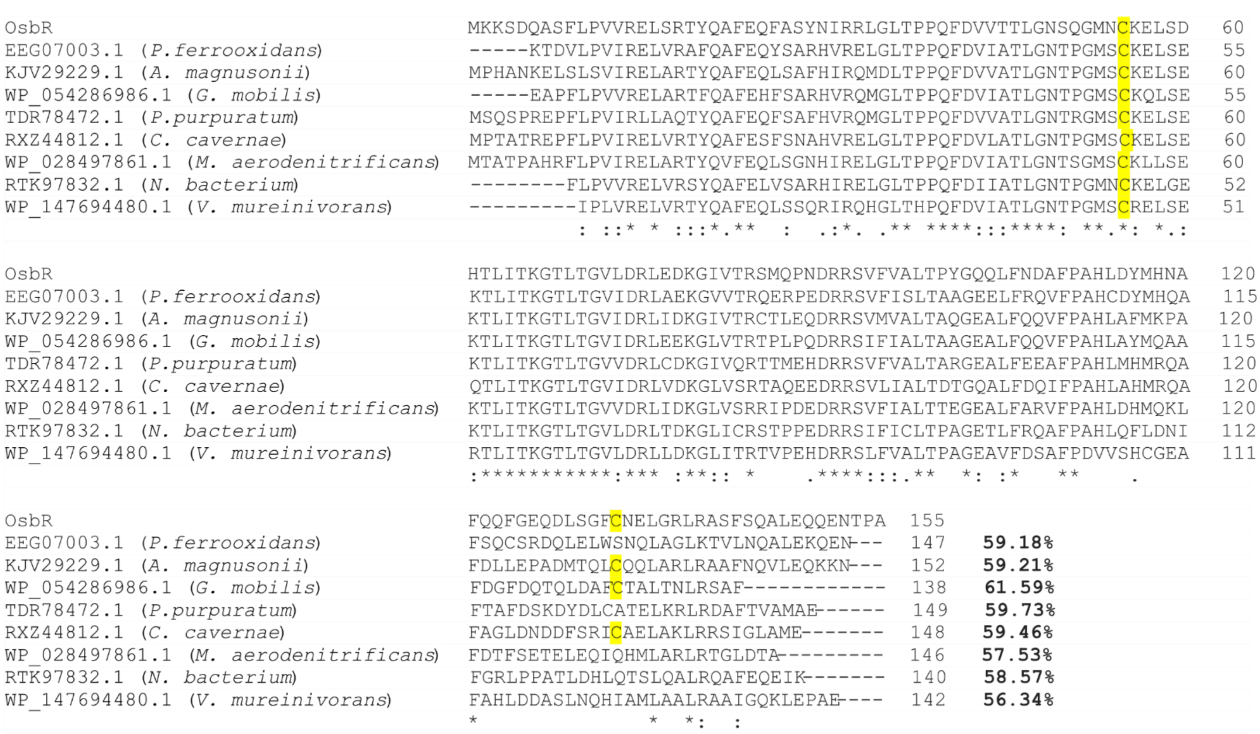
The MarR family regulator OsbR controls oxidative stress response, anaerobic nitrate respiration, and biofilm formation in *Chromobacterium violaceum*

Additional File 1. Figures S1-S2.

A



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OsbR                                   MKKSDQASFLPVVRELSRTYQAFEQFASYNIRRLGLTPPQFDVVTTLGNSQGMNCKELSD   60
EEG07003.1 (P.ferrooxidans)            -----KTDVLPVIRELVRAFQAFEQYSARHVRELGLTPPQFDVIATLGNTPGMSCKELSE   55
KJV29229.1 (A. magnusonii)             MPHANKELSLSVIRELARTYQAFEQLSAFHIRQMDLTPPQFDVVATLGNTPGMSCKELSE   60
WP_054286986.1 (G. mobilis)            -----EAPFLPVVRELARTFQAFEHFSARHVRQMGLTPPQFDVIATLGNTPGMSCKQLSE   55
TDR78472.1 (P.purpuratum)              MSQSPREPFLPVIRLLAQTYQAFEQFSAFHVRQMGLTPPQFDVVATLGNTRGMSCKELSE   60
RXZ44812.1 (C. cavernae)               MPTATREPFLPVIRELVRTYQAFESFSNAHVRELGLTPPQFDVLATLGNTPGMSCKELSE   60
WP_028497861.1 (M. aerodenitrificans)  MTATPAHRFLPVIRELARTYQVFEQLSGNHIRELGLTPPQFDVIATLGNTSGMSCKLLSE   60
RTK97832.1 (N. bacterium)              --------FLPVVRELVRSYQAFELVSARHIRELGLTPPQFDIIATLGNTPGMNCKELGE   52
WP_147694480.1 (V. mureinivorans)      ---------IPLVRELVRTYQAFEQLSSQRIRQHGLTHPQFDVIATLGNTPGMSCRELSE   51
                                                : ::* * :::*.**  :  .:*. .** ****:::****: **.*: *.:

OsbR                                   HTLITKGTLTGVLDRLEDKGIVTRSMQPNDRRSVFVALTPYGQQLFNDAFPAHLDYMHNA   120
EEG07003.1 (P.ferrooxidans)            KTLITKGTLTGVIDRLAEKGVVTRQERPEDRRSVFISLTAAGEELFRQVFPAHCDYMHQA   115
KJV29229.1 (A. magnusonii)             KTLITKGTLTGVIDRLIDKGIVTRCTLEQDRRSVMVALTAQGEALFQQVFPAHLAFMKPA   120
WP_054286986.1 (G. mobilis)            KTLITKGTLTGVIDRLEEKGLVTRTPLPQDRRSIFIALTAAGEALFQQVFPAHLAYMQAA   115
TDR78472.1 (P.purpuratum)              KTLITKGTLTGVVDRLCDKGIVQRTTMEHDRRSVFVALTARGEALFEEAFPAHLMHMRQA   120
RXZ44812.1 (C. cavernae)               QTLITKGTLTGVIDRLVDKGLVSRTAQEEDRRSVLIALTDTGQALFDQIFPAHLAHMRQA   120
WP_028497861.1 (M. aerodenitrificans)  KTLITKGTLTGVVDRLIDKGLVSRRIPDEDRRSVFIALTTEGEALFARVFPAHLDHMQKL   120
RTK97832.1 (N. bacterium)              KTLITKGTLTGVLDRLTDKGLICRSTPPEDRRSIFICLTPAGETLFRQAFPAHLQFLDNI   112
WP_147694480.1 (V. mureinivorans)      RTLITKGTLTGVLDRLLDKGLITRTVPEHDRRSLFVALTPAGEAVFDSAFPDVVSHCGEA   111
                                       :***********:*** :**:: *    .****:::.**  *: :*   **    .

