SUPPLEMENTAL INFORMATION

The EutQ and EutP Proteins are Novel Acetate Kinases Involved in Ethanolamine Catabolism: Physiological Implications for the Function of the Ethanolamine Metabolosome in *Salmonella enterica.*

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Running title: novel acetate kinases of *Salmonella*

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Keywords: bacterial metabolism, ethanolamine catabolism, acetate kinases, metabolosome, bacterial microcompartments, acetate activation, substrate-level phosphorylation

Supplemental figures

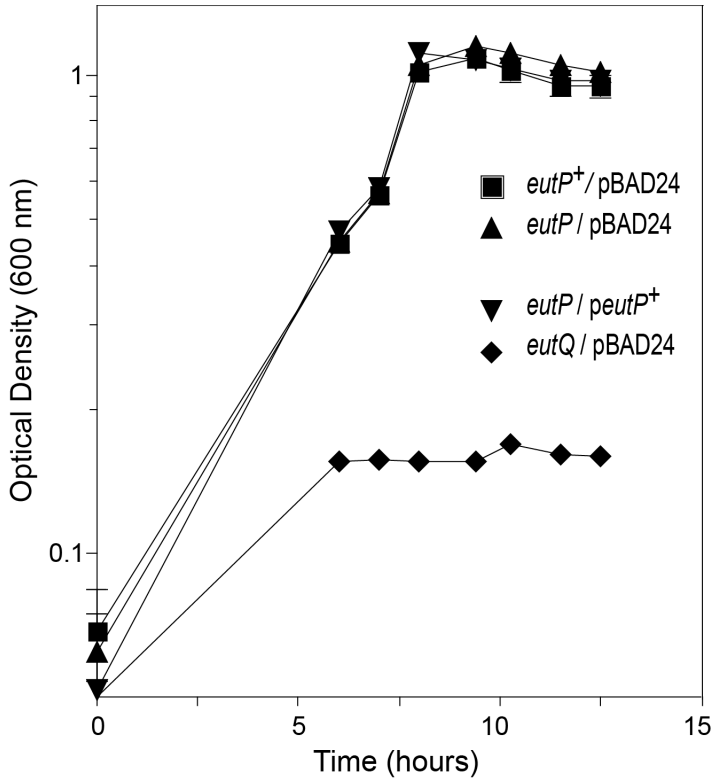

Figure S1. Comparison of the growth behavior displayed by Δ*eutP* and Δ*eutQ* strains under anoxic conditions. All strains were grown under anoxic conditions in minimal medium (1) supplemented with ethanolamine as the source of carbon and energy and sodium tetrathionate as electron acceptor. Concentrations of these supplements and additional information about the composition of the medium are described under *Experimental procedures*.

