

Chapter 5

Phenazines and other redox active antibiotics promote microbial mineral reduction

* Adapted from Hernandez, Kappler & Newman, 2004, *A&EM.*, Vol. 70, p.921-928.

5.1. Introduction

Microbial reductive dissolution of minerals can significantly affect the chemistry of soils and sedimentary environments by making inorganic compounds such as iron more available to plants and other organisms and by mobilizing adsorbed nutrients and/or pollutants into the aqueous phase (2, 11, 30). Which organisms in the environment most affect iron cycling has been the subject of several studies (6, 44), and diverse bacteria are known that can reduce but not grow on iron including *Aerobacter aerogenes*, *Bacillus polymyxa*, *Escherichia coli*, *Pseudomonas sp.* and *Serratia marcescens* (31). With the discovery that some bacteria can respire iron (hydr)oxides (32, 41) attention has focused mainly on the importance of dissimilatory iron reducing bacteria (DIRB) and the molecular strategies they take to reduce minerals (4, 7, 13, 29, 35, 40). Evidence supporting the release of small organic molecules by DIRB for the purpose of mineral dissolution has been accumulating, and it is thought that this process may be most significant in environments where bacteria form biofilms on mineral surfaces (19, 42, 43).

It has been appreciated for some time that naturally present complex organic molecules such as humic substances (**Fig 1a**) and their functional surrogate, anthraquinone-2,6-disulfonate (AQDS) (**Fig 1b**), can stimulate iron reduction by serving as electron shuttles between microbes and minerals (33). Whether natural products produced by soil and/or sedimentary bacteria can play a similar role has been unexplored. *Pseudomonas*, *Streptomyces*, *Sorangium*, *Arthrobacter*, *Nocardia*, *Burkholderia*, *Brevibacterium* and other bacterial genera are commonly found in the soil and are major producers of antibiotics (36). Among the antibiotics that they produce are those that possess redox activity, such as complex glycopeptidic antibiotics like bleomycin (**Fig. 1c**) and the numerous multicolored phenazine pigments (23, 54) (**Fig. 1d**). Structurally, these molecules resemble humic substances and AQDS in their aromatic ring structure and redox-active functional groups.

To date, most ecological discussions of phenazines have focused on their crucial role in suppressing fungal pathogens of plants such as *Fusarium oxysporum f. sp. radicle-lycopersici* and *Gaeumannomyces graminis var. tritici* (9). However, there has also been some discussion of a role for phenazines in aiding the ecological competence of *Pseudomonads* in the rhizosphere (37, 45) presumably due to their ability to generate reactive oxygen species that kill other organisms (17). Appreciable concentrations of phenazines have been detected in the rhizosphere (e.g., phenazine carboxylic acid (PCA) produced by *P. fluorescens* strain 2-79 was found to be present in concentrations of 27 to 43 ng/g of root with adhering soil) (53), and the expression of phenazine biosynthetic genes in organisms growing on tomato root seedlings and bean plants roots has been measured (10, 48). While little is known about the abundance of bleomycin in the soil,

this compound has been the subject of intense biochemical scrutiny due to its effectiveness as an anticancer agent, attributed to its ability to coordinate Fe which then transfers electrons to DNA via an activated oxygen molecule, resulting in DNA cleavage (18).

Given that both phenazines and bleomycin can function as electron carriers (in fact, phenazines are known to be part of electron transport chains in methanogenic archaea) (1), we hypothesized that redox active antibiotics might promote reduction of minerals by serving as electron shuttles between microbes and minerals. To test this hypothesis, we chose to work primarily with the soil isolate *P. chlororaphis* PCL1391 that produces phenazine-1-carboxamide (**fig 1d**), and the DIRB, *Shewanella oneidensis* MR-1.

