{Fe}(\text{OH})_3 + 7 \text{H}^+$ (Widdel et al. 1993) and produces poorly crystalline ferric oxyhydroxides (Kappler and Newman 2004). Photoferrotrophs are widespread throughout many environmental ecosystems which contain ferrous iron, bicarbonate

and organic carbon (Caiazza et al. 2007; Cockell et al. 2011; Crowe et al. 2008; Ehrenreich and Widdel 1994; Hegler et al. 2012; Jiao et al. 2005; Salka et al. 2011; Waidner and Kirchner 2007). Therefore, their requirement for Fe and C potentially plays an important role in modern-day environmental biogeochemical carbon and iron cycling. In addition, their aptitude for anaerobic photoautotrophic Fe(II) oxidation may have played a leading role in the deposition of Precambrian banded iron formations (BIFs) (Crowe et al. 2008; Kappler et al. 2005; Konhauser et al. 2002; Posth et al. 2008; Posth et al. 2013).

Several phototrophic strains isolated from the environment are capable of ferrous iron oxidation including: the green sulphur bacterium *Chlorobium ferrooxidans* strain KoFox (Heising et al. 1999), the purple sulphur bacterium *Thiodictyon* sp. strain F4 (Croal et al. 2004) and the purple non-sulphur bacteria *Rhodobacter ferrooxidans* sp. strain SW2 (Ehrenreich and Widdel 1994) and *Rhodopseudomonas palustris* strain TIE-1 (Jiao et al. 2005). So far, the role these phototrophic Fe(II)-oxidizers play in environmental iron and carbon redox cycling is largely unknown.

Purple non-sulphur bacteria (PNSB) such as those in the *Rhodopseudomonas* genus are well known for their metabolic flexibility (Oda et al. 2008) which enables them to oxidize a variety of inherently different electron donors including organic compounds, hydrogen and hydrogen sulphide (Zinder and Dworkin 2006). PNSB are found in habitats that naturally contain inorganic electron donors as well as organic carbon. For instance, ferrous iron concentrations in freshwater lake sediments are approximately $200 \pm 70 \mu\text{M}$ (Gerhardt et al. 2005), yet organic acids like acetate are present in

*Address correspondence to Andreas Kappler, Geomicrobiology, Center for Applied Geosciences, University of Tübingen, Sigwartstraße 10, D-72076 Tübingen, Germany; E-mail: andreas.kappler@uni-tuebingen.de

the range of 50 to 680 μM , and lactate has been measured at around 100 μM (Melton and Kappeler unpublished data; Montag and Schink personal communication, September 2012). As a consequence, the role of PNSB in the carbon cycle is defined not only through phototrophic carbon dioxide fixation, but also by their ability to oxidize organic acids as alternative electron donors. So far, it is unknown whether they exhibit a preference or sequential order to electron donor oxidation when presented with multiple electron donors simultaneously.

The purple non-sulphur alphaproteobacterium *Rhodospseudomonas palustris* strain TIE-1 was isolated from School Street Marsh in Woods Hole, MA, in the presence of light with Fe(II) as electron donor (Jiao et al. 2005). The three-gene *pio* operon is instrumental to Fe(II) oxidation in this strain (Bose and Newman 2011; Jiao and Newman 2007). Like other PNSB, strain TIE-1 oxidizes many different electron donors under anoxic conditions in the presence of light including organic acids (e.g. lactate, acetate) and inorganic electron donors such as Fe(II) and H_2 (Jiao et al. 2005). This makes strain TIE-1 an ideal candidate to investigate metabolic preference of PNSB.

To determine under which conditions *R. palustris* strain TIE-1 oxidizes Fe(II) in the presence of organic compounds such as lactate and acetate, this study focuses on the cultivation of *R. palustris* strain TIE-1 both in the presence of either Fe(II), acetate or lactate, or with combinations of two or three of these electron donors. We used substrate concentrations typically employed in laboratory studies in the range of 10–20 mM lactate and acetate, and 4–6 mM Fe(II) which have been shown to support growth of other *Rhodospseudomonas palustris* strains (Albers and Gottschalk 1976; Jiao et al. 2005; McKinlay and Harwood 2010).

Materials and Methods

Media, Culture Conditions, and Experimental Setup

Two liters of growth medium for strain TIE-1 (Jiao et al. 2005) were prepared, which also included the addition of 1 mL·L⁻¹ trace element solution, 1 mL·L⁻¹ vitamin B12 solution and 10 mL·L⁻¹ of an altered vitamin solution (Ehrenreich and Widdel 1994) which contained 50 mg·L⁻¹ riboflavin. The vitamin solution was diluted 10-fold compared to the original recipe. One liter of the medium was amended with Fe(II)Cl₂ to reach a final concentration of 10 mM Fe(II) and was left to equilibrate and form iron carbonate and iron phosphate precipitates for 48 hours (Hohman et al. 2010). Subsequently, the medium was filtered in an anoxic glovebox under N₂, resulting in a clear Fe(II)-containing medium of approximately 5 mM dissolved Fe(II). The other liter of the medium was not amended with Fe(II) and was therefore not filtered and Fe(II)-free. Various combinations of acetate and lactate were added to the non-Fe(II)-containing medium and to the Fe(II)-containing medium (Table 1).

