Supplemental Information

A genome-wide analysis of PhoP~P binding to chromosomal DNA reveals several novel features of the PhoPR-mediated phosphate limitation response in Bacillus subtilis.

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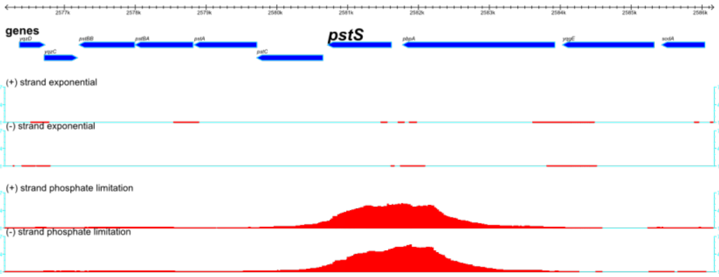
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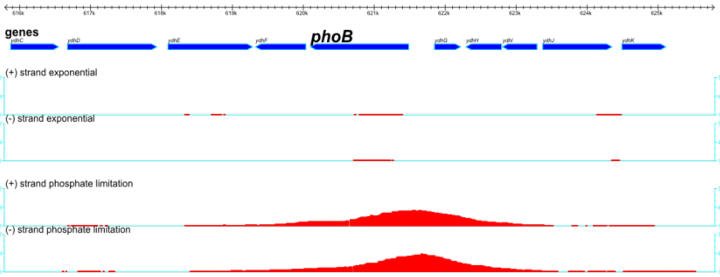
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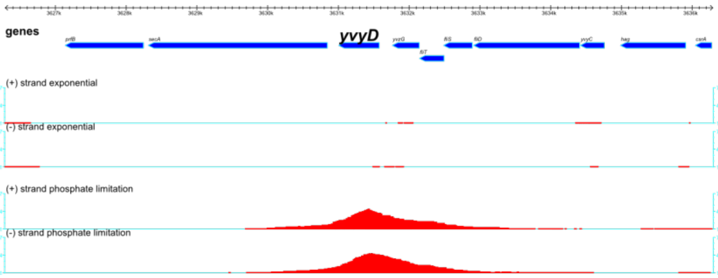
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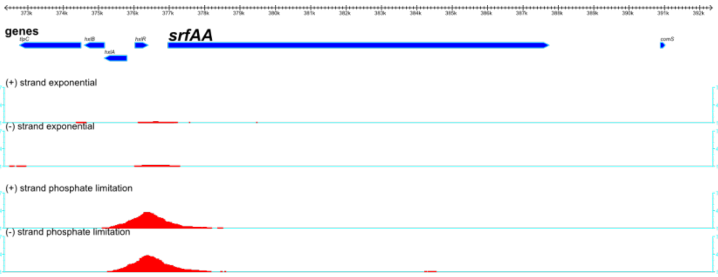
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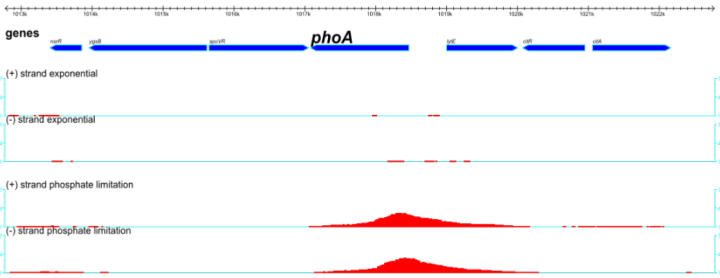
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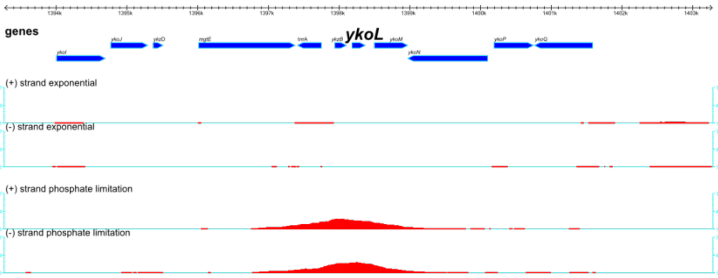
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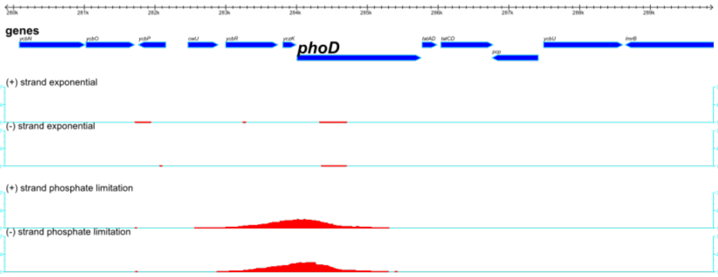
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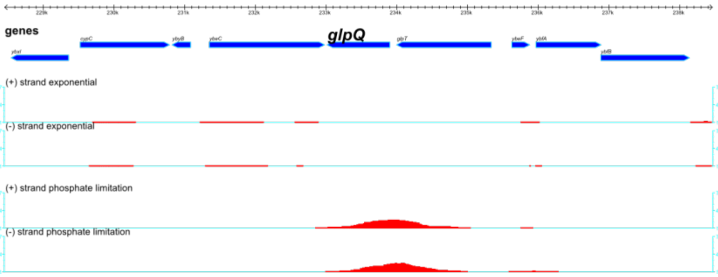
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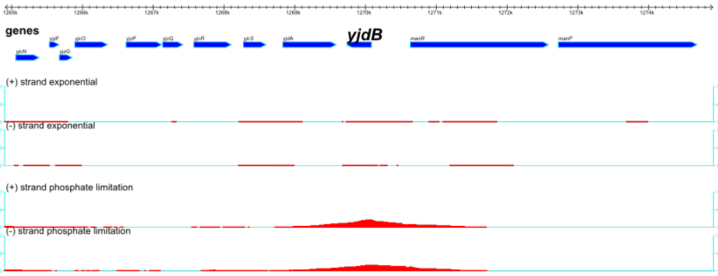
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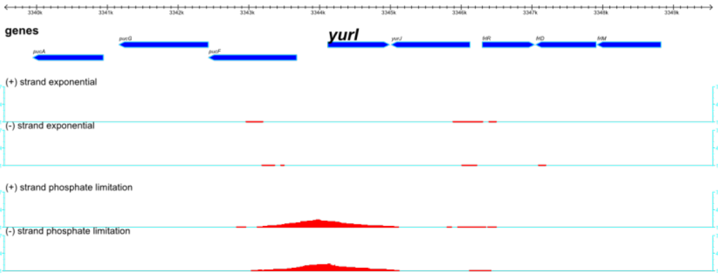
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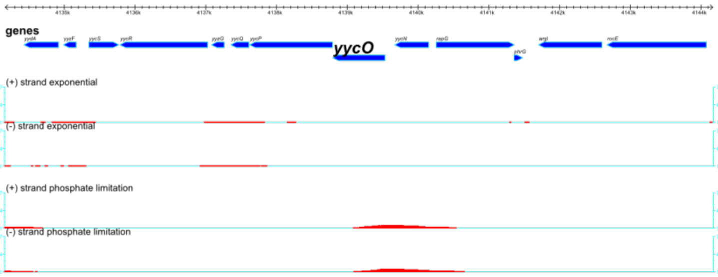
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(-) strand exponential
(+) strand phosphate limitation
(-) strand phosphate limitation

Fig S1. PhoP~P binding to the promoters of PhoPR regulon operons *in vivo*. Binding of PhoP~P to chromosomal DNA was determined by ChIP on chip analysis using a tiled array. Chromosomal loci are named in each panel. The genetic organization and direction of transcription of genes in the region are represented by blue arrows with the dimensions shown above in kilobases. The intensity (peak height) and extent (peak length) of PhoP~P binding is represented by the red peak whose position corresponds to that region of the chromosomal locus (blue arrows). The fold enrichment of PhoP~P binding is shown on the blue vertical axes located on the left and right of each panel. (+)/(-) strand exponential: shows PhoP~P binding on the plus (+) and minus (-) DNA strands at this locus in cells growing exponentially in LPDM; (+)/(-) strand phosphate limitation shows PhoP~P binding on the plus (+) and minus (-) DNA strands at this locus in phosphate limited cells.

Sequences at the newly identified chromosomal loci to which PhoP~P binds:

Previously characterized PhoP~P Promoters (examples):

>*tuaA* (positively regulated by PhoPR)
AAACAAATAGGACTTGTATATAAAATAGTAAAAGAGTA**TTAACA**ACAT**TTATCA**GAA**AAAACA**CGTAA**TTCACA**C
TTC**TTAACA**TACCA**TTTACA**TCCAA**TTAACA**TCCGTCTGCTAAACTGACTGGC**AT**

>*tagA* (negatively regulated by PhoPR)
TTCATTCAAT**TGTAAA**AT**TGGAAA**CATATTTAAATTTAA**TGTAAA**GATAA**TGTTAA**TCCTT**TGTTGA**AGATTTAT
GTTAAAATATAAGG**TA**GCTTGAT

Newly identified chromosomal loci to which PhoP~P binds in vivo:

1. *>S1052/PipA* (positively regulated by PhoPR)
TGCTGGGCGGCTGTCCGGGTAATTCCAGTAGATTGTTCTC**TTGACA**AGTGTT**TTTTCA**TTTACA**AGTTCA**CCTCT
G**GCTGCA**TCTA**AGCTCA**AGGTGTTTTCGGTCTTAAT**GTGACT**CTCTCCCTTTTT**TTCTCT**CAT**TTTACT**ATATAT
CG**GATTCA**TATAATC**TTTACA**TTTC**TTTAAA**ATTACCTCAATAAAGCA

2. > 3’ end of the *phoPR operon* * (positively regulated by PhoPR)
TAATG**TTTACA**AAGGT**TTAACA**ATA**TCTTCA**TGCACT**GTTAAA**CCTATGCTGGTAGGATGTAGTAGAACCCCTTC
ATCCAAGGAGCCAATTTTGACGGCGGGAACCTATTTGTGTTCCCG*TCCTTTTTTGTGTCTT*CTC

3. *>yqgSTUV*
GCGTTATCGGAGGCGCTTGGATCGCTAAAAA**TGAATG**GC**TGAAAC**ATCAAAAT**TGTTAA**AATTG**TGTAAA**TGAAA
T**TGATTT**TTTGT**TGTGCT**CAGGTTAAGATTTAATTTGAT**GTGTTAA**TGAGAA**TGTTGG**GAATAGAC**TG**
ATTTTTT**TGAGCG**TGC

4. *>ygzD*
CAGTACCGCTCTGACTTTTTTTATATTACATGTTTATTTATTTTT**CGTCAA**GGGAA**TATAAA**AATTT**TGTCGA**AA
AAA**AGTTAT**CTTTT**TGTAAA**GTTGTTA**AGTTCC**ATTTACATAA**TGTTAA**GTATAC**TATACA**ATATAA**T**C
AATCGA

5. *>wapA*
AAAAA**TGTAAA**AAGGCCTATGCGGCCTTTTTT**TGTTTT**AGGTCAAT**TGACTC**TCGCTAATCCTTAAAATAAGATA
AATTTTCTAGAAAAATATTGTAA**TGATAT**TTCAG**TCTAGT**TAAGATTAT**TG**A**GTA**AATAT

6. *>comQ*
AAAGCATTGATCAACTCGATAAATACAATTATGCAA**TGAAAA**TTTCG**TGAAAA**AGACT**TGGAAA**CAAG**TCTTTT**T
TTT**CGTTCT**ACCGATACAATAAA**TGGATA**AAG**TATTAT**ATGAT**TGTTAA**AAAACGAAAAACCTGC**TGT**
CCTTTAAA**TGTCCC**ATTT**AGTAAA**ATGGA

7. *>dacA*
ATAAAT**TGTTAC**AAATTAAAAACATT**TGACAG**GGTCTC**TGACTC**TGTCTATTTTTTTATACTGATTA**TGAT**
AAAATTTATA**C**TGTTG**TGCATT**GAAAA**TGTCCT**TAACGGCTTAATTATAGA**TGAAGA**AAA**TGAAAT**ACGGAG

8. >*codV*

GGTAT**TGATTG**CAACC**TGAGCG**CGATT**TGTGAT**ACCATTTAAAA**G**CCCTTCCTGGGGAGGTATTAGGC**ATG**GAGAA**TGTTAA**GAATTT**CGTAAA**GTTA

9. >*rapK*

AACCCCATACCCTTCTCT**TGATTG**AGGAG**GGTATT**TTTTT**CGTTTT**TTCAAA**TGAAAA**AAT**TGCAGA**AAAC**TTTACA**TCCT**TTTACA**ATTTTTTCAATGACTA**TGTTAT**GAT**TGTTTT**CGAGAG

10. >*pgsB*

TAAAACTTAATATT**TGTTCA**AGAAA**TCTTCA**TCCATATT**TGTGAA**GACTT**TGTCAA**AAAAAG**AGTGAA**AACCTTAAATTTTTCAATTATATATACAATTTACAATTAGATTTCTTT**TGATAT**TTTTAT**TGCTAa**CTTCGGAT**TGTTCA**TGATAATCTATCTA**TGTAAA**CGG

11. >*ggaAB*

AATGAGTATAGATAAGATATCAGATCTGTAAAAAATACACATCAA**TGCAAC**GATAT**TGTCAA**AAAGTG**CTGATG**ATTTACATTTTTAAAGAGCCTTAACTTTTATAGGAACATA**TGCTAT**TAT**TGTTTA**TTATT**A**ATGGAA**CGTAAA**AAATA**TGTCGA**AA**TGATAA**ACAATCAGTTAAGGATTTT**TGTTTT**TTCTA**AGTTGG**AAA

12. >*yomG**

AATAAAACATCATGA**AGTTAT**CAATGT**TGATTC**CAATAAAAAAT**TGATCA**CTTCA**AGTATT**TCG**GGTTAT**GAA**TGTTAT**GACCA**AGTAAA**TGAAG**AGTATG**TGA**TTTTAA**CAAGA**GGTAAA**AACAGAATTAAAATAAATGGTGAG**TGCTAT**ATTCGATTAAAATACCGATACAAATA**TTTATA**GGAGGA**TGTATTTTGTTAA**ATA**AGTTTA**AAC

Figure S2. The sequence at the newly identified chromosomal loci to which PhoP~P binds. The sequences marked with an asterisk (*yomG* and 3'end of *phoR*) are not located at promoter regions. For illustration, the sequences at the *tuaA* and *tagA* promoters are given to show the orientation of binding sites relative to the initiation point of transcription (bases in red underlined), at promoters that are activated (*tuaA*) and repressed (*tagA*) by PhoP~P. Putative PhoP~P binding sequences in the activating (TTTACA) and repressing (TGTAAA) orientations are marked in bold underlined. Sequences in larger typeface are those designated as promoter motifs by Nicolas *et al.*, (1). Start and stop codons are shown in yellow and green respectively while the putative *phoPR* terminator is shown by inverted arrows (stem-loop) followed by a run of T bases (italicized). It is evident that only the motifs at the S1052/*pipA* locus and the 3'end of *phoPR* are in the orientation associated with promoter activation with all others in the orientation associated with repression.

Reference

1. **Nicolas P, Mäder U, Dervyn E, Rochat T, Leduc A, Pigeonneau N, Bidnenko E, Marchadier E, Hoebeke M, Aymerich S, Becher D, Bisicchia P, Botella E, Delumeau O, Doherty G, Denham EL, Fogg MJ, Fromion V, Goelzer A, Hansen A, Härtig E, Harwood CR, Homuth G, Jarmer H, Jules M, Klipp E, Le Chat L, Lecointe F, Lewis P, Liebermeister W, March A, Mars RA, Nannapaneni P, Noone D, Pohl S, Rinn B, Rügheimer F, Sappa PK, Samson F, Schaffer M, Schwikowski B, Steil L, Stülke J, Wiegert T, Devine KM, Wilkinson AJ, van Dijl JM, Hecker M, Völker U, Bessières P, Noirot P.** 2012. Condition-dependent transcriptome reveals high-level regulatory architecture in *Bacillus subtilis*. Science **335**:1103-1106.

Supplementry Table 1. Oligonucleotides used in this study

Primer #	Primer name	Sequence
	16SBsu5'qPCR	5’-GTCAGCTCGTGTCGTGAGATG-3’
	16SBsu3'qPCR	5’-CAACTGAATGCTGGCAACTAAGATC-3’
	phoA chip F	5’-CCAATCGCCGCTGTTTCTG-3’
	phoA chip R	5’-TGGCCTTTTCTGTTTGCTGAAG-3’
	phoP qPCR F	5’-GGGAAGTAAATGCGAGAGTCAAAG-3’
	phoP qPCR R	5’-TTACGATATGGCCTTCCATTTC-3’
	phoR qPCR F	5’-GCGGATGAGAAAGGCATCTC-3’
	phoR qPCR R	5’-GCCTGTACGGGTCACCAGATAC-3’
	PHOP5PET	5’-GGAATTCCATATGAACAAGAAAATTTTAGTTG-3’
	PHOP3PET	5’- CGGCTCGAGTTCATTCATTTTTGGCTCC-3’
	tagA qPCR F	5’-GGTGTAGTCATGATGTCACGTTTG-3’
	tagA qPCR R	5’-TGTTAGCAACGCCCAGAAGTTC-3’
p40	PhoRF(NcoI)	5’-ATGCCATGGCCAACAGCCTTGCCATTGATCTG-3’
p41	PhoRR(XhoI)	5’-CCGCTCGAGGGCGGACTTTTCAGCGG-3’
oLS148	polA EMSA fwd	5’- TCTTTACCGTGACGCTGAAACGG-3’
oLS149	polA EMSA rev	5’- GACTGTTTCCGTCTACAAGCAC-3’
oLS202	3' phoR up fwd IF (B)	5’-CGCGGATCCCATTAACCTACACGCCTGAGGG-3’
oLS203	3' phoR up rev IF	5’-CATTAGGCGGACTTTTCAGCG-3’
oLS204	3' phoR do fwd IF	5’-CGCTGAAAAGTCCGCCTAATGACGGCGGGAACCTATTTGTGTTCCC-3’
oLS205	3' phoR do rev IF (H)	5’-CCCAAGCTTCGTCGGTTTTTCATCCTCAAGC-3’
oLS230	phoD qPCR fwd	5’- GCAGCATACCAGGCATACTATGAAC-3’
oLS231	phoD qPCR rev	5’- TGCATATCTGGGCCATTCG-3’
oLS232	glpQ qPCR fwd	5’- AAGCCTGGTAAAAGTCCATCAATTAC-3’
oLS233	glpQ qPCR rev	5’- CATCTGTCATTGACGCCATTTG-3’
oLS242	3' phoR up rev MB	5’ACTACATCCTACCAGCATAGGCTGCAGAGTGCACCATGGTATGAATTCACCTTATCGATC ATTAGGCGGACTTTTCAGCG-3’

oLS243	3' phoR do fwd MB	5’-CCTATGCTGGTAGGATGTAGTCTCGAGCCTTCATCCAAGGAGCC-3’
oLS365	mls dif fwd	5’- GTGAATACCTACTTCCTAGAATATATATTATGTAAACTGATCCTTTAACTCTGGCAACCCTC-3’
oLS366	mls dif rev	5’- CTTGCATATTAGTTTACATAATATATATTCTAGGAAGTGCCGACTGCGCAAAAGACATAATCG-3’
oLS664	yomG qPCR fwd	5’- GTCTATTGATAGCAGCGGACGTATAC-3’
oLS665	yomG qPCR rev	5’- TTTTTCCGGATCTTCTGGCTTAG-3’
oLS666	pgsB qPCR fwd	5’- GATCCGGGAGCAATGAGAATTC-3’
oLS667	pgsB qPCR rev	5’- CGGCAAACCCATTAACAAAGTG-3’
oLS670	comQ qPCR fwd	5’- AAATGCAGCACTTTCCTTGTATACAG-3’
oLS671	comQ qPCR rev	5’- TGCAATCAGACTTCCACATTTTAATC-3’
oLS672	dacA qPCR fwd	5’- CGGTTCTTGCGGATCACTTG-3’
oLS673	dacA qPCR rev	5’- AGTTCGGCATGTCCATTTCATC-3’
oLS831	gfp rev (BamHI)	5’ – CGCGGATCCCTATTATTTGTATAGTTCATCCATGCC – 3’
oLS834	S1052 qPCR fwd	5’- GGGAGACAGAAATGGAAAAAACG-3’
oLS835	S1052 qPCR rev	5’- GGCGACAGCTTAATTCGATAGC-3’
oLS836	S1052 up fwd	5’-AACGGGTCATAATTGAAAAGGC-3’
oLS837	S1052 up rev (dif)	5’-TATATTCTAGGAAGTAGGTATTCACATCTTATCATTGCTTTATTGAGG-3’
oLS838	S1052 do fwd (dif)	5’-TATATTATGTAAACTAATATGCAAGGGCTGCCTTTTTGATTTAAG-3’
oLS839	S1052 do rev	5’-ACCAGATACACTAGAATTAGGC-3’
oLS845	PpipA fwd	5’-CCGCGGGCTTTCCCAGCAAATGCTTTTCATCATTTGTTCCG-3’
oLS846	PpipA rev	5’-GTTCCTCCTTCCCACCTTTATTGGCTTTATTTCATCTTATC-3’
oLS849	S1052 fwd (SphI)	5’ -ACATGCATGCCCTCAATAAAGCAATGATAAG- 3’
oLS850	S1052 rev (BamHI)	5’-CGCGGATCCCTTAAATCAAAAAGGCAGCC-3’
oLS870	gfp fwd	5’-ATGCGTAAAGGAGAAGAACTTTTCAC-3’
oLS872	pipA-GFP fwd 2 (E)	5’-CGGAATTCTTACCTCAATAAAGCAATGATAAG-3’
oLS873	pipA-GFP rev 2	5’-GTGAAAAGTTCTTCTCCTTTACGCATCACAGTTAAGTAGACCGCTTCTG-3’
oLS874	pipA fwd (H)	5’- CCCAAGCTTTTACCTCAATAAAGCAATGATAAG-3’
oLS875	pipA rev (B)	5’- CGCGGATCCCTTTCTGACGGCAGAAATCAG-3’
OE254		5 - CGCGGATCCCGCGCATGCCGCTTAATTAAACCACTTTGTTAACGCTT-3’
OE255		5’- CCGGAAGCTTTTACATTTTAACGATATCTAGAAAAT-3’