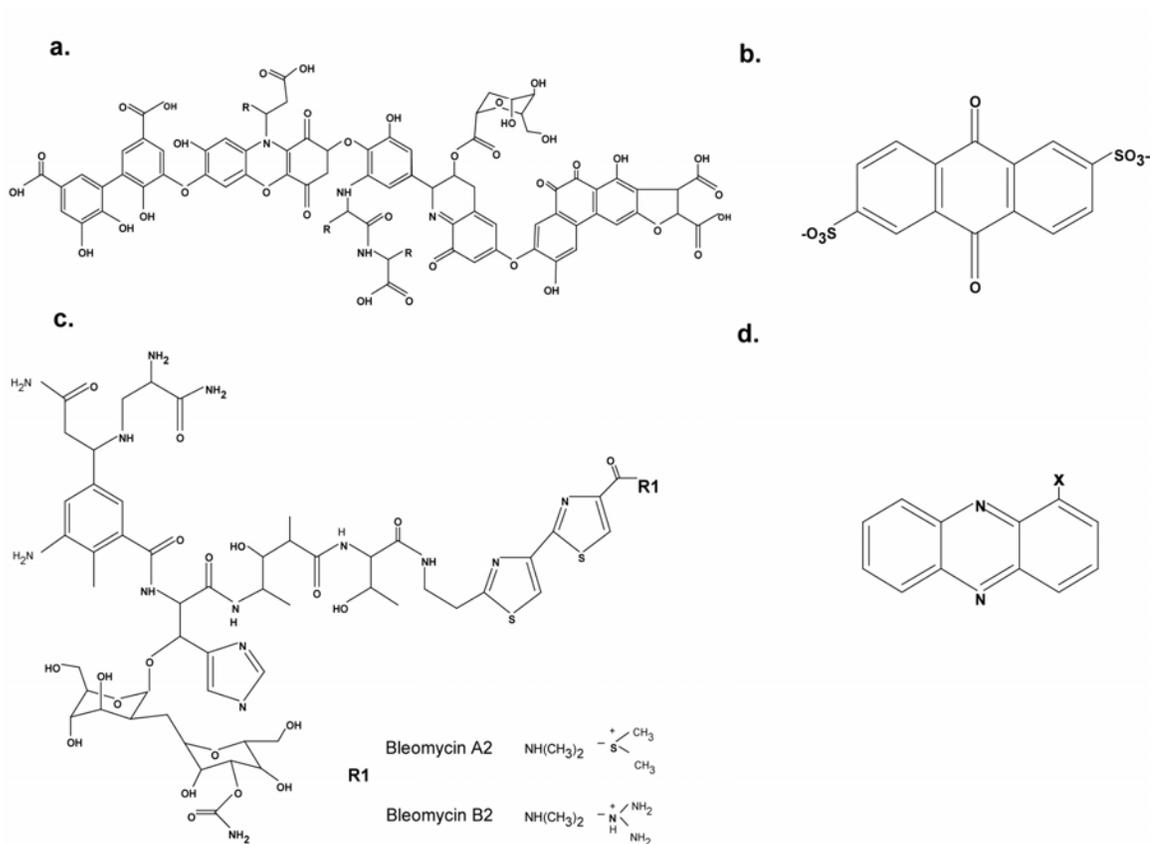


Figure 5.1. Structure of **a.**) a representative humic substance molecule, after those described in the literature (49) **b.**) anthraquinone-2,6-disulfonate (AQDS) **c.**) bleomycin and **d.**) a generic phenazine. If X= CONH₂ : phenazine-1-carboxamide (PCN); if X= COOH : phenazine-1-carboxylic acid (PCA).

5.2 Materials and methods

5.2.1 Bacterial strains and culture conditions

The bacterial strains *P. chlororaphis* PCL1391 and *P. chlororaphis* PCL1119 used in this study were obtained from G. Bloemberg (Institute of Biology, Leiden University, The Netherlands). PCL1391 was isolated from a tomato field in Spain and PCL1119 is a transposon generated mutant with a disruption in *phzB*, one of the genes in the core operon required for the biosynthesis of phenazines, making it defective in phenazine production (10). *Shewanella oneidensis* MR-1 was isolated from Oneida lake, New York (41). Cultures were grown in LB, King's A (a medium designed to elicit phenazine production) (24), Basic Medium with low phosphate (0.1 mM), HEPES 10 mM and 20 mM glucose as the carbon source (38), or LML medium (0.01% peptone, 0.02% yeast extract, 20 mM lactate, 10 mM HEPES) as appropriate at 30°C. Other strains used in this study included additional gamma proteobacteria grown on LB (*E. coli* JM109, *P. fluorescens* WCS365, *P. aeruginosa* PAO1 and *V. cholera* El Tor) and *Geobacter* sp. Dfr1 and Dfr2 (grown as described by Straub et al., ref. 52).

5.2.2 Chemicals

AQDS (anthraquinone-2,6-disulfonate), phenazine and bleomycin were purchased from Sigma-Aldrich. Pyocyanine was prepared from phenazine-methosulfate by photochemical oxidation as previously described (25).

5.2.3 Purification of phenazines and HPLC analysis.

Phenazine-1-carboxamide (PCN) was purified from green precipitates in culture supernatants or from Cl_3CH extracts after solvent evaporation by multiple recrystallizations from hot water. Purity was assessed by high-pressure liquid chromatography (Beckman System Gold) with a C18 Symmetry-Prep column (Waters; 7.8×150 mm) in a gradient of water/0.01%TCA (solvent A)–acetonitrile/0.01%TCA (solvent B): 0–1 min 100% A, 1–7 min linear gradient to 100% B, 7–9 min 100% B, 9–12 min linear gradient to 100% A, 12-15 min 100% A) and a flow rate of 2 ml/min. Retention times for PCN and PCA were 7.45 and 8.17 minutes, respectively.