The initial total organic acid concentration was either 20 mM acetate, 20 mM lactate or a mix of 10 mM acetate

Table 1. Overview of experimental setups containing different combinations of acetate, lactate and ferrous iron that were added to TIE-1 inocula used during this study

	TIE-1 medium without ferrous iron		
	Acetate	Lactate	TIE-1 inoculum
Biotic 2×	20 mM	—	Yes
Sterile 2×	20 mM	—	—
Biotic 2×	—	20 mM	Yes
Sterile 2×	—	20 mM	—
Biotic 2×	10 mM	10 mM	Yes
Sterile 2×	10 mM	10 mM	—
	TIE-1 medium with 5 mM ferrous iron		
Biotic 2×	—	—	Yes
Sterile 2×	—	—	—
Biotic 2×	20 mM	—	Yes
Sterile 2×	20 mM	—	—
Biotic 2×	—	20 mM	Yes
Sterile 2×	—	20 mM	—
Biotic 2×	10 mM	10 mM	Yes
Sterile 2×	—	—	—

All setups were performed in duplicate and accompanied by the appropriate duplicate sterile controls.

plus 10 mM lactate. Set-ups were prepared in duplicates of 50 mL each, sealed with butyl rubber stoppers and flushed with an N₂/CO₂ (90:10) headspace. Duplicate sterile set-ups containing no inoculum were run to check for chemical oxidation. *Rhodospseudomonas palustris* strain TIE-1 was kindly provided by Dianne Newman's group (Caltech) and was pre-cultured for phototrophic growth with H₂ as the electron donor by inoculation into the non-Fe(II)-containing medium (described above) with a headspace of H₂/CO₂ gas (80:20), and incubated at 23°C for 10 days. The culture was transferred twice and cultured under the same conditions before inoculation (10⁷ cells·mL⁻¹) into the main experiment from the exponential phase. The incubation time was 22 days at 23°C under constant light (True Light 15 W/5500 K).

Analytical Methods

From each culture bottle, 2-mL samples were taken regularly in an anoxic glovebox (100% N₂) for 21 days (Figure 1). Total Fe(II)/Fe(III), dissolved Fe(II)/Fe(III), cell numbers and organic acids were analyzed. All samples, biotic and abiotic, were treated identically. The headspace was regularly replenished with N₂/CO₂ gas (90:10), and the pH stayed at around 7 throughout the experiment [regularly measured inside the glovebox with pH indicator strips (Merck)]. Total iron samples were first dissolved anoxically in 1 M HCl by shaking (at 180 rpm) for 24 h before quantification. The supernatant of centrifuged samples (5 min at 15,400 g) was stabilized in 1 M anoxic HCl for dissolved iron measurements and quantified directly after sample collection. Iron was measured by the spectrophotometric ferrozine assay (Stookey 1970) in a microtiter plate reader with a 5% error

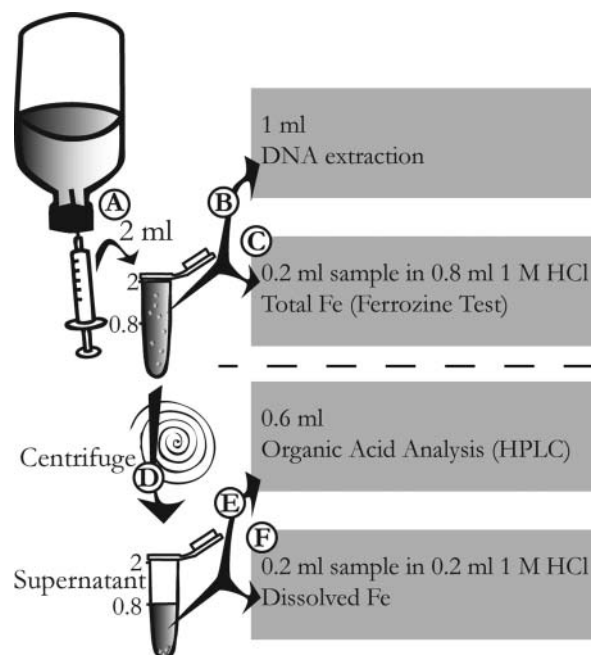


Fig. 1. Sampling scheme of *R. palustris* strain TIE-1 cultures performed within an anoxic glovebox. A) Bottles were set up in duplicates and a 2 ml sample was taken from each bottle at each timepoint with an anoxic needle and syringe. B) 1 ml from the sample was pipetted into a sterile Eppendorf tube for DNA extraction. C) 0.2 ml sample was dissolved in 0.8 ml of 1 M anoxic HCl in order to stabilize the iron redox speciation and measure the total Fe content. D) The remaining sample was centrifuged after which the supernatant was sampled for E) Organic acid analyses and F) the dissolved Fe phase speciation was stabilized in 0.2 ml 1 M HCl.

(Flashscan 550, Analytik Jena AG Germany). DNA was extracted from 1 mL filtered culture ($0.2 \mu\text{m}$ polyethersulfone membrane filter) for cell number quantification with an ultra-clean microbial DNA isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). Quantitative polymerase chain reactions (qPCR) were performed using general bacterial 16S rRNA primers to quantify the total number of cells over time (as in Emmerich et al. 2012), calibrated with a plasmid standard of a clone 16S rRNA fragment. Acetate and lactate were quantified by high performance liquid chromatography (Bio Rad Aminex HPX-87H Ion Exclusion Column and 5 mM H_2SO_4 eluent).

