Chapter 1
INTRODUCTION

1.1 Motivation

Iron is a major component of the Earth's crust and constitutes a key nutrient for most forms of life. Depending on pH and presence/absence of oxidized compounds such as molecular oxygen, iron is present as either ferrous (II) or ferric iron (III). Reduction of iron(III) to iron(II) in anoxic sedimentary and soil environments is largely microbially catalyzed and a significant amount of carbon turnover is due to this metabolism (4, 7). By coupling the oxidation of organic substrates to the reduction of Fe(III), dissimilatory iron reducing bacteria (DIRB) can directly or indirectly participate in bioremediation of organic and metal contaminants (3, 5). The product of microbial iron(III) reduction, i.e., Fe(II), has a very high affinity for iron oxide surfaces and when bound to them its reactivity increases and it can participate in reductive transformations of a variety of pollutants and other metals.

Given the importance and potential of this metabolism a detailed molecular understanding is necessary for the successful implementation of bioremediation strategies.

Under oxic and neutral pH conditions, iron is stable in its oxidized form (Fe(III)) and is mostly present as near insoluble iron(III)(hydr)oxides (1). The extremely low solubility of iron(III) minerals posses a problem for its acquisition for assimilation in oxic environments and for dissimilation in anoxic environments. Although this process has been widely studied for more than two decades, the mechanism(s) of bacterial electron

transfer to minerals for the purpose of respiration or even just reductive dissolution for assimilation are yet not completely understood (2, 6). For years the prevailing paradigm was that direct contact between microbes and minerals was necessary for iron respiration and that siderophores were produced to solubilize and acquire Fe(III) in oxic environments. Recently it was shown that humic substances, a class of polymeric and redox-active organic molecules ubiquitously present in the environment, can mediate and dramatically accelerate iron reduction opening the possibility that an important portion of bacterially catalyzed iron reduction processes is indirect . Whether microbes produce organic redox-active molecules for the purpose of electron shuttling and if so in what conditions, was mostly unexplored.

1.2 Research objectives and scope

The main objective of my research was to explore mechanisms of mineral reduction mediated by bacterially produced organic redox-active molecules (electron shuttles), mainly as an indirect mechanism of iron respiration but also as a potential mechanism for iron acquisition.

For that purpose two model organisms were used: the DIRB *Shewanella oneidensis* MR-1, whose genome has been sequenced, and the soil isolate and phenazine producer, *Pseudomonas chlororaphis* PCL1391.

Chapter 2 introduces the challenges of mineral respiration and presents the background in extracellular electron transfer. Chapter 3 answers the question of whether *Shewanella* MR-1 can reduce iron(III) without directly being in contact with the iron mineral and describes the development of ferrihydrite-coated porous glass beads as a

means to test for iron reduction at a distance. Chapter 4 discusses experiments designed to test whether a secreted small molecule derived from the menaquinone biosynthetic pathway in MR-1 is a electron shuttle to minerals as it was previously suggested. Chapter 5 interrogates whether other redox-active metabolites produced by soil bacteria, e.g., phenazines or bleomycin, can be used as mediators for iron reduction by the producer strain *Pseudomonas chlororaphis* PCL1391, but also by the DIRB *Shewanella oneidensis* MR-1. Chapter 6 summarizes the results of these studies and identifies the open questions for future research.

1.3 References

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