Concentration of PCN and PCA was estimated using an extinction coefficient (ϵ_{368}) of 17,600 at 368 nm that was calculated by measuring the absorption spectra of a PCN and of a phenazine solution of known concentration and assuming the same extinction coefficient for phenazine and PCN at 250 nm (5).

Determination of presence and concentration of phenazines in cultures was performed by extracting 0.9 ml culture supernatant twice with an equal volume of chloroform, evaporating the organic phase and resuspending the residue in 200 μl of acetone. Acetone fractions were analyzed by HPLC after filtration through 0.2 μm pore filter tubes (nylon-membrane).

5.2.4 Fe(III) reduction assays

P. chlororaphis cells were grown overnight in LB medium, washed and resuspended at about 10⁷ cells/ml in 48 ml bottles with 25 ml KA medium plus 10 mM poorly crystalline iron (hydr)oxide [Fe(OH)₃] (34). Tubes were capped and incubated in a rotary shaker at 30°C. When required, the tubes were amended with purified PCN (~40 µM) from a concentrated stock in hot water. Samples were taken regularly to determine the number of colony forming units (CFUs) by plating 100 µl of serial dilutions of the sample onto LB-agar plates. Fe(II) was determined in acidified culture samples with the ferrozine assay (50), both before (for total Fe(II)) and after filtration (for dissolved Fe(II)) through a 0.2 µm pore filter. Soluble Fe(III) was calculated by determining total soluble iron as Fe(II) after a reduction step with NH₄OH and subtracting the dissolved Fe(II) concentration.

Shewanella oneidensis MR-1 cells were inoculated at about 10⁶ cells/ml in LML medium in anaerobic glass tubes with 30 mM Fe(OH)₃ as the electron acceptor from an overnight aerobically-grown LB culture and incubated at 30 °C. PCN (10 µM), filtered spent supernatants (to a final 1:10 dilution), AQDS (10 µM) and bleomycin (15-20 µM) were added as required. Reduction of Fe(OH)₃ was determined by the ferrozine assay. Cell counts were done as previously described (34).

Abiotic reduction of Fe(OH)₃ was accomplished by reducing PCN with a Pd catalyst and H₂, and then mixing reduced PCN with iron citrate or poorly crystalline iron (hydr)oxide in a 80:15:5% N₂:CO₂:H₂ glove-box.

5.2.5 Pyocyanine reduction assays

Pyocyanine reduction was measured by adapting a previously described method (12). *E. coli* JM109, *P. fluorescens* WCS365, *P. aeruginosa* PAO1 and *V. cholera* El Tor were harvested from late exponential/early stationary phase LB culture, washed twice in saline solution (0.85% NaCl) and resuspended in the same volume of LML medium. At the start of the experiment, 50 μ l of this cell suspension were added to 2 ml of an anoxic solution (pH 7.5) containing 10 mM HEPES, 4 mM MgCl₂, 2 mM (each) lactate, succinate and pyruvate, and 2 μ g/ml pyocyanine. The mix was prepared in the anaerobic chamber and placed in air-tight cuvettes to prevent pyocyanine reoxidation by oxygen during the absorption measurements. Pyocyanine reduction was followed by the characteristic change of the absorption spectrum accompanying the transformation of the oxidized to the reduced form. The decrease in absorption at 690 nm was measured compared to a reference cuvette containing a cell suspension lacking pyocyanine, in a Beckman Coulter DU7400 spectrophotometer. The optical density at 550 nm (OD₅₅₀) of each cell suspension was measured and the rates were standardized to an OD₅₅₀ of 0.1.

5.2.6 Toxicity experiments

Shewanella oneidensis MR-1 cells were grown aerobically in LB overnight or anaerobically in LB/fumarate (20 mM) for a day and were inoculated in fresh medium at OD₆₀₀ \approx 0.05. Different amounts of PCN were added and the tubes (duplicates each) were incubated under the same conditions as the precursor cultures. Samples (200 μ l) were taken periodically, and cell growth was determined by measuring the optical density at 600 nm in a microtiter plate reader (Opsys MR, Dynex Technologies).