Results

Oxidation of Fe(II), Acetate or Lactate as Single Electron Donors by R. palustris TIE-1

To assess the use of individual substrates for later comparison with combined substrate configurations, *R. palustris* was grown first with single electron donors. Oxidation of Fe(II) started immediately after inoculation (Figure 2A). After a lag-phase of 14 days, the cell density increased exponentially to 1.24×10^8 cells/mL (Figure 2A). Acetate utilization

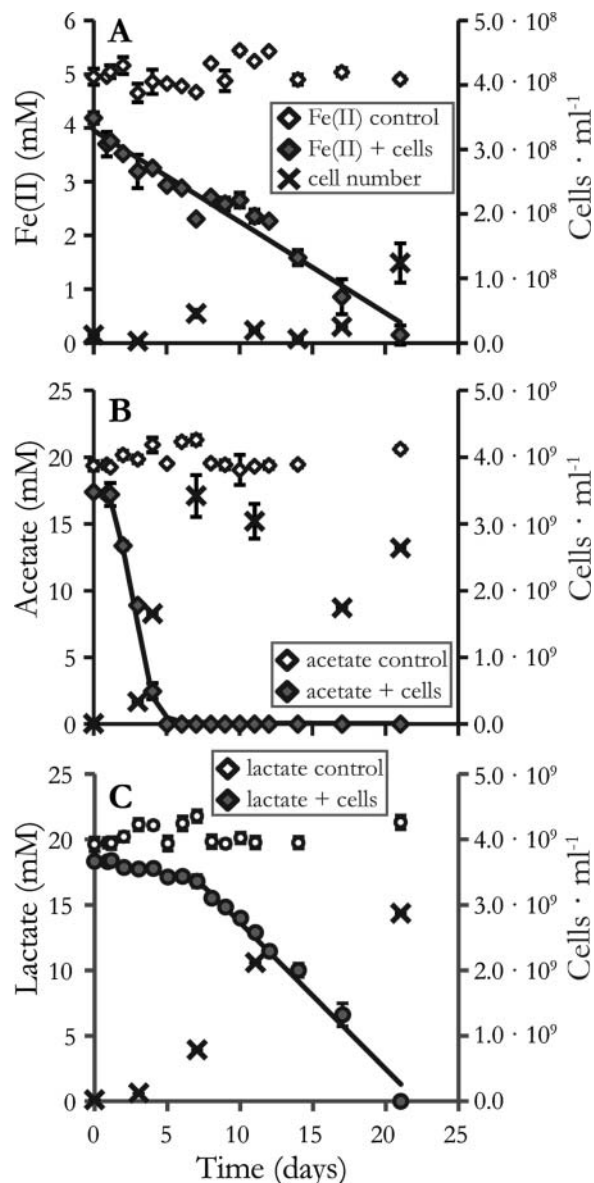


Fig. 2. Growth of *R. palustris* strain TIE-1 with single electron donors. Shown are electron donor concentrations and cell numbers over time. Closed symbols signify the average of duplicate set-ups and open symbols denote sterile controls. Crosses indicate cells per mL. Error bars denote the maximum and minimum of duplicate assays. A) 5 mM Fe(II); B) 20 mM acetate; C) 20 mM lactate.

exhibited a lag phase of 0.9 days (Figure 2B) and afterwards proceeded to increase linearly to a maximum of 3.4×10^9 cells/mL (Figure 2B). Lactate utilization proceeded immediately (Figure 2C), and maintained the initial rate throughout the 21 days of the experiment. The distinct slope increase after 7 days is presumably due to an increase in cell numbers, rather than an increase in oxidation rate per cell. This was confirmed by calculating the maximum Fe(II) oxidation rate per cell which was $5.37 \times 10^{-10} \pm 1.38 \times 10^{-11} \mu\text{mol} \cdot \text{cell}^{-1} \cdot \text{day}^{-1}$, during the first 10 days and $6.76 \times 10^{-10} \pm 6.60 \times 10^{-11} \mu\text{mol} \cdot \text{cell}^{-1} \cdot \text{day}^{-1}$ during the last 10 days. Growth with this substrate yielded an increase in cell density

proportional to lactate utilization (Figure 2C) with a maximum density of 2.9×10^9 cells/mL (Figure 2C). In the uninoculated control setups, the substrate concentrations remain the same throughout the incubation time (Figure 2).

Utilization of Binary Combinations of Electron Donors by *R. palustris* Strain TIE-1

Simultaneously providing an organic acid and ferrous iron resulted in different oxidation patterns for the acetate and ferrous iron and lactate and ferrous iron scenarios (Figure 3).

Acetate and Fe(II)

In the presence of Fe(II), acetate oxidation proceeded after a lag-phase of 0.9 days (Figure 3A), similar to when offered as the only electron donor (Figure 2B) and ferrous iron oxidation followed after acetate oxidation. Ferrous iron oxidation did not start before all acetate disappeared, resulting in a 7-day delay. Cell numbers increased after a 3-day lag phase to a maximum density of 2.7×10^9 cells/mL (Figure 3A). In absence of TIE-1 inoculum, the substrate concentrations remained stable throughout the incubation time (Figure 3B).

Lactate and Fe(II)

In the presence of Fe(II) and lactate, lactate consumption proceeded instantly and continued throughout the 21 days when offered in combination with ferrous iron. In contrast to ferrous iron oxidation in the presence of acetate, iron oxidation in the presence of lactate proceeded immediately. The

distinct increase in the Fe(II) oxidation rate after 10 days is presumably due to an increase in cell numbers rather than to an increase in the oxidation rate per cell (Figure 3B). The cell density increased after a lag-phase of 3 days until it reached a maximum of 2.4×10^9 cells/mL (Figure 3B). Ferrous iron oxidation occurred parallel to lactate oxidation from the beginning of the experiment. In absence of TIE-1 inoculum, the substrate concentrations remained stable throughout the incubation time (Figure 3D).

Acetate and lactate

When the two organic electron donors acetate and lactate were offered simultaneously, acetate consumption commenced after a short lag phase of 0.9 days (Figure 4A). Lactate consumption proceeded immediately and acetate and lactate were used simultaneously; however, acetate was fully consumed before lactate. Cells grew after a lag phase of 3 days to a maximum density of 3.9×10^9 cells/mL (Figure 4A). In absence of TIE-1 inoculum, the substrate concentrations remained stable throughout the incubation time (Figure 4B).

