

## **Adaptation to growth on Fe(II) in the photoferrotroph *R. palustris* TIE-1: a proteomic and electron microscopy analysis**

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Photoferrotrophy, the oxidation of Fe(II) by anoxygenic photosynthetic bacteria, is suggested to have been a key contributor to Earth's biosphere prior to the evolution of oxygenic photosynthesis and is still found in modern environments. All known organisms which can perform photoferrotrophy show remarkable metabolic flexibility and can utilize a wide range of organic and inorganic electron donors yet the cellular adaptations required to oxidize Fe(II) are not well constrained. In this study, we used a combination of quantitative proteomics and cryo transmission electron microscopy to compare cells of the photoferrotroph *Rhodospseudomonas palustris* TIE-1 grown phototrophically with either Fe(II), hydrogen or acetate. We established that photoferrotrophy requires strict control of intracellular cation concentrations, utilizing mechanisms to control both efflux of Fe(II) and associated Cu ions, plus influx of complexed iron via siderophores. Additionally, growth on Fe(II) results in alterations of the cell membrane including changes in the production of peptidoglycan and hopanoids. Using knock out mutants, a specific role for HpnN and HpnH hopanoid-related proteins was investigated. Photoferrotrophy did not require a specific response to phosphorus stress as might be expected following co-precipitation of iron and phosphorus in minerals. Furthermore, we observed that photoautotrophy differs from photoheterotrophy in terms of the development of thylakoid membranes which is likely related to an observed increase in pigments such as bacteriochlorophyll a.

Together these results suggest that the main adaptation required to perform photoferrotrophy, in addition to an enzymatic mechanism for autotrophy and Fe(II) oxidation, is the induction of numerous defences against toxic concentrations of metal ions and harmful reactions in the cell.