Supplementary material

Westbye *et al*

Materials and methods

Construction of a *ΔphoB* mutant. For the construction of the in-frame *ΔphoB* strain SBKOB16, the N-terminal and C-terminal regions of *phoB* (*rcc03498*) and flanking regions were amplified using the primers KOB1-F (ACCCTGTTCG**GAGCTC**GACCCGATCG) and KOB1-R (TGTTGCGCAGGATCCATCGATCAGCT), and KOB2-F (AATGGCGGCGCGGATCCCGTG) and KOB2-R (A*TCTAGA*TGCAGGAAATGGCGGGGGCG) After digestion with BamHI (underlined), the two DNA fragments were ligated, re-amplified with KOB1-F and KOB2-R, and after digestion with SacI (**bold**) and XbaI (*italic*) cloned into the suicide plasmid pZJD29A that encodes gentamicin resistance and the *sacB* counter-selection marker (Z. Jiang and C. E. Bauer, personal communication). The resultant plasmid was conjugated from *E. coli* S17-1 λ *pir* into *R. capsulatus* SB1003, and single-crossover recombinants were obtained by gentamicin selection on RCV plates. After growth in RCV broth for ~20 generations, second-crossover recombinants containing the desired mutation were obtained by plating on RCV agar medium containing 5% (wt/v) sucrose. The correct mutation was confirmed by the absence of the WT PCR-band and by DNA sequencing.

Alkaline phosphatase activity. Alkaline phosphatase activity was measured by the method of Wende et al (34). Fifty µl of culture were centrifuged, the cell pellet washed in 500 µl dH_2O and resuspended in 150 µl dH_2O. Six hundred µl of phosphatase buffer (0.1 M glycine-NaOH [pH 10], 0.1 M NaCl, 5 mM *p*-nitrophenyl phosphate) were added and the mixture heated at 37 °C

24 until a faint yellow color appeared. A volume of 275 µl of 0.5 M NaOH was added to stop the
25 reaction and the samples centrifuged at 16,000 rcf for 90 s. Absorption of the supernatant at 405
26 nm was measured and the activity calculated by the formula:
27 $U = \frac{A_{405}}{time[min] \times 1.85 \times 10^{-2} [umol^{-1} cm^{-1}] \times 1cm}$ and normalised to culture turbidity at 660 nm. Sterile
28 growth medium treated as above was used as blank.

29 **Results**

30 **A phosphate starvation response is not required for RcGTA release**

31 The *R. capsulatus* genome sequence encodes one PhoB homologue (*rcc03498*; 48% identical in
32 amino acid sequence to the *E. coli* MG1655 PhoB). A *ΔphoB* mutant did not induce alkaline
33 phosphatase (Fig. S1A), indicating that the *R. capsulatus* PhoB homologue regulates alkaline
34 phosphatase and presumable other Pho regulon genes. However little or no difference was
35 observed in RcGTA transduction frequency or RcGTA capsid production (Figs. S1B and S1C).
36 Similarly, RcGTA release did not require a near-complete depletion of phosphate from the
37 culture medium, or an operon encoding homologues of the *E. coli* PstSCAB phosphate
38 transporter (data not shown).

39 **Figures**

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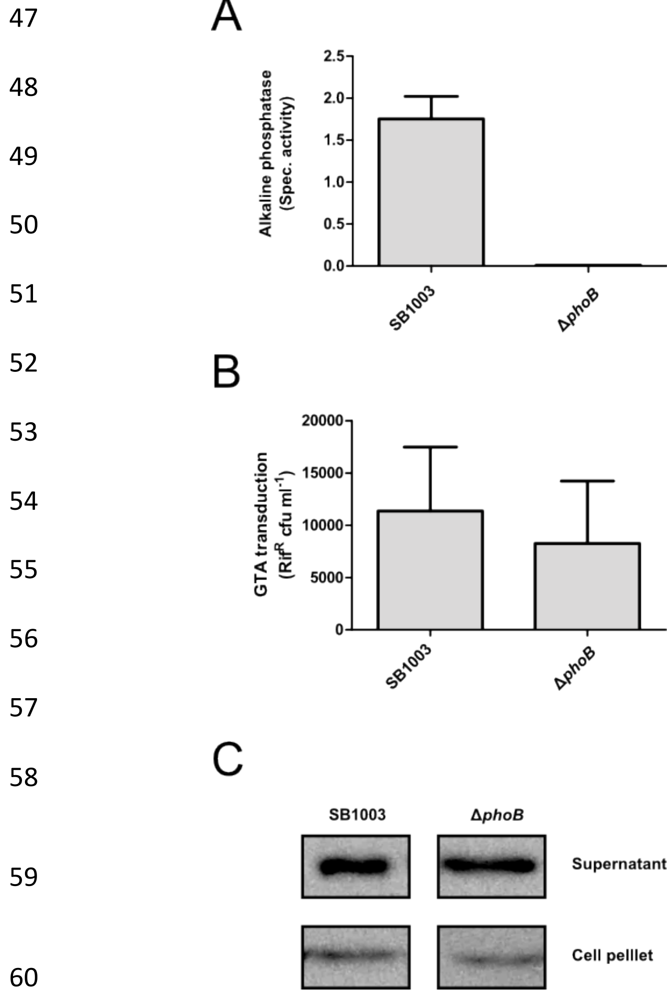