Substrate Utilization in the Simultaneous Presence of Fe(II), Acetate and Lactate

When *R. palustris* strain TIE-1 was provided with Fe(II), acetate and lactate simultaneously, all electron donors were oxidized in parallel (Figure 4B). Ferrous iron and lactate oxidation proceeded immediately, whilst acetate oxidation

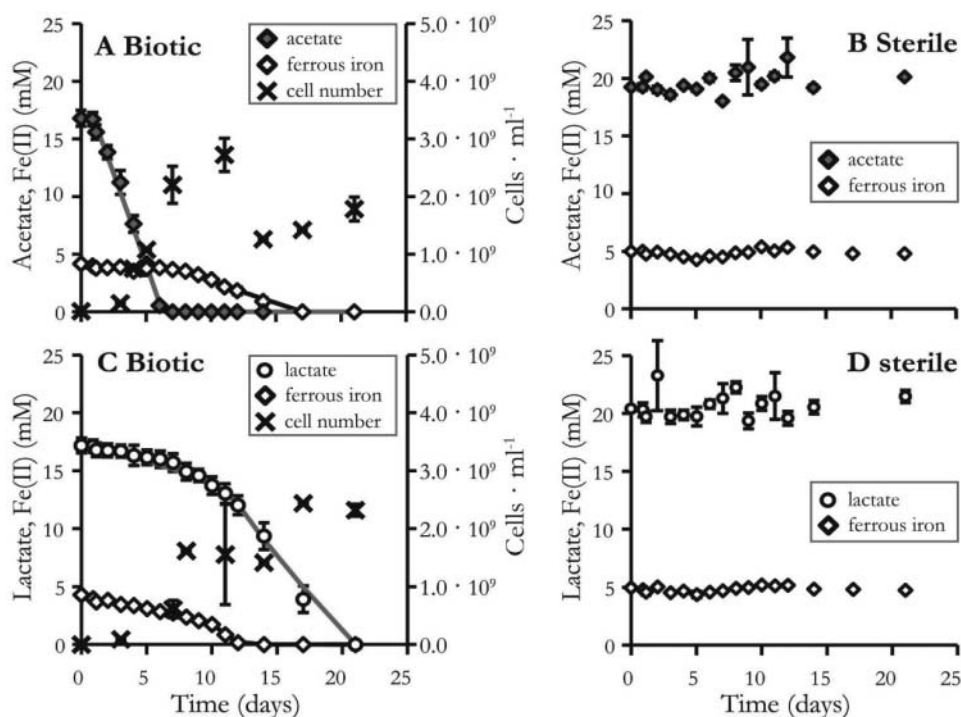


Fig. 3. Growth of *R. palustris* strain TIE-1 with binary combinations of electron donors. Shown are electron donor concentrations and cell numbers over time. Closed symbols signify the average of duplicate set-ups and open symbols denote sterile controls. Crosses indicate cells per mL. Error bars denote the maximum and minimum of duplicate assays. A) 5 mM Fe(II) + 20 mM acetate; B) 5 mM Fe(II) + 20 mM lactate.

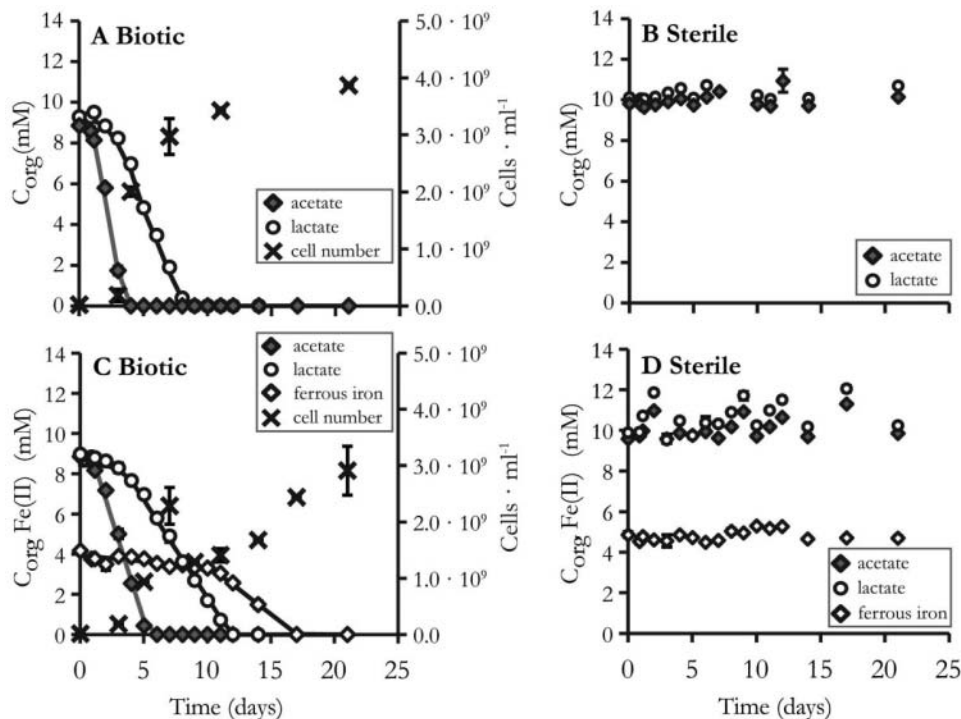


Fig. 4. Growth of *R. palustris* Strain TIE-1 with two organic acids and with all three electron donors tested. Shown are electron donor concentrations and cell numbers over time. Closed symbols signify average of duplicate set-ups and open symbols denote sterile controls. Crosses indicate cells per mL. Error bars denote the maximum and minimum of duplicate assays. A) 10 mM acetate + 10 mM lactate B) 10 mM acetate + 10 mM lactate + 5 mM Fe(II).

started after a short lag phase of 0.9 days, similar to all other setups containing acetate. Cell density followed a linear increase without a lag-phase to a maximum of 2.9×10^9 cells/mL at the end of the experiment (Figure 4B).