5.3 Results

5.3.1 Mineral reduction by *Pseudomonas chlororaphis* strains

P. chlororaphis PCL1391 (wild type, WT) and *P. chlororaphis* PCL1119 (the *phzB* mutant) were grown in medium amended with poorly crystalline manganese or iron (hydr)oxides in microtiter plates. After a day, mineral reduction and phenazine production in the presence of the WT but not the *phzB* mutant was evident by the dissolution of the manganese mineral, the change of color of the iron mineral (from a deep orange to a darker brown), and the appearance of small green crystals, most likely phenazine-1-carboxamide (PCN) precipitates (see Methods). To quantify these observations, Fe(III) mineral reduction was measured; Fe(II) production was only detected in the WT culture (**Fig. 2a**). However, CFUs were similar in both cultures, indicating that Fe(III) mineral reduction did not have an impact on cell growth under these conditions (**Fig. 2b**). About half of the reduced iron in the WT cultures was in solution as shown by its presence in the filtrate fraction (data not shown); the remaining part was likely adsorbed to the mineral and/or the cells, as well as re-precipitated as Fe(II)-minerals.

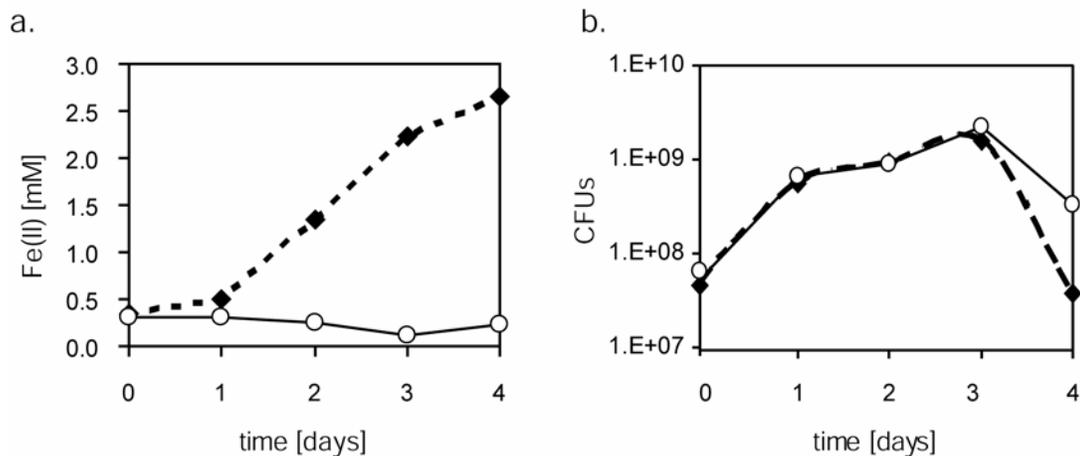


Figure 5.2 Total Fe(II) produced in culture **a.**) and colony forming units (CFU's) **b.**) of *P. chlororaphis* PCL1391 (WT) (diamonds) and PCL1119 (*phzB* mutant) (circles) grown in the presence of poorly crystalline iron (III) hydr(oxides). Data is representative of at least 3 independent experiments.

Extraction and quantification of phenazines in the WT culture showed that PCN production was correlated with cell numbers and its concentration was constant once the cells reached stationary phase. Up to 20 μM PCN and 5 μM PCA was produced by the WT (**Fig. 3a**). No phenazines were detected in the *phzB* mutant culture. The amount of PCN produced by the WT was positively correlated with the amount of oxygen initially present in the system (defined by the size of the headspace of the bottle) and varied with the medium used in the experiment (e.g., KA, LB, or BM-glucose), but a similar trend was observed under all conditions. The final concentration of Fe(II) (3 mM) (**Fig. 3b**) was about 120 times higher than the total phenazine concentration (25 μM). Because two electrons are available per reduced phenazine molecule and only one electron is required to reduce each iron ion, the phenazines must have been cycled at least 60 times to account for the measured iron reduction. Addition of PCN to the *phzB* mutant restored the ability of the strain to reduce the iron mineral, indicating that PCN is essential for the reduction process (**Fig. 3b**). Again, the amount of iron reduced per amount of phenazine added was high, demonstrating redox shuttling of phenazines between the bacteria and the minerals. That phenazines were serving as electron shuttles was confirmed by control experiments in which chemically reduced PCN was found to reduce and solubilize Fe(III) minerals in the absence of bacteria. Moreover, no soluble Fe(III) could be detected in the

filtrate fractions of WT cultures, suggesting that chelation of Fe(III) is not a significant mechanism for phenazine stimulation of Fe(III) mineral reduction.

When the ability of the WT and *phzB* mutant to reduce soluble Fe(III) citrate was measured, the *phzB* mutant was able to reduce approximately half the amount of Fe(III) as the WT; this implies that a mechanism other than phenazine production confers the ability to reduce dissolved Fe(III) (data not shown). Neither the WT nor the *phzB* mutant were able to grow anaerobically with Fe(III) as the only electron acceptor, however, regardless of the form provided.

