Supplementary Information for:

***Vibrio cholerae* phosphatases required for the utilization of nucleotides and extracellular DNA as phosphate sources.**

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SUPPLEMENTARY EXPERIMENTAL PROCEDURES

RNA purification and qRT-PCR

For MOPS grown cultures, bacteria were pre-grown first in LB and then in MOPS-glucose medium supplemented with 10 mM KH_2PO_4 and then washed twice in no phosphate MOPS, as described for the growth curves. Following washing, the bacteria were resuspended in MOPS-glucose medium supplemented with the desired source of phosphate, if any. Bacteria were incubated in the test medium for two hours after which aliquots were removed and mixed 1:2 with RNAprotect. After 5 minutes of incubation at room temperature, the bacteria were spun at 16,000 x g for 10 minutes. The supernatant was aspirated and the invisible pellet stored at -20 or -80°C. Alternatively, bacteria were spun down, resuspended in 100 µl of PBS and mixed with 1 ml of RNALater and stored overnight at 4°C. It is important to note that while we saw an increase in transcription of *phoX*, *cpdB*, and *ushA* after incubation in no phosphate when RNAprotect was used, we did not see this increase when using RNALater.

RNA was prepared and qRT-PCR done using methods described previously (McDonough et al., 2014). Briefly, following isolation of the RNA using the RNeasy Mini Kit (Qiagen), any contaminating DNA in the RNA prep was digested using the TURBO DNA-free kit (Ambion, Life Technologies). cDNA was synthesized from 0.25 to 1 µg of DNA-free RNA using an iScript cDNA Synthesis Kit (Bio-Rad). DNA contamination was assessed through the use of control samples lacking reverse transcriptase. cDNA was diluted 5-fold in DEPC treated water and 5 µl was used as a template for qRT-PCR. SYBR green qRT-PCR was conducted as described previously (Pratt et al., 2009). Refer to Table 2 for the primers used in these experiments. All C_t values were corrected for primer efficiency as determined by a standard curve using genomic DNA template. Transcript levels were normalized to the housekeeping gene, *rpoB*, within each sample.

Table S1.

VC number	Gene annotation[a]	Predicted function[a]	Method of identification	Predicted localization	Homology to other nucleotidases/phosphatases[b]
VC2174	*ushA*	UDP-sugar hydrolase/ 5'nucleotidase	Transposon screen	Periplasmic[c], Predicted secretion signal sequence[d]	79% to *E. coli* UshA, 41% to *C. glutamicum* UshA, 72% to *S. oneidensis* UshA
VC2562	*cpdB*	2'3'cyclic phosphodiesterase	Homology to *V. cholerae* UshA and genome annotation search	Periplasmic[c]	25% to *V. cholerae* UshA, 81% to *E. coli* CpdB, 80% to *Yersinia* spp. CpdB
VCA0545		5'nucleotidase, putative	Homology to *V. cholerae* UshA and genome annotation search	Periplasmic[c]	23% to *V. cholerae* UshA, 62% to *S. oneidensis* UshA
VCA0608		Manganese-dependent 5'nucleotidase, putative; Haloacid dehalogenase-like hydrolase	Genome annotation search	Cytoplasmic[c], Extra-cytoplasmic[e]	

a) Heidelberg *et al.*, 2000

b) BLAST: Altschul *et al.*, 1997; Altschul *et al.*, 2005

c) PSORT: (http://www.psort.org/psortb/index.html)

d) SignalP: (http://www.cbs.dtu.dk/services/SignalP-4.1/) Nakai and Kanehisa 1991; Petersen et al., 2011

e) TMHMM: Krogh et al., 2001

Table S2.

	WT			Δ*ushA*		
	Slope[a]	r^2	95% CI[b]	Slope[a]	r^2	95% CI[b]
5'dAMP[c]	5.4	0.99	5.0 to 5.7	0.0083	0.013	-0.030 to 0.045
5'dGMP	2.5	0.95	2.1 to 2.9	-0.021	0.13	-0.059 to 0.017
5'dCMP	4.5	0.96	3.9 to 5.1	0.0024	0.0027	-0.024 to 0.029
5'TMP	4.2	0.99	4.0 to 4.5	0.0024	0.0015	-0.026 to 0.031

a) The slope is correlated with the rate of nucleotidase activity. However, since these are crude enzyme assays, it does not suggest precise enzymatic rates.

b) 95% confidence interval of the slope, as calculated by Prism 5.

c) The slope, r^2, and 95% CI for the WT were caluclated using fthe first three data points only.

Table S3.

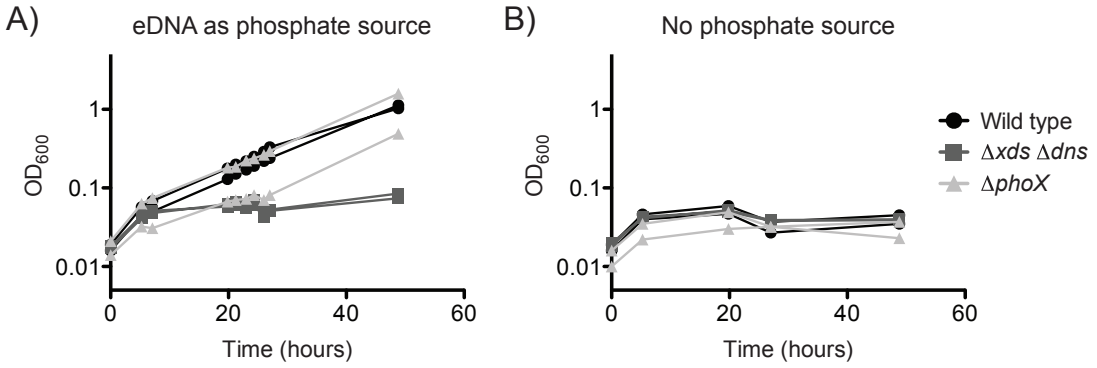
	WT			Δ*cpdB*		
	Slope[a]	r^2	95% CI[b]	Slope[a]	r^2	95% CI[b]
3'AMP	0.70	0.87	0.57 to 0.83	0.025	0.59	0.010 to 0.040
3'dGMP	0.15	0.91	0.10 to 0.19	0.035	0.81	0.018 to 0.053
3'CMP	-0.00041	0.0004	-0.022 to 0.021	-0.0088	0.061	-0.028 to 0.011
3'TMP	0.10	0.98	0.085 to 0.12	0.028	0.80	0.014 to 0.042

a) The slope is correlated with the rate of nucleotidase activity. However, since these are crude enzyme assays, it does not suggest precise enzymatic rates.

b) 95% confidence interval of the slope, as calculated by Prism 5.

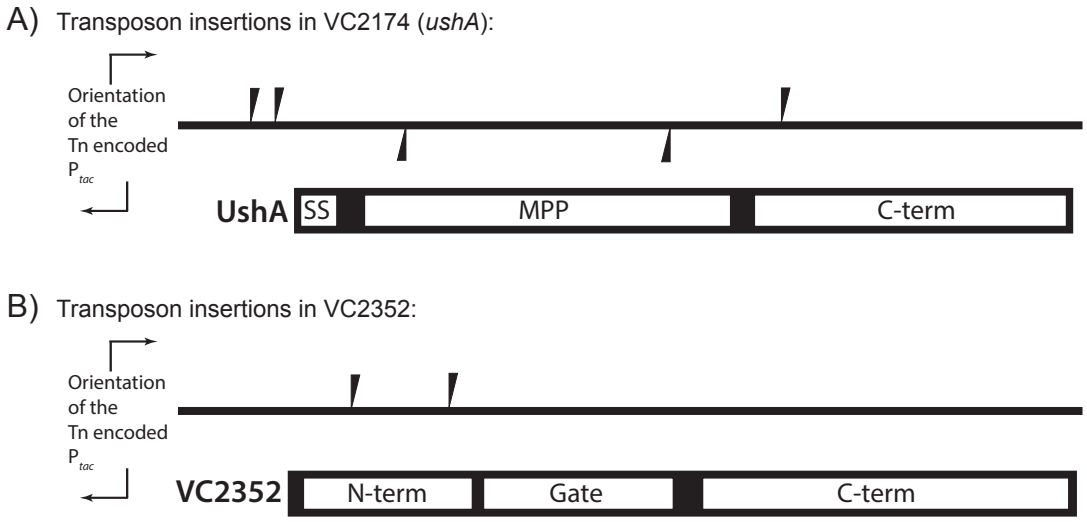
FIGURES

Supplementary Figure 1. *phoX* is not required for growth on eDNA as a source of phosphate.



After overnight growth in a MOPS-glucose medium (10 mM KH_2PO_4), strains were back diluted to an OD_{600} ~0.05 and grown to mid-exponential phase in the same medium. The bacteria were washed two times in MOPS medium containing no phosphate and inoculated into 2 ml MOPS-glucose media cultures with either A) sheared salmon sperm DNA consisting of 0.5 mM phosphate or B) no phosphate. Strains were grown at 37°C with aeration. Shown is a representative graph of the mean of two biological replicates assayed on the same day. The growth assay was performed thrice, with a total of six biological replicates; each experiment exhibited the same results.

Supplementary Figure 2. Transposon insertions in *ushA* and VC2352.



Seven total Tn insertions were identified in A) *ushA* and B) VC2352. A single line represents each open reading frame, and the Tn insertions are represented by triangles. Triangles above the line indicate that the Tn-encoded Ptac promoter is oriented in the same direction as the gene, those below the line are oriented opposite the gene. The predicted protein domains are shown beneath the gene. SS = secretion signal sequence, MPP = metallophosphatase domain, Gate = involved in determining nucleoside substrate specificity.