Discussion

Simultaneous and Sequential Oxidation of Fe(II) and Organic Electron Donors

The order of electron donor consumption depended on the identity of the organic electron donors and the presence of ferrous iron. The observations from Figures 2–5 are not due to any bias towards a certain metabolism, as the pre-culture was grown photoautotrophically with hydrogen, an electron donor not present in these culture experiments.

It has been recognized since 1994, shortly after the discovery of photoferrotrophy (Widdel et al. 1993), that *Rhodospirillum rubrum* strain SW2 first oxidizes acetate (1 mM) followed by ferrous iron (10 mM) (Ehrenreich and Widdel 1994), and that PNSB growth is greatly enhanced by ferrous iron in the presence of an organic co-substrate (Heising and Schink 1998). More recently, it was shown that the addition of hydrogen impedes ferrous iron oxidation by photoferrotrophs and that the extent of inhibition was dependent on the bicarbonate concentration present (Croal et al. 2009). This has prompted the assumption that there could be a kinetic factor in the chemical competition for electron donation, i.e.,

when the electron acceptor (e.g., the bicarbonate or CO_2) is limiting, H_2 may kinetically outcompete Fe(II) for oxidation by the electron transport chain (Croal et al. 2009). Another PNSB, *R. capsulatus*, is able to survive on a photomixotrophic metabolism with Fe(II)-NTA and acetate using the electrons from Fe(II) and deriving carbon both from organic sources and from fixation of inorganic carbon (Kopf and Newman 2012).

The incorporation of lactate into biomass via the citric acid cycle requires its oxidation from lactate to pyruvate catalyzed by lactate dehydrogenase. Pyruvate can then be further decarboxylated to acetyl CoA by pyruvate dehydrogenase, or carboxylated to oxaloacetate by pyruvate carboxylase (Campbell and Reese 2002). Acetate, on the other hand, is converted to acetyl CoA by one enzyme called acetyl CoA synthase (Campbell and Reese 2002). Ferrous iron is oxidized in *R. palustris* through the three-gene *pio* operon (Jiao and Newman 2007). The oxidation of ferrous iron yields 1 mol of electrons per mol of ferrous iron that is oxidized. Organic compounds on the other hand are able to provide a larger amount of mol electrons per mol of organic compound oxidized. One mol of acetate oxidation yields 8 mol electrons, yet one mol of lactate oxidation yields 12 mol electrons. Therefore, it is possible that a preferential use of an organic substrate can be masked due to its excess supply of electrons compared to ferrous iron, i.e., Fe(II) could be depleted earlier only due to the small amount of electrons it can supply.

We found that *R. palustris* strain TIE-1 clearly exhibits a preference towards the organic electron rich donor acetate, as

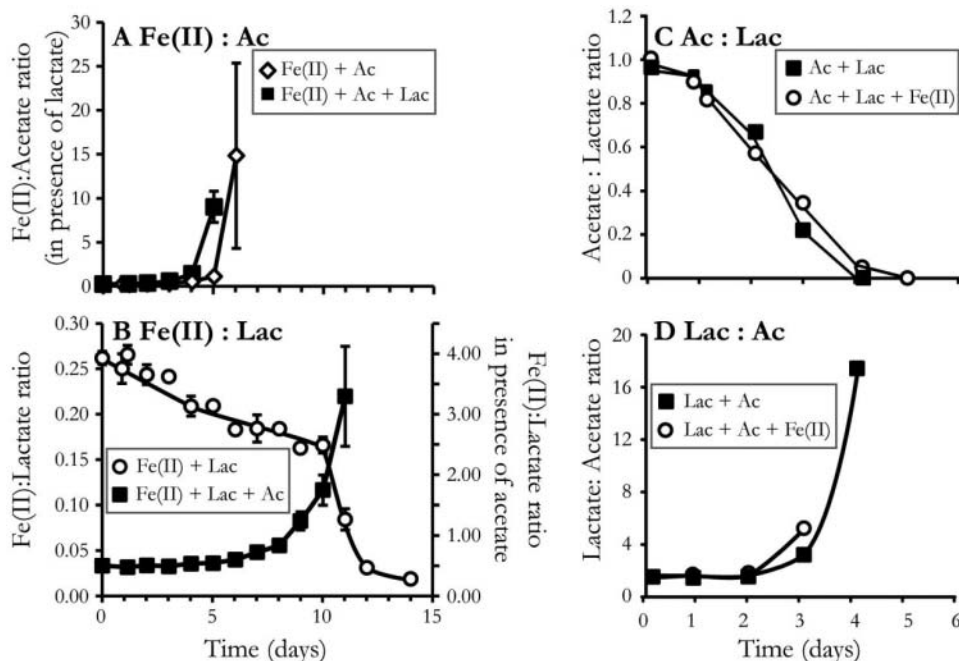


Fig. 5. Ratio of the molar concentrations of remaining substrate over time. A) Fe(II):acetate ratio for the setups ferrous iron with acetate and ferrous iron with acetate and lactate. B) Fe(II):lactate ratio for the setups ferrous iron with lactate and ferrous iron with lactate and acetate. C) acetate:lactate ratio for the setups containing acetate and lactate in the absence and presence of ferrous iron. D) lactate:acetate ratio for the setups containing lactate and acetate in the absence and presence of ferrous iron. The dataset in panels A and D stops after a few days, due to complete depletion of acetate and lactate causing the ratio to go to infinity.

its consumption precedes the electron poor ferrous iron oxidation (Figure 3A). There is no clear preference of lactate over ferrous iron, as they disappear simultaneously from the solution (Figure 3C). However, as ferrous iron is completely consumed before lactate, this may represent a slight preference for ferrous iron over lactate which could be due to a kinetic effect caused, e.g., by the difference in the number of electrons in ferrous iron and lactate, properties of the different enzymes needed for lactate or Fe(II) oxidation, or uptake mechanisms in both processes.