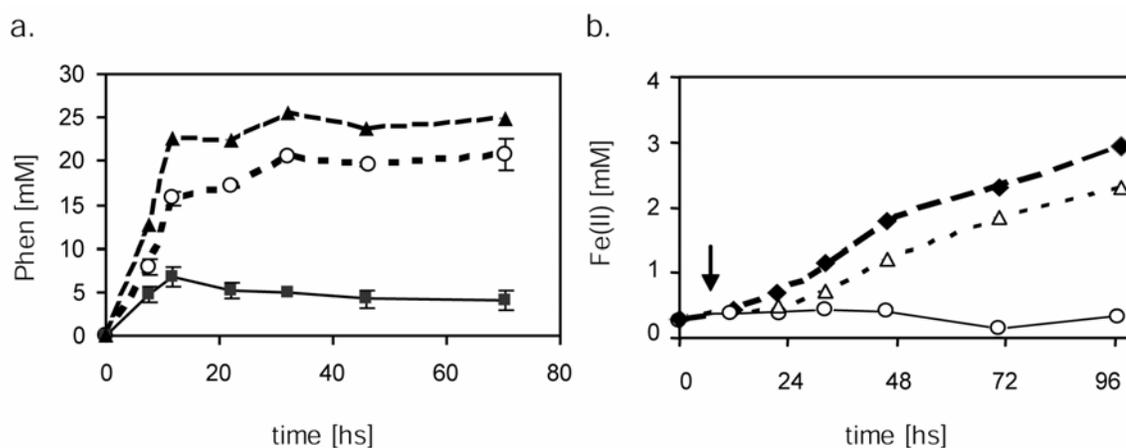


Figure 5.3 a.) Total phenazine production (triangles) in a PCL1391 culture representing the sum of phenazine-1-carboxamide (circles) and phenazine-1-carboxylic acid (squares). Data represent the average of two independent extractions of each time point in the same experiment; bars show the data range. b.) Total Fe(II) production by PCL1391 (WT) (diamonds), PCL1119 (*phzB* mutant) (circles) and PCL1119 with PCN added (40 μ M; at time point indicated by arrow) (triangles). Data is representative of at least 2 independent experiments.

5.3.2 Redox active antibiotics stimulate Fe(III)-reduction by diverse bacteria

Because phenazines proved to be good electron shuttles to poorly crystalline iron (hydr)oxide for the *Pseudomonas* producer species, we decided to test their ability to stimulate iron reduction by other organisms that do not produce these compounds. For this purpose, a representative DIRB, *Shewanella oneidensis* MR-1 was chosen.

Anaerobic cultures of MR-1 were grown on poorly crystalline iron hydr(oxides) with and without added PCN and compared to a positive control where AQDS, that is known to act as an electron shuttle, was added. Notably, stimulation of iron reduction by the addition of 10 μ M PCN was comparable to that observed by the addition of 10 μ M AQDS (Fig. 4a). MR-1 grew more rapidly in the presence of either PCN or AQDS than it did when provided with Fe(III) hydr(oxide) alone (Fig. 4b). When shuttles were added, fine grained magnetite formed rapidly, coinciding with fast iron reduction; in contrast, magnetite accumulated more slowly when shuttles were omitted. Other phenazines tested (e.g., pyocyanine, phenazine methosulfate and phenazine) stimulated Fe(III) mineral reduction by MR-1 in the same way. When PCN was added to cultures of different DIRB (e.g., *Geobacter* strains Dfr1 and Dfr2 (51)), acceleration of iron reduction was also observed (data not shown). Because reduced phenazines react abiotically with insoluble Fe(III) minerals, any strain that can reduce phenazines has the potential to promote mineral reduction. Diverse gamma proteobacteria that we tested, including *E. coli* JM109, *P. fluorescens* WCS365, *P. aeruginosa* PAO1, and *V. cholera* strain El Tor, were able to reduce pyocyanine.

To determine whether phenazines play a significant role in extracellular electron transfer that is differentiable from the contribution of other small molecules released by *P*

chlororaphis PCL1391, we tested whether Fe(III) mineral reduction by *S. oneidensis* MR-1 could be stimulated by spent supernatants from the WT and spent supernatants from the *phzB* mutant. Fig. 4c shows that rapid and significant production of Fe(II) by *S. oneidensis* MR-1 occurs in the presence of spent supernatants from the WT, whereas spent supernatants from the *phzB* mutant had a reduced effect that was evident only at later stages in the experiment. Because *P. chlororaphis* PCL 1119 does not produce phenazines, these results suggest that phenazines are the primary natural products released by *P. chlororaphis* PCL1391 that promote iron reduction by *S. oneidensis* MR-1; however, it is likely that other bacterially produced compounds in the culture can stimulate iron reduction as well.

