Regulation of anaerobic respiratory pathways in Dinoroseobacter shibae
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Introduction
Within the great family of Rhodobacteraceae one major subdivision are the marine Roseobacter. Our model organism Dinoroseobacter shibae DFL122 is capable of perform nitrate respiration and subsequent denitrification under anaerobic conditions. Little is known about the regulatory network involved in anaerobic adaptation so far. Genome analysis of D. shibae resulted in the identification of seven members of the Crp/Fnr-family of transcription factors. Beside one FnrL-homologue which conserved cysteine residues predicted to coordinate a [4Fe-4S]2−-cluster, six Dnr-like regulators were found. Protein alignments grouped three of the regulators (DnrD, DnrE and DnrF) within the Dnr-subgroup. To study the role of these regulators for anaerobic adaptation we started to create knock-out mutants and analyzed the anaerobic growth of the mutant strains. Moreover, we produced and purified Dnf and studied Protein/DNA interactions with potential regulatory sequences of target genes identified by virtual footprint and transcriptome analysis (Laass et al., unpublished).

Predicted regulatory network

Growth defect of D. shibae ∆dnrF and ∆fnrL mutant strains
We created D. shibae knock-out mutants of fnrL, dnrF and dnrE genes. Growth analyses under anaerobic denitrifying conditions revealed a clear growth defect of the D. shibae ∆dnrF and ∆fnrL mutant strain indicating their role for anaerobic adaptation.

Specific binding of DnrF to a predicted Dnr binding motif within the napD promoter
In transcriptome analysis the anaerobic expression of the napD operon encoding the nitrate reductase was found Dnf dependent. We produced and purified Dnf and performed Electromobility shift assays with a 75 bp napD promoter fragment containing the predicted Dnr binding sequence TTGAC-N4-GTCAA. Increasing amounts of DnrF protein resulted in the formation of two retard bands representing two DnrF/DNA complexes (A). Kinetic analyses with a Hill coefficient = 1 result in a Kd = 3,45 µM which equals 0.84-fold Protein concentration.

Conclusion
We established a robust knock-out system for D. shibae. In addition we were able to identify and confirm a Dnr binding motif (D).