When all three electron donors were present simultaneously, the electron-rich substrate lactate was consumed before Fe(II) (Figure 4C), indicating a preference for lactate. This could be due to similarities in the pathways of acetate and lactate oxidation, where enzymes needed for the citric acid cycle following the conversion of acetate to acetyl CoA can immediately also process the acetyl CoA or oxaloacetate formed from lactate. These enzymes may not be activated as fast in the absence of acetate. The sequential preference of acetate over Fe(II) utilization (Figure 3A) is somehow annulled by lactate (Figure 4C). Thus, lactate allows the co-oxidation of Fe(II) and acetate. Interestingly, the ratio between remaining ferrous iron and acetate in solution is not affected by the presence of lactate, illustrating that acetate is consumed before Fe(II), regardless of the influence of lactate (Figure 5A).

In contrast, the ratio between remaining ferrous iron and lactate is affected by the presence of acetate (Figures 5A, 5B). In the absence of acetate, the Fe(II):

lactate ratio becomes smaller over time, meaning that more lactate remains and Fe(II) is consumed faster than lactate. In turn, when acetate is present simultaneously to Fe(II), the Fe(II):lactate ratio becomes larger over time meaning that more Fe(II) remains and lactate is consumed faster than Fe(II). This means that in the presence of acetate, lactate is consumed before Fe(II), yet Fe(II) may be preferred in the absence of acetate and shows that acetate biases the electron donor preference towards the organic compounds. The ratio of acetate to lactate and vice versa, is however not influenced by the presence of iron(II) (Figure 5CD). Consequently, the preferential order of electron donor consumption for *R. palustris* comes to: acetate > lactate > Fe(II).

Pathways for Electron and Carbon Flow During Oxidation of Fe(II) in the Presence and Absence of Acetate and Lactate

When strain TIE-1 oxidized Fe(II) in the absence of acetate and lactate, it grew photoautotrophically to a maximum cell density of 1.24×10^8 cells \cdot ml $^{-1}$ (Figure 2A). The incorporation of carbon into biomass and cell growth during heterotrophic growth can either come straight from the organic carbon through anaplerotic processes fuelled by intermediates from the TCA cycle, or from substrate oxidation followed by CO $_2$ fixation through the Calvin cycle. Thus, the organic substrates acetate and lactate simultaneously act as electron donors and carbon source. The production of

biomass depends not only on the number of reducing equivalents available or rate of substrate oxidation, but also on the mechanisms and pathways required for the synthesis of cellular biomass.

The redox state of carbon in *R. palustris* biomass is slightly negative at -0.5 (calculated from Carozzi and Sacchi 2001), and the carbon in acetate and lactate has an average redox state of 0; therefore, reducing equivalents are necessary to convert the acetate and lactate to cellular biomass. These reducing equivalents can be generated from partial oxidation of the organic compound to CO_2 through the TCA cycle. Thus, the organic compounds are diverged within the cell where they can either be incorporated into biomass directly or can be oxidized to CO_2 . It has been determined through carbon labelling experiments with *R. palustris* strain CGA009 that when acetate is provided as the sole electron donor, 22% of acetate is oxidized to CO_2 , of which 68% is fixed in the Calvin cycle (McKinlay and Harwood 2010). The other 78% of the acetate is incorporated into biomass through the glyoxylate cycle. *R. palustris* is unable to utilize acetate phototrophically in the absence of a functional Calvin cycle (Laguna et al. 2011).

Assuming that 100% of the electrons harvested through Fe(II) oxidation are used to fix CO_2 , theoretically less of the organic substrate needs to be oxidized in order to provide sufficient electrons for biomass production when iron is provided in addition to an organic substrate. However, as acetate and Fe(II) are consumed sequentially (Figure 3A), the electrons from Fe(II) are probably not used to incorporate acetate into biomass. In fact, the cell yield is lower when Fe(II) is provided in addition to acetate (Figures 2B and 3A), highlighting a possible toxic effect of Fe(II) on cells (Carlson et al. 2013; Poulain and Newman 2009) or the need to invest carbon and electrons into exopolysaccharide (EPS) synthesis to protect the cell from Fe(III) mineral encrustation (Miot et al. 2009a, 2009b). In the case of lactate and Fe(II), both substrates are consumed in parallel (Figure 3C), pointing towards a facilitation of lactate incorporation. However, as was the case with Fe(II) plus acetate, there was a decrease of the cell yield in the presence of Fe(II) (Figures 2C and 3C). In addition, when ferrous iron was added to an organic electron donor, this caused a decrease in the acetate oxidation rate, yet the lactate oxidation rate seemed to increase (Figure 3), in comparison to the organic carbon oxidation rate in absence of ferrous iron (Figures 2B, 2C).

When all three electron donors were present simultaneously, all substrates were oxidized in parallel which may point towards a similar Fe(II) facilitation of lactate oxidation, as when Fe(II) and lactate are oxidized in parallel. However, the ratio between remaining Fe(II) and lactate in solution was influenced by the presence of acetate (Figure 5B), meaning that in the presence of acetate, lactate was preferred over iron, yet in the absence of acetate, lactate accumulated in the medium solution and more iron was oxidized first. Also, acetate oxidation appeared to be independent of electrons gained from Fe(II) oxidation.