Figure S1. RcGTA production is independent of PhoB.

A: Alkaline phosphatase activity of WT strain SB1003 and SB1003 *ΔphoB* grown in RCVm medium containing 0.5 mM KPO_4. **B:** Transduction frequencies using culture supernatants from WT strain SB1003 and SB1003 *ΔphoB* grown in RCVm medium containing 0.5 mM KPO_4. **C:** Western blots of WT strain SB1003 and SB1003 *ΔphoB* culture supernatant and cell pellet fractions probed using GTA capsid protein antiserum; cultures grown in RCVm medium containing 0.5 mM KPO_4. Error bars represent standard deviation of three biological replicates. Samples were normalised to culture turbidity at 660 nm.

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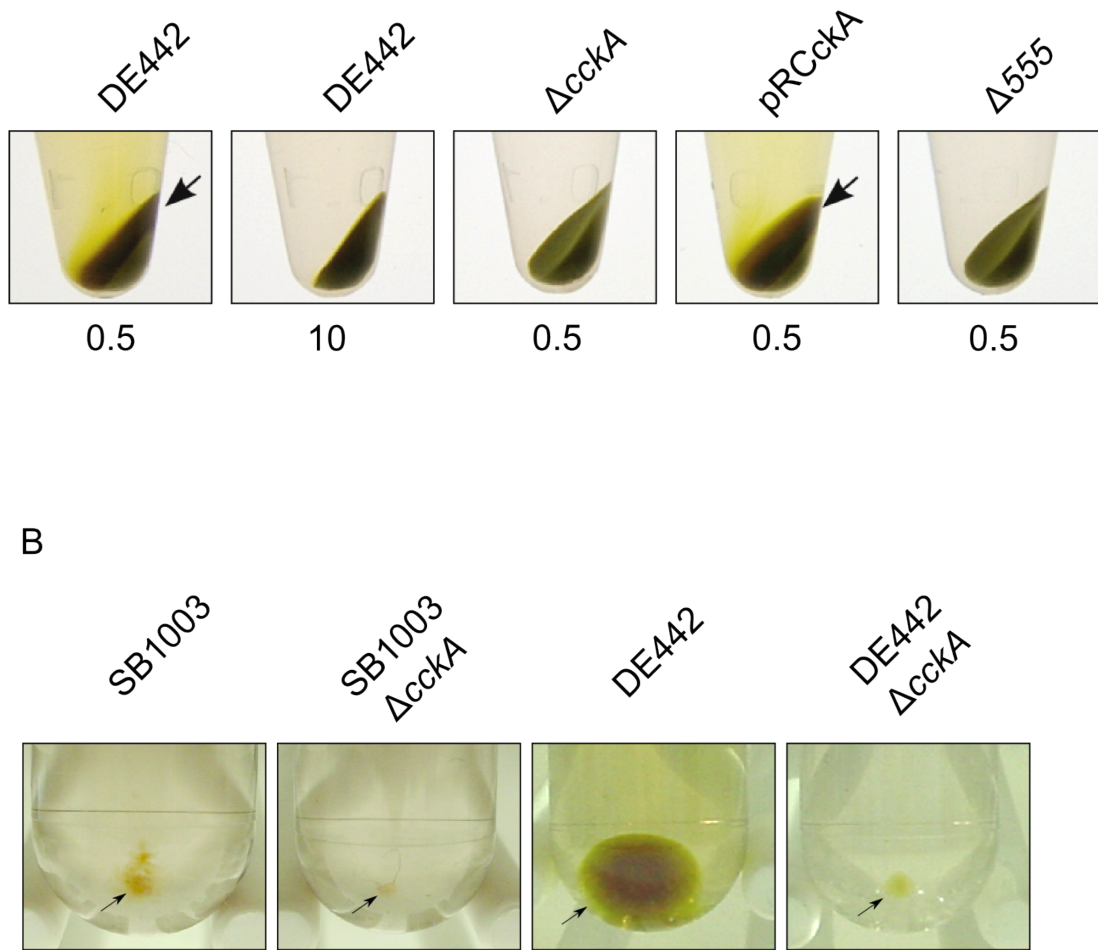

