SUPPLEMENTAL MATERIAL

Binding of HapR and VqmA to the *hapR* promoter in the absence or presence of c-di-GMP

Method

To prepare pure HapR and VqmA proteins the corresponding open reading frames were amplified from C7258 using primer pairs 5'-GATCGCATATGGACGCATCAATCGAAAAACG/5'-GATCGGCTCTTCAGCACGCGTTCTTATAGATACACAGCA and 5'-GGTGGTCATATGCTTGGTATCAATA/5'-CGGGATCCTTACTGGGCGACAACGTCAAC, respectively. The corresponding PCR products were cloned and expressed using the IMPACT^{TM} (for HapR) and pMAL protein fusion and purification systems (for VqmA). Proteins were purified following the manufacturer's instructions (New England BioLabs, Inc.). Binding of HapR and the MBP (maltose binding protein)-VqmA fusion to the *hapR* promoter was investigated using electrophoresis mobility shift assays and the DIG Gel Shift Kit (Roche Applied Sciences). To this end, specific regions within the *hapR* promoter containing the reported HapR and VqmA binding sites were amplified using the primer pairs 5'-GTTGCACATTTTTCACCCAACAG/5'-TTCGATTGATGCGTCCATAGGG and 5'-CTTCAACTGTGTGTTTCATACCAT/5'-TTCGATTGATGCGTCCATAGGG, respectively. The PCR products were confirmed by DNA sequencing. As a result, we obtained a 157 bp *hapR* promoter fragment containing the HapR binding site (*hapR*p^{HapR}) and a 332 bp fragment containing the published VqmA binding site (*hapR*p^{VqmA}).

Results

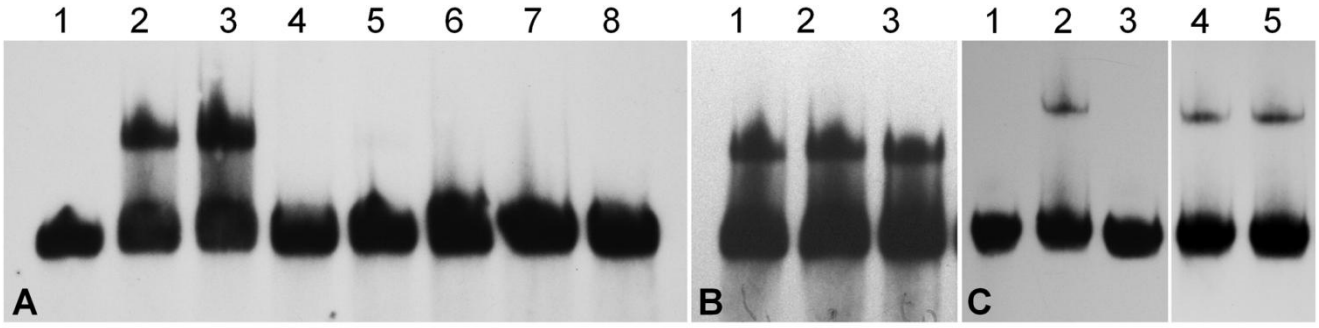

Fig.S1. A. HapR binding to wild type promoter. 10 ng of DIG-labeled *hapR*p^{HapR} DNA was reacted with 0 ng (lane 1), 5 ng (lane 2) and 15 ng (lane 3) of HapR protein as well as 15 ng of HapR in the presence of a 100-fold excess of unlabelled *hapR*p^{HapR} competitor DNA (lane 4)**.** Lanes 5-8 are as described above but using *hapR*p^{HapR} DNA containing the A→G mutation (*hapR*p*) reported to eliminate auto repression. **B. Effect of c-di-GMP on HapR binding.** 10 ng of labeled *hapR*p^{HapR} DNA was reacted with 5 ng of HapR protein in the presence of 0 (lane 1), 1 nmol (lane 2) and 10 nmol (lane 3) of c-di-GMP. **C. Binding of VqmA**. 1 ng of DIG-labeled *hapR*p^{VqmA} DNA was reacted with 0 ng (lane 1), 500 ng of MBP-VqmA (lane 2), 500 ng of MBP-VqmA plus 100-fold unlabelled competitor DNA (lane 3), 500 ng of MBP-VqmA plus 1 nmol c-di-GMP (lane 4) and 500 ng of the MBP-VqmA fusion protein digested with Factor Xa protease that cleaves off the MBP portion (lane 5).

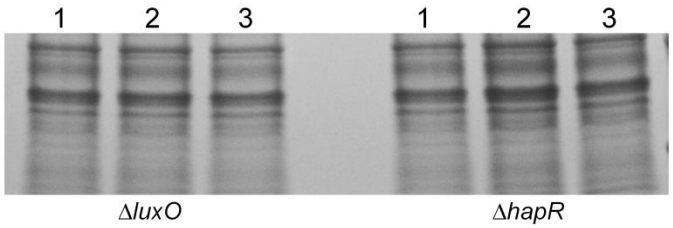

Fig. S2. Loading control for Fig. 4B. Lanes are as indicated in **Fig. 4B**. A parallel gel receiving the same samples was stained with Coomassie to confirm that similar amounts of protein were loaded in each well.