Conclusions and Implications

Though the organic acid concentrations in this study were high in comparison to environmental concentrations, they are well within the range used for cultivation of PNSB (Albers and Gottschalk 1976; Jiao et al. 2005; McKinlay and Harwood 2010). Our results thus allow us to draw conclusions on the electron donor preference and carbon flow of PNSB under typical laboratory cultivation conditions. The preferential growth substrate tested in this study with *R. palustris* was acetate, which is commonly available in most habitats of phototrophic bacteria. Other strains of PNSB have also been shown to promote organic carbon acquisition through phototrophic Fe(II) oxidation (Caiazza et al. 2007) and promote growth on Fe(II) oxidation by addition of an organic co-substrate (Ehrenreich and Widdel 1994; Heising and Schink 1998). *R. palustris* strain TIE-1 grew photoautotrophically with ferrous iron only (Figure 2A), as has been shown before for other PNSBs (Ehrenreich and Widdel 1994; Jiao et al. 2005). Although this behavior is observed in the presence of relatively high concentrations of carbon and iron, it is at present not clear whether their electron donor preference would be the same at lower substrate concentrations typically present in the environment. This illustrates the need for experiments with lower substrate concentrations in environmental studies, which closer mimic the natural environment.

Environmental Fe(II) concentrations are subject to constant fluctuations caused by day–night cycles (Gerhardt et al. 2005) and mechanical mixing (Chubarenko et al. 2003; Gerhardt and Schink 2005), which influence the redox zonation. Algal blooms (Peeters et al. 2007) and seasonal temperature variations control the organic carbon influx into the sediments. Thus, the ability to harvest electrons from various donors allows PNSB to survive in heterogeneous environments which is of great significance to the microbially mediated iron cycle. As growth with ferrous iron was possible in parallel with lactate consumption but sequential to acetate utilization, the environmental role of phototrophic Fe(II)-oxidizers may be constrained by the identity and concentration of organic substrates present simultaneously. As a consequence, microbial iron cycling in may be constrained in photic zones rich in bioavailable organic material.

Acknowledgments

Special thanks to Kirsten Oswald, Ellen Struve, Annika Rudolph, Karin Stögerer, and Theda Himpel for their valuable analytical contributions to this research.

Funding

This study was funded by a grant from the Deutsche Forschungsgemeinschaft (DFG) to A.K. (KA1736/16-1), a Marie Curie ERG grant to C.S. (PERF04-GA-2008-239252) and a Landesgraduiertenförderung fellowship to E.D.M. (GZ 11.2_7631.2/Melton).