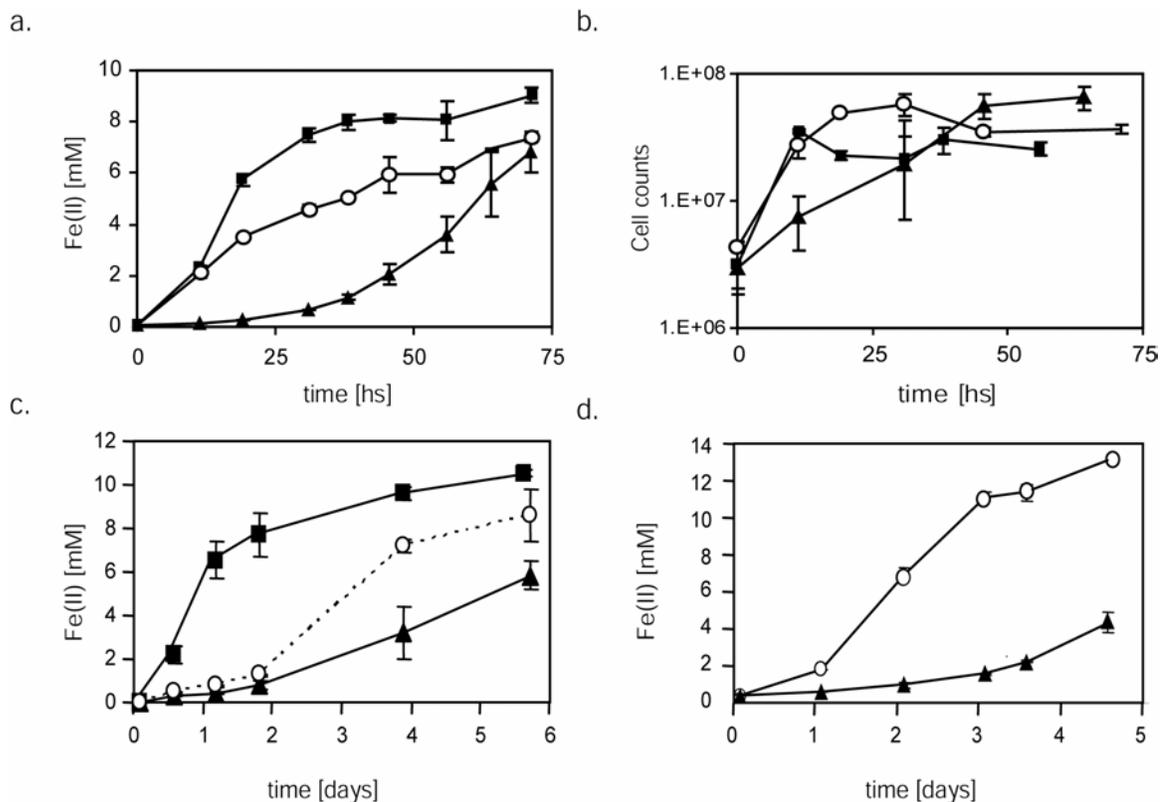


Figure 5.4 Total Fe(II) produced a.) and cell growth achieved b.) by the reduction of poorly crystalline iron (hydr)oxide by *S. oneidensis* MR-1 in the absence (triangles) or presence of 10

μM AQDS (squares) or $10 \mu\text{M}$ PCN (circles). **c.**) Total Fe(II) produced by the reduction of poorly crystalline iron (hydr)oxide by *S. oneidensis* MR-1 in the absence (triangles) or presence of a final 1:10 dilution of filtered culture supernatant from the WT strain PCL1391 (squares) or the mutant PCL1119 (circles). **d.**) Total Fe(II) produced by the reduction of poorly crystalline iron (hydr)oxide by *S. oneidensis* MR-1 in the absence (triangles) or presence (circles) of 15-20 μM bleomycin. Data represent the average value of duplicate experiments, with bars showing the data range.

In addition to phenazines, bleomycin, a glycopeptidic redox-active antibiotic produced by *Streptomyces verticillus* (**Fig 1c**), also greatly increased the rate of Fe(III) mineral reduction by MR-1 cultures (**Fig. 4d**). Controls with bleomycin in the absence of cells showed no iron reduction (data not shown).