81 **Figure S2 Effects of culture medium phosphate concentration on cell lysis.**

82 **A**: RcGTA release correlates with formation of a pigmented culture supernatant and
83 semitransparent layer on top of cell pellet (arrow). Initial phosphate concentration (mM) of
84 growth medium is indicated. **B**: Ultracentrifugation of cleared culture supernatant. All samples
85 were from cultures grown in RCVm medium containing phosphate concentrations as indicated
86 (**A**), or 0.5 mM KPO_4 (**B**).

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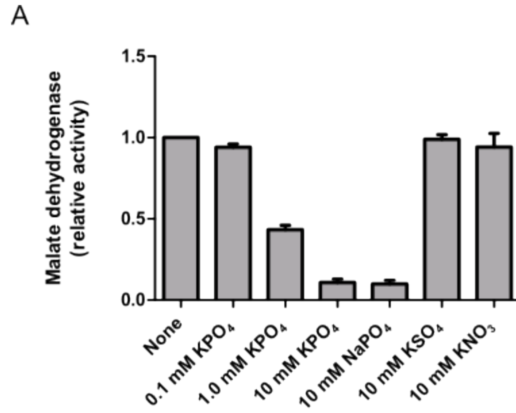

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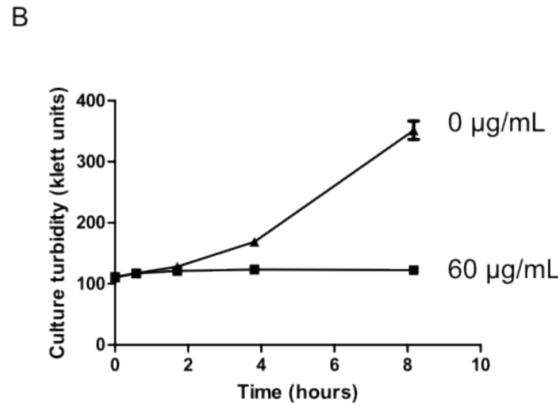

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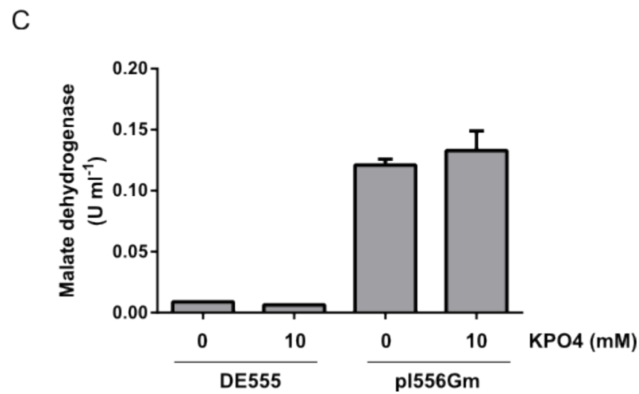

101 **Figure S3 Inhibition of cell lysis by phosphate and growth inhibition by Gentamicin.**

102 **A**: Malate dehydrogenase activity of supernatant of DE442 grown in 10 mM KPO_4 resuspended

103 in RCVm containing indicated salts. **B:** Growth curves of DE442 grown in 10 mM KPO_4

104 resuspended in RCVm containing 10 mM KPO_4 and 0 or 60 ug/mL gentamicin sulphate. **C:**

105 Malate dehydrogenase activity of supernatant of DE555 and DE555 complemented with

106 pI556Gm grown in 10 mM KPO_4 resuspended in RCVm containing 0.5 or 10 mM KPO_4.Cells

107 were resuspended to an OD_{660} of 5.0 (**A**) or 0.5 (**B**). Error bars represents standard deviation of

108 three biological replicates.