References

- Albers H, Gottschalk G. 1976. Acetate metabolism in *Rhodopseudomonas gelatinosa* and several other Rhodospirillaceae. Arch Microbiol 111:45–49.
- Bose A, Newman DK. 2011. Regulation of the phototrophic iron oxidation (pio) genes in *Rhodopseudomonas palustris* TIE-1 is mediated by the global regulator, FixK. Mol Microbiol 79:63–75.
- Caiazza NC, Lies DP, Newman DK. 2007. Phototrophic Fe(II) oxidation promotes organic carbon acquisition by *Rhodobacter capsulatus* SB1003. Appl Environ Microbiol 73:6150–6158.
- Campbell NA, Reese JB. 2002. Biology 6th edition. San Francisco: Benjamin Cummings.
- Carlozzi P, Sacchi A. 2001. Biomass production and studies on *Rhodopseudomonas palustris* grown in an outdoor, temperature controlled, underwater tubular photobioreactor. J Biotechnol 88:239–249.
- Carlson HK, Clark IC, Blazewicz SJ, Iavarone AT, Coates JD. 2013. Fe (II) oxidation is an innate capability of nitrate-reducing bacteria involving abiotic and biotic reactions. J Bacteriol 195:3260–3268. doi:10.1128/JB.00058-13.
- Chubarenko I, Chubarenko B, Bauerle E, Wang Y, Hutter K. 2003. Autumn physical limnological experimental campaign in the Island Mainau littoral zone of Lake Constance. J Limnol 62:115–119.
- Cockell CS, Kelly LC, Summers S, Marteinson V. 2011. Following the kinetics: Iron-oxidizing microbial mats in cold Icelandic volcanic habitats and their rock-associated carbonaceous signature. Astrobiology 11:679–694.
- Croal LR, Hao Y, Kappler A, Newman DK. 2009. Phototrophic Fe(II) oxidation in an atmosphere of H₂: implications for banded iron formations. Geobiology 7:21–24.
- Croal LR, Johnson CM, Beard BL and Newman DK. 2004. Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria. Geochim. Cosmochim. Acta 8:1227–1242.
- Crowe SA, Jones C, Katsev S, Magen C, O'Neill AH, Sturm A, Candiell DE, Haffner GD, Mucci A, Sunby B, Fowle DA. 2008. Phototrophs thrive in an Archean Ocean analogue. Proc Natl Acad Sci USA 105:15938–15943.
- Davison W, Seed G. 1983. The kinetics of the oxidation of ferrous iron in synthetic and natural waters. Geochim Cosmochim Acta 47: 67–79.
- Druschel GK, Emerson D, Sutka R, Suchecki P, Luther GW. 2008. Low-oxygen and chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing microorganisms. Geochim Cosmochim Acta 72:3358–3370.
- Ehrenreich A, Widdel F. 1994. Anaerobic oxidation of ferrous iron by purple bacteria, a new-type of phototrophic metabolism. Appl Environ Microbiol 60:4517–4526.
- Emmerich, M, Bhansali A, Lösekann-Behrens T, Schröder C, Kappler A, Behrens S. 2012. Abundance, distribution, and activity of Fe (II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of Lake Kasin, Southern Russia. Appl Environ Microbiol 78:4386–4399.
- Gerhardt S, Brune A, Schink B. 2005. Dynamics of redox changes of iron caused by light-dark variations in littoral sediment of a freshwater lake. Biogeochem 74:323–339.
- Gerhardt S, Schink B. 2005. Redox changes of iron caused by erosion, resuspension and sedimentation in littoral sediment of a freshwater lake. Biogeochem 74:341–356.
- Hegler F, Lösekann-Behrens T, Hanselman K, Behrens S and Kappler A. 2012. Influence of seasonal and geochemical changes on the geomicrobiology of an iron carbonate mineral water spring. Appl Environ Biol 78:7185–7196.
- Heising S, Richter L, Ludwig W and Schink B. 1999. *Chlorobium ferrooxidans* sp. Nov., a phototrophic green sulphur bacterium that oxidizes ferrous iron in coculture with “*Geospirillum*” sp. Strain. Arch Microbiol 172:116–124.
- Heising S, Schink B. 1998. Phototrophic oxidation of ferrous iron by a *Rhodomicrobium vannielli* strain. Microbiology 144:2263–2269.
- Hohman C, Winkler E, Morin G, Kappler A. 2010. Anaerobic Fe(II)-oxidizing bacteria show As resistance and immobilize As during Fe (III) mineral precipitation. Environ Sci Technol 44:94–101.
- Jiao Y, Kappler A, Croal LR, Newman DK. 2005. Isolation and characterization of a genetically traceable photoautotrophic Fe(II)-oxidizing bacterium, *Rhodopseudomonas palustris* strain TIE-1. Appl Environ Microbiol 71:4487–4496.
- Jiao Y, Newman DK. 2007. The pio operon is essential for phototrophic Fe(II) oxidation in *Rhodopseudomonas palustris* TIE-1. J Bacteriol 189:1765–1773.
- Kappler A, Newman DK. 2004. Formation of Fe(III)-minerals by Fe (II)-oxidizing photoautotrophic bacteria. Geochim Cosmochim Acta 68:1217–1226.
- Kappler A, Pasquero C, Konhauser KO, Newman DK. 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. Geology 33:865–868.
- Kappler A, Straub KL. 2005. Geomicrobiological cycling of iron. Re. Mineral Geochem 59:85–108.
- Konhauser KO, Hamade T, Raiswell R, Morris RC, Ferris FG, Southam G, Canfield DE. 2002. Could bacteria have formed the Precambrian banded iron formations? Geology 30: 1079–1082.
- Konhauser KO, Kappler A, Roden EE. 2011. Iron in microbial metabolisms. Elements 7:89–93.
- Kopf SH, Newman DK. 2012. Photomixotrophic growth of *Rhodobacter capsulatus* SB1003 on ferrous iron. Geobiology 10: 216–222.
- Laguna R, Tabita FR, Alber BE. 2011. Acetate-dependent photoheterotrophic growth and the differential requirement for the Calvin-Benson-Bassham reductive pentose phosphate cycle in *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris*. Arch Microbiol 193:151–154.
- McKinlay JB, Harwood CS. 2010. Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. Proc Natl Acad Sci USA 107:1169–11675.
- Miot J, Benzerara K, Morin M, Kappler A, Bernard S, Obst M, Féraud F, Skouri-Panet F, Guigner J.M, Posth N, Galvez M, Brown Jr GE, Guyot F. 2009b. Iron biomineralization by neutrophilic iron-oxidizing bacteria. Geochim Cosmochim Acta 73:696–711.
- Miot J, Benzerara K, Obst M, Kappler A, Hegler F, Schädler S, Bouchez C, Guyot F, Morin G. 2009a. Extracellular iron biomineralization by photoautotrophic iron-oxidizing bacteria. Appl Environ Microbiol 75:5586–5591.
- Oda Y, Larimer FW, Chain PSG, Malfatti S, Shin MV, Vergez LM, Hauser L, Land ML, Braatsch S, Beatty JT, Pelletier DA, Schaefer A, Harwood CS. 2008. Multiple genome sequences reveal adaptations of a phototrophic bacterium to sediment microenvironments. Proc Natl Acad Sci USA 105:18543–18548.
- Peeters F, Straile D, Lorke A, Ollinger D. 2007. Turbulent mixing and phytoplankton spring bloom development in a deep lake. Limnol Oceanogr 52:286–298.
- Posth NR, Hegler F, Konhauser KO, Kappler A. 2008. Alternating Si and Fe deposition caused by temperature fluctuations in Precambrian oceans. Nat Geosci 1:703–708.
- Posth, N.R.; Konhauser, K.O. and Kappler, A. 2013. Microbiological processes in BIF deposition. Sedimentology 60:1733–1754.
- Poulain AJ, Newman DK. 2009. *Rhodobacter capsulatus* catalyzes light-dependent Fe(II) oxidation under anaerobic conditions as a potential detoxification mechanism. Appl Environ Microbiol 75:6639–6646.
- Salka I, Cuperova Z, Masin M, Kobizek M, Grossart HP. 2011. Rhodoferrax-related pufM gene cluster dominates the aerobic anoxygenic phototrophic communities in German freshwater lakes. Environ Microbiol 13:2865–2875.

- Stookey LL. 1970. Ferrozine —A new spectrophotometric reagent for iron. *Anal Chem* 42:779–781.
- Straub KL, Benz M, Schink B, Widdel F. 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Appl Environ Microbiol* 62:1458–1460.
- Waidner LA, Kirchman DL. 2007. Aerobic anoxygenic phototrophic bacteria attached to particles in turbid waters of the Delaware and Chesapeake estuaries. *Appl Environ Microbiol* 73:3936–3944.
- Weber KA, Urrutia MM, Churchill PF, Kukkadapu RK, Roden EE. 2006. Anaerobic redox cycling of iron by freshwater sediment microorganisms. *Environ Microbiol* 8:100–113.
- Widdel F, Schnell S, Heising S, Ehrenreich A, Assmus B, Schink B. 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature* 362:834–836.
- Zinder SH, Dworkin M. 2006. Morphological and physiological diversity. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K, Stackebrandt E, editors. *The Prokaryotes*, 3rd ed. New York: Springer, chapter 1.7.