

Supplementary Data

DNA binding properties of YbaB, a putative nucleoid associated protein from *Caulobacter crescentus*.

Parul Pal¹, Malvika Modi¹, Shashank Ravichandran², Ragothaman M Yennamalli² and Richa Priyadarshini^{1*}

¹ Department of Life Sciences, School of Natural Sciences, Shiv Nadar University, Gautam Buddha Nagar, Uttar Pradesh, India

² Department of Bioinformatics, School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur, Tamil Nadu, India

ORCID:

Richa Priyadarshini: 0000-0002-8613-9438

Ragothaman M. Yennamalli: 0000-0002-3327-1582

* Corresponding author

Correspondence and reprints:

Dr. Richa Priyadarshini

Department of Life Sciences, School of Natural Sciences, Shiv Nadar University, Gautam Buddha Nagar, Uttar Pradesh, India.

Ph no: +91-120-3819100 Ext. 220

Email: richa.priyadarshini@snu.edu.in (RP)

Plasmid Construction

pPAL1: *ybab_{cc}* was amplified using primers Fwd: 5'-CCCCCGGTACCAGGAGGATGAAAGACCTCGGCGGCC-3' and Rev: 5'-CCCCCCTGCAGTTAGAACTTCATCCCGGGCAG-3'. The resulting fragment was ligated with vector pJS14 equally treated with KpnI and PstI.

pPAL2: *ybab_{cc}* was amplified using primers Fwd: 5'-CCCCCGGTACCATGAAAGACCTCGGCGGCC-3' and Rev: 5'-CCCCCGCTAGCTTAGAACTTCATCCCGGGCAG-3'. The resulting fragment was ligated with vector pXCFPN-5, equally treated with KpnI and NheI.

pPAL3: *ybab_{cc}* gene was amplified using primers Fwd: 5'-CCCCCGGATCCATGAAAGACCTCGGCGGCC-3' and Rev: 5'-CCCCCGAGCTCTTAGAACTTCATCCCGGGCAG-3'. The resulting fragment was ligated into vector pET28b, equally treated with SacI and BamHI.

pPAL4: *ybab_{cc}* was amplified using primers Fwd: 5'-CCCCCATATGATGAAAGACCTCGGCGGCC-3' and Rev: 5'-CCCCCGGTACCTTAGAACTTCATCCCGGGCAG-3'. The resulting fragment was ligated with vector pJS14, equally treated with NdeI and KpnI.

pPAL5: truncated *ybab_{cc}* gene was amplified using primers Fwd: 5'-CCCCCGGATCCATGAAAGACCTCGGCGGCC-3' and Rev: 5'-CCCCCGAGCTCATGACCTCGCCCTCGCCGGG-3'. The resulting fragment was ligated into vector pET28b, equally treated with SacI and BamHI.

Strain Construction

RP40: *E. coli* wild type strain MG1655 was transformed with pPAL1 recombinant vector.

RP41: BL21 (DE3) pLysS was transformed with pPAL3 recombinant vector.

RP42: S17-1 carrying pPAL2 was mated with CB15N

RP43: S17-1 carrying pPAL4 was mated with CB15N

RP44: BL21 (DE3) pLysS was transformed with pPAL5 recombinant vector.

RP45: *C. crescentus ftsI(ts)* was transduced with RP42 lysate

RP46: *E. coli* wild type strain MG1655 was transformed with empty pBAD18 vector.

Figure legends

Figure S1. YbaB_{Cc} localization in wildtype CB15N. RP42 cells (CB15N *xylX*:: pPAL2) carrying CFP-fused YbaB_{Cc} under the control of *P_{xyl}* promoter were cultured at 30°C in PYE medium till 0.1 OD and then induced with 0.3% xylose. Samples were taken at multiple time points (4h,6h,8h,10h,12h) and imaged on agarose-padded glass slides. The figure shows images from 4h sample. At all time-points, YbaB_{Cc} was found to be dispersed throughout the cell length. The scale bar represents 5µM.

Figure S2. The Electrostatic surface of YbaB_{Cc} protein (shown here in surface representation) has a patch of positively charged surface (colored blue) that is most likely to interact with DNA. The sequence range is from Arg51 to Ile71. Image made using APBS and PyMOL.

Figure S3: Confirmation of purified YbaB_{Cc} protein. RP41 cells were used to overexpress YbaB_{Cc} protein via IPTG induction to carry out purification of the full length YbaB_{Cc} protein. (A) Coomassie stained SDS-PAGE gel showing overexpression of YbaB_{Cc} from pet28b plasmid. Lane 1 carries protein ladder. Protein band (13kDa, arrow) is present in sample lanes: Whole cell lysate (lane 2), Pellet fraction (lane 3), Supernatant fraction (lane 4), lanes 5 and 6 are flowthrough and wash buffer samples collected from protein purification process and lanes 7-9 show elution fractions carrying purified protein. (B) Western Blot showing 13kDa band corresponding to YbaB_{Cc}. (C) Dot Blot of purified YbaB_{Cc}. 5 µl each of concentrated purified YbaB_{Cc}, 1:5, 1:10 dilutions of the protein and Anti-his antibody (control) were spotted onto a nitrocellulose membrane and probed with anti-his antibody.

Figure S4: Confirmation of purified truncated YbaB_{Cc} protein. Western Blot showing 11kDa band from whole cell lysate (WCL), pellet, supernatant and elution fractions.