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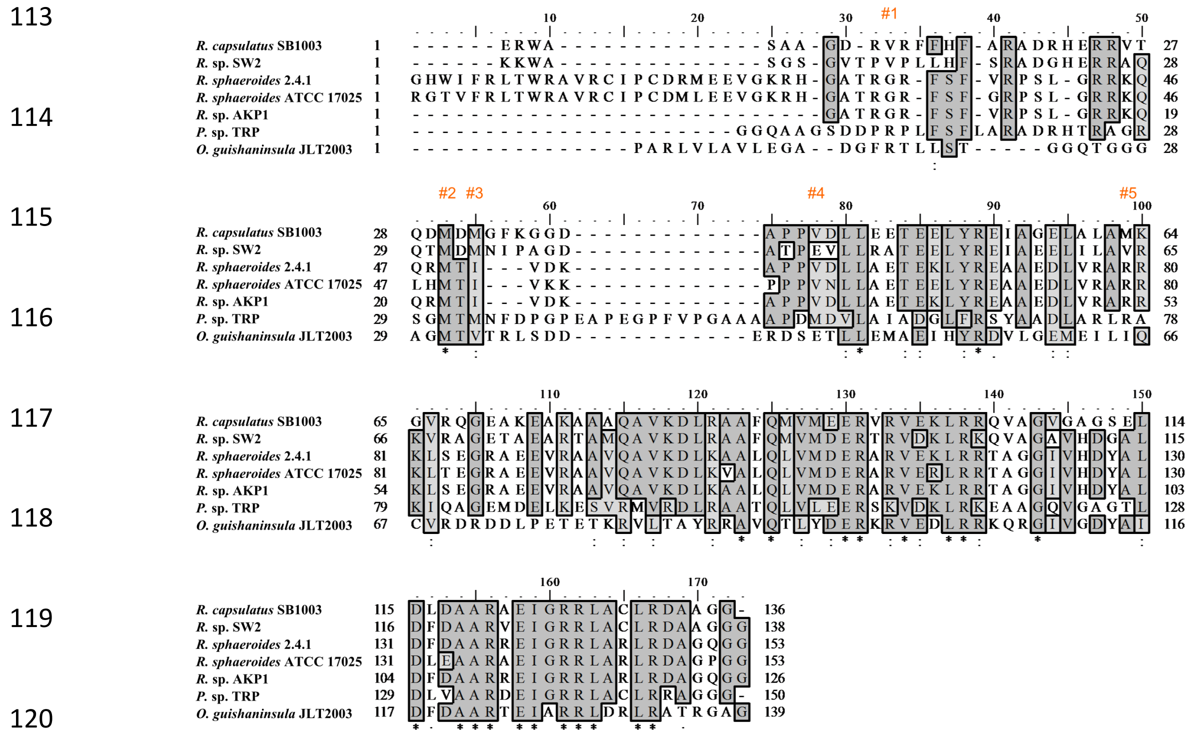

Figure S4 Conserved residues in RcGTA orf *g1*

Multiple sequence alignment of N-terminally extended protein sequences of RcGTA orf *g1* and selected top BlastP hits. Sequences included are *Rhodobacter capsulatus* SB1003 (YP_003577834.1), *Rhodobacter* sp. SW2 (WP_008032392.1 / Rsw2DRAFT_2979), *Rhodobacter sphaeroides* 2.4.1 (YP_352526.1), *Rhodobacter sphaeroides* ATCC 17025 (YP_001167279.1), *Rhodobacter* sp. AKP1 (WP_009564490.1), *Paracoccus* sp. TRP (WP_010394130.1) , *Oceaniovalibus guishaninsula* JLT2003 (WP_007427541.1 / OCGS_2389). BlastP using annotated RcGTA orf *g1* was performed to the NCBI refseq_protein database. The coding sequence of selected top BlastP hits were extended upstream from the annotated translational start codon by 30 amino acids or until stop codon was encountered. Sequences were aligned using ClustalO 1.1.0 and graphical representation created in Bioedit

132 7.0.5.3 using 70% threshold for shading. Amino acids of putative start codons from Fig. 2 are

133 indicated in orange.

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