5.3.3 Inhibitory concentrations of phenazines on MR-1.

Phenazines are known to have antibacterial properties, therefore the effect of adding PCN to growing MR-1 cultures was tested. PCN reduced the growth rate and yield of MR-1 growing aerobically in LB and anaerobically in LB/fumarate as follows. Aerobically grown cells eventually reached stationary phase cell densities comparable to controls without PCN up to a concentration of $75 \mu\text{M}$. When PCN was added at a concentration of $150 \mu\text{M}$, the cell yield was only 25% that of the no PCN control, and growth of *S. oneidensis* MR-1 was completely inhibited at concentrations equal to or greater than $300 \mu\text{M}$ PCN. *S. oneidensis* MR-1 cells growing anaerobically on fumarate generally reached lower stationary phase cell density than the aerobically grown cultures. The inhibitory effect of PCN in cultures growing on fumarate increased with increasing concentrations of added PCN. Small amounts of PCN (e.g., $2.4 \mu\text{M}$) reduced the final cell

density of the culture to 77% that of the no PCN control and the final cell density achieved in the presence of 300 μM added PCN was 6% relative to the no PCN control. No inhibitory effects were seen for *P. chlororaphis* PCL1391 growing aerobically in LB with up to 300 μM PCN.

5.3.4 Thermodynamic constraints on phenazine-mediated iron mineral reduction.

To assess the conditions under which iron mineral reduction by phenazines would be thermodynamically feasible, we constructed an E_h /pH diagram. Total iron was assumed to be 10^{-5} M, carbonate species were neglected for simplicity, and a value of $E_h^{\circ} = -114$ mV for PCN was taken from the literature (14). Given these parameters, the reaction between PCN and $\text{Fe}(\text{OH})_3$ is clearly favorable at pH 7 and lower, but becomes unfavorable at higher pH (≥ 8) (Fig. 5). As shown in Fig. 5, the more Fe^{2+} accumulates in the system, the smaller the pH range becomes in which phenazines can act as an electron shuttle. On the other hand, in the presence of more reduced phenazines relative to oxidized phenazines in the system, electron transfer via phenazines is also possible at higher Fe^{2+} concentrations and/or higher pH values.

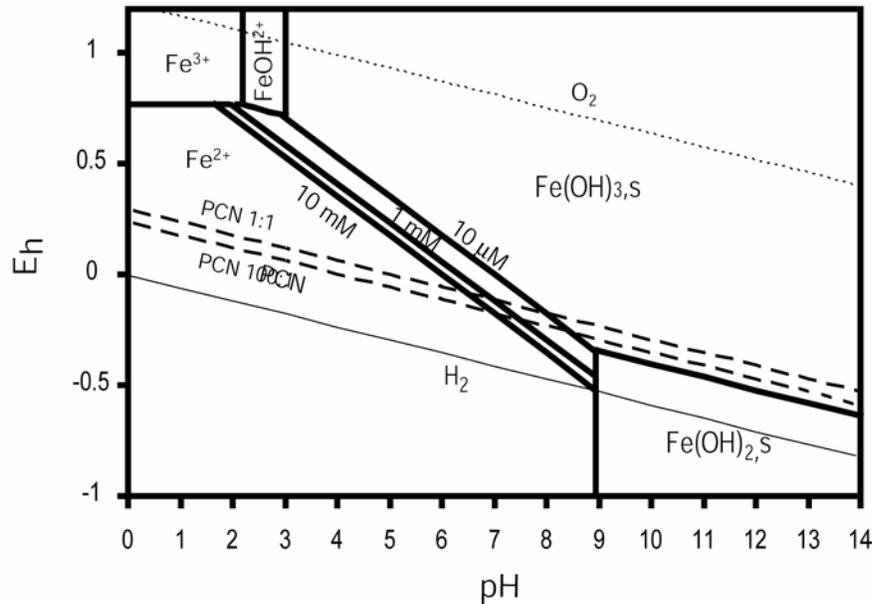


Figure 5.5 E_h /pH diagram showing the thermodynamic stability region of iron species and PCN as a function of pH and E_h . The dashed lines represent the redox potential for the PCN redox couple at a ratio $PCN_{red}/PCN_{oxid}=1:1$ and $100:1$, respectively. To draw the $Fe(OH)_3/Fe^{2+}$ boundary line, a concentration of Fe^{2+} of $10 \mu M$, $1 mM$ and $10 mM$ was assumed. H_2 and O_2 lines frame the stability field for water.

5.4 Discussion

That common soil bacteria such as *Pseudomonas* species can reduce (but not grow on) iron minerals has been appreciated for a long time (31), however, the mechanism(s) responsible for this phenomenon have been unknown. Here we show that phenazines and other redox active antibiotics produced by soil bacteria can serve as electron shuttles, being reduced microbiologically and subsequently oxidized by poorly crystalline iron and manganese (hydr)oxides.

