Biochemical characterization and functional analysis of the iron responsive regulator RirA from Dinoroseobacter shibae

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Introduction
The Roseobacter clade of marine bacteria represents one of the major lineages of near surface waters in the global oceans. According to the highly oxygenated sea water at pH 7 the main portion of iron is the insoluble ferric form, the soluble ferrous form is limited. Therefore, a fine-tuned regulation of iron homeostasis is necessary for marine organisms.

The role of RirA for iron-dependent adaptation
The growth rates of wild-type D. shibae DFL12(white) and the rirA mutant strain (red) were compared in salt water medium in the presence of 15 µM Fe(II)SO₄ (solid line) or without addition of iron to the medium (dotted line). The rirA mutant strain showed a reduced growth compared to the wild type strain.

Samples for RNA preparation were taken from mid log growth phase and the RirA regulon was defined by DNA array hybridization.

RirA coordinates an oxygen sensitive Fe-S cluster using four conserved cysteine residues as ligands
UV/Vis spectra of the protein solution were first recorded under anoxic conditions. An absorption maximum at 420 nm, typical for Fe-S cluster containing proteins, was observed for recombinant D.shibae RirA (solid black line). The Fe-S cluster appears to be oxygen labile, since exposure to air reduced the absorption drastically (dashed black line).

To identify the cysteine residues essential for Fe-S cluster formation each of the four conserved cysteine residues of RirA was changed to an alanine residue by site directed mutagenesis of the corresponding gene. The RirA mutant proteins were produced and purified under anaerobic conditions. The RirA mutant proteins C17A (red line), C91A (green line), C99A (blue line) and C150A (orange line) showed no longer absorption at 420 nm suggesting a role of these cysteine residues in Fe-S cluster coordination.

Conclusion
The rhizobial iron regulator protein RirA from D. shibae is able to measure iron availability by coordination of a [3Fe-4S]⁺⁺ cluster as cofactor and regulates target genes in response to iron limitation.