OsbR                                   FQQFGEQDLSGFCNELGRLRASFSQALEQQENTPA   155
EEG07003.1 (P.ferrooxidans)            FSQCSRDQLELWSNQLAGLKTVLNQALEKQEN---   147   59.18%
KJV29229.1 (A. magnusonii)             FDLLEPADMTQLCQQLARLRAAFNQVLEQKKN---   152   59.21%
WP_054286986.1 (G. mobilis)            FDGFDQTQLDAFCTALTNLRSAF------------   138   61.59%
TDR78472.1 (P.purpuratum)              FTAFDSKDYDLCATELKRLRDAFTVAMAE------   149   59.73%
RXZ44812.1 (C. cavernae)               FAGLDNDDFSRICAELAKLRRSIGLAME-------   148   59.46%
WP_028497861.1 (M. aerodenitrificans)  FDTFSETELEQIQHMLARLRTGLDTA---------   146   57.53%
RTK97832.1 (N. bacterium)              FGRLPPATLDHLQTSLQALRQAFEQEIK-------   140   58.57%
WP_147694480.1 (V. mureinivorans)      FAHLDDASLNQHIAMLAALRAAIGQKLEPAE----   142   56.34%
                                       *              *  *:  :
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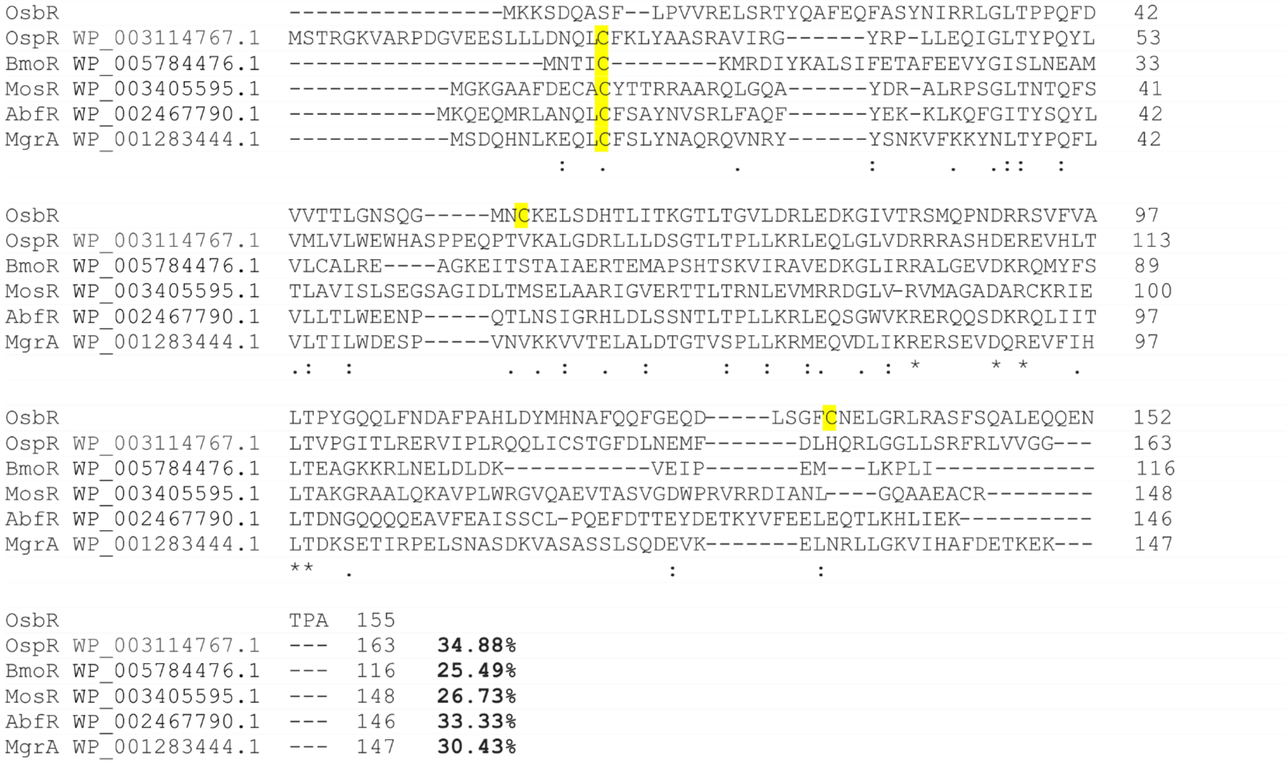
B



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OsbR                 ----------------MKKSDQASF--LPVVRELSRTYQAFEQFASYNIRRLGLTPPQFD   42
OspR WP_003114767.1  MSTRGKVARPDGVEESLLLDNQLCFKLYAASRAVIRG------YRP-LLEQIGLTYPQYL   53
BmoR WP_005784476.1  -------------------MNTIC--------KMRDIYKALSIFETAFEEVYGISLNEAM   33
MosR WP_003405595.1  ------------MGKGAAFDECACYTTRRAARQLGQA------YDR-ALRPSGLTNTQFS   41
AbfR WP_002467790.1  -----------MKQEQMRLANQLCFSAYNVSRLFAQF------YEK-KLKQFGITYSQYL   42
MgrA WP_001283444.1  ------------MSDQHNLKEQLCFSLYNAQRQVNRY------YSNKVFKKYNLTYPQFL   42
                                         :  .         .         :     .  .::  :

OsbR                 VVTTLGNSQG-----MNCKELSDHTLITKGTLTGVLDRLEDKGIVTRSMQPNDRRSVFVA   97
OspR WP_003114767.1  VMLVLWEWHASPPEQPTVKALGDRLLLDSGTLTPLLKRLEQLGLVDRRRASHDEREVHLT   113
BmoR WP_005784476.1  VLCALRE----AGKEITSTAIAERTEMAPSHTSKVIRAVEDKGLIRRALGEVDKRQMYFS   89
MosR WP_003405595.1  TLAVISLSEGSAGIDLTMSELAARIGVERTTLTRNLEVMRRDGLV-RVMAGADARCKRIE   100
AbfR WP_002467790.1  VLLTLWEENP-----QTLNSIGRHLDLSSNTLTPLLKRLEQSGWVKRERQQSDKRQLIIT   97
MgrA WP_001283444.1  VLTILWDESP-----VNVKKVVTELALDTGTVSPLLKRMEQVDLIKRERSEVDQREVFIH   97
                     .:  :           . . :  .  :     :  :  :.  . : *     * *   .

OsbR                 LTPYGQQLFNDAFPAHLDYMHNAFQQFGEQD-----LSGFCNELGRLRASFSQALEQQEN   152
OspR WP_003114767.1  LTVPGITLRERVIPLRQQLICSTGFDLNEMF-------DLHQRLGGLLSRFRLVVGG---   163
BmoR WP_005784476.1  LTEAGKKRLNELDLDK-----------VEIP-------EM---LKPLI------------   116
MosR WP_003405595.1  LTAKGRAALQKAVPLWRGVQAEVTASVGDWPRVRRDIANL----GQAAEACR--------   148
AbfR WP_002467790.1  LTDNGQQQQEAVFEAISSCL-PQEFDTTEYDETKYVFEELEQTLKHLIEK----------   146
MgrA WP_001283444.1  LTDKSETIRPELSNASDKVASASSLSQDEVK-------ELNRLLGKVIHAFDETKEK---   147
                     **  .                       :          :

OsbR                 TPA  155
OspR WP_003114767.1  ---  163   34.88%
BmoR WP_005784476.1  ---  116   25.49%
MosR WP_003405595.1  ---  148   26.73%
AbfR WP_002467790.1  ---  146   33.33%
MgrA WP_001283444.1  ---  147   30.43%
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Fig. S1 OsbR alignment. a. Clustal Omega alignment of *C. violaceum* OsbR with highly similar MarR family transcriptional regulators. Proteins of *Pseudogulbenkiania ferrooxidans* (EEG07003.1), *Aquitalea magnusonii* (KJV29229.1), *Gulbenkiania mobilis* (WP_054286986.1), *Paludibacterium purpuratum* (TDR78472.1), *Crenobacter cavernae* (RXZ44812.1), *Microvirgula aerodenitrificans* (WP_028497861.1), *Neisseriaceae bacterium* (RTK97832.1), *Vogesella mureinivorans* (WP_147694480.1) found by BLASTp analysis. High conserved Cys55 and less conserved Cys133 residues are indicated in yellow. Percentages indicate protein identity with OsbR accordingly with NCBI BLAST. **b.** Clustal Omega alignment of OsbR with *Pseudomonas aeruginosa* OspR, *Bacteroides fragilis* BmoR, *Mycobacterium tuberculosis* MosR, *Staphylococcus epidermidis* AbfR, and *Staphylococcus aureus* MgrA. Conserved cysteines are indicated in yellow, as well as both OsbR cysteine residues. Percentages indicate protein identity with OsbR accordingly with NCBI BLAST.

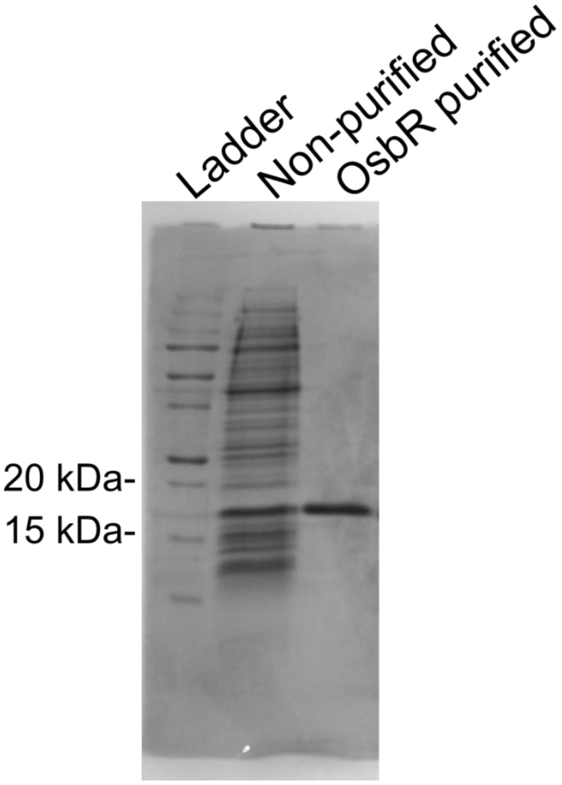

Fig. S2 His-OsbR purification by affinity chromatography. The His-OsbR was overexpressed in *E. coli* BL21(DE3) with IPTG (Non-purified). The purification of His-OsbR in a Ni-NTA column presented high efficiency and purity (OsbR purified). His-OsbR has approximately 17 kDa.