Phenazine production has been well-studied in a variety of organisms because of their function in the bio-control of plant pathogens and as virulence factors in the human host (9, 23). It is noteworthy that the environmental factors that are known to stimulate phenazine production make sense in the context of Fe(III) mineral reduction. These factors include: low phosphate (15), oxygen limitation (26), and the presence of Fe(III) (26, 27) and organic components of root and/or seedling exudates such as fructose, ribose, and citric acid (Chin-A-Woeng, T.F, personal communication). Microaerophilic or anaerobic zones have been reported to occur in rhizosphere environments and soil micropockets (22). Oxygen regulation of phenazine production thus may ensure that phenazines are produced under the most favorable conditions for mineral reduction. Moreover, oxygen tensions drop when bacteria are at high cell density (e.g., in biofilms), and quorum sensing appears to be an additional mechanism for the regulation of phenazine production (8, 28, 46, 56). Regulating phenazine production by quorum sensing may limit competition by non-producer strains that could benefit directly or indirectly from the production of phenazines.

There are multiple ways phenazines may impact ecological fitness: as antibiotics (3), as accessory respiratory pigments (16), and as agents of mineral reduction as shown in this study. With respect to the latter role, it seems likely that phenazines may act in concert with siderophores to make iron bioavailable. Although siderophores are thermodynamically able to dissolve iron minerals due to their high binding constants for Fe(III), kinetically, this can be a very slow process and it is questionable whether siderophores alone can explain iron acquisition in the environment (21, 55). Evidence in support of this hypothesis comes from studies of *Pseudomonas mendocina*, where

unidentified low molecular weight reductants and siderophores were thought to account for iron acquisition from minerals (20).

Our thermodynamic calculations show that phenazine-mediated reduction of poorly crystalline iron (hydr)oxides would be favored at $\text{pH} \leq 7-8$, whereas higher pH values would make the process endergonic. If Fe(II) accumulates, as happened in our experimental system, the reaction will reach equilibrium and further reduction will not occur. On the other hand, if the Fe(II) produced is scavenged by active uptake, binding to chelators, reoxidation by oxygen, or precipitation as a mineral phase such as siderite, FeCO_3 , phenazine-mediated Fe(III) reduction will be favored. Although our model does not explicitly include carbonate species, their presence would enhance Fe(III) reduction because siderite precipitation would maintain dissolved Fe(II) at low levels, and this would affect the potential of the Fe(II)/Fe(III) redox couple so as to favor Fe(III) reduction by phenazine. The redox potential of the specific redox active antibiotic produced, the ratio of reduced:oxidized antibiotic, and the redox potential of the mineral are key parameters that constrain whether reduction occurs. For example, PCA is reported to have a lower redox potential than PCN (47) and thus it would be expected to reduce minerals at higher pH and higher Fe(II) concentrations compared to PCN. Due to their lower redox potentials compared to poorly crystalline ferric hydr(oxide)(39), other common Fe(III) mineral phases in soils, like hematite and goethite, would be predicted to be reduced by phenazines only at lower pH. Because adsorption of reductants to minerals is required for electron transfer to occur, the specific chemical structure of the reductant is also important since it will determine its adsorption properties and thus the rate of reductive dissolution.

In addition to promoting iron reduction by the producer strains, redox-active antibiotics may positively impact iron reduction and/or acquisition by other microorganisms. The stimulation of Fe(III) reduction by *S. oneidensis* MR-1 and *Geobacter* species by phenazines and bleomycin suggests that DIRB may reduce *in situ* produced antibiotics when soils and sediments become anaerobic. Because these compounds are more bioavailable than ferric minerals, organisms that can reduce them may gain more energy per unit time, thus providing them with a growth advantage. From the literature, we can estimate that phenazine concentrations in the rhizosphere may be on the order of a tenth of a μM to several μM , assuming 1 g of soil fills a volume of 1 ml (52). Although it is difficult to know exactly how much will be bioavailable (i.e., not adsorbed onto minerals or natural organic matter), it seems likely that at these concentrations, phenazines may function more effectively as electron shuttles than antibiotics. Moreover, any bacterium that can reduce phenazines or bleomycin—regardless of whether it is a DIRB—can be expected to indirectly promote Fe(III) reduction.

In summary, our results show that redox-active natural products produced by soil bacteria can function as electron shuttles to minerals, and can also be utilized by non-antibiotic producing species. This suggests that the production and cycling of redox-active antibiotics may impact bacterial and plant access to iron and a variety of other nutrients such as phosphate, trace metals and organics that are associated with mineral phases. Such nutrient mobilization may provide an ecological advantage for the producer strains—a possibly important and under-appreciated role for redox active antibiotics that can now be tested. Moreover, given their facility for electron transfer, it is important to

consider whether redox-active antibiotic producing strains can derive energy from their reduction; we are currently exploring this possibility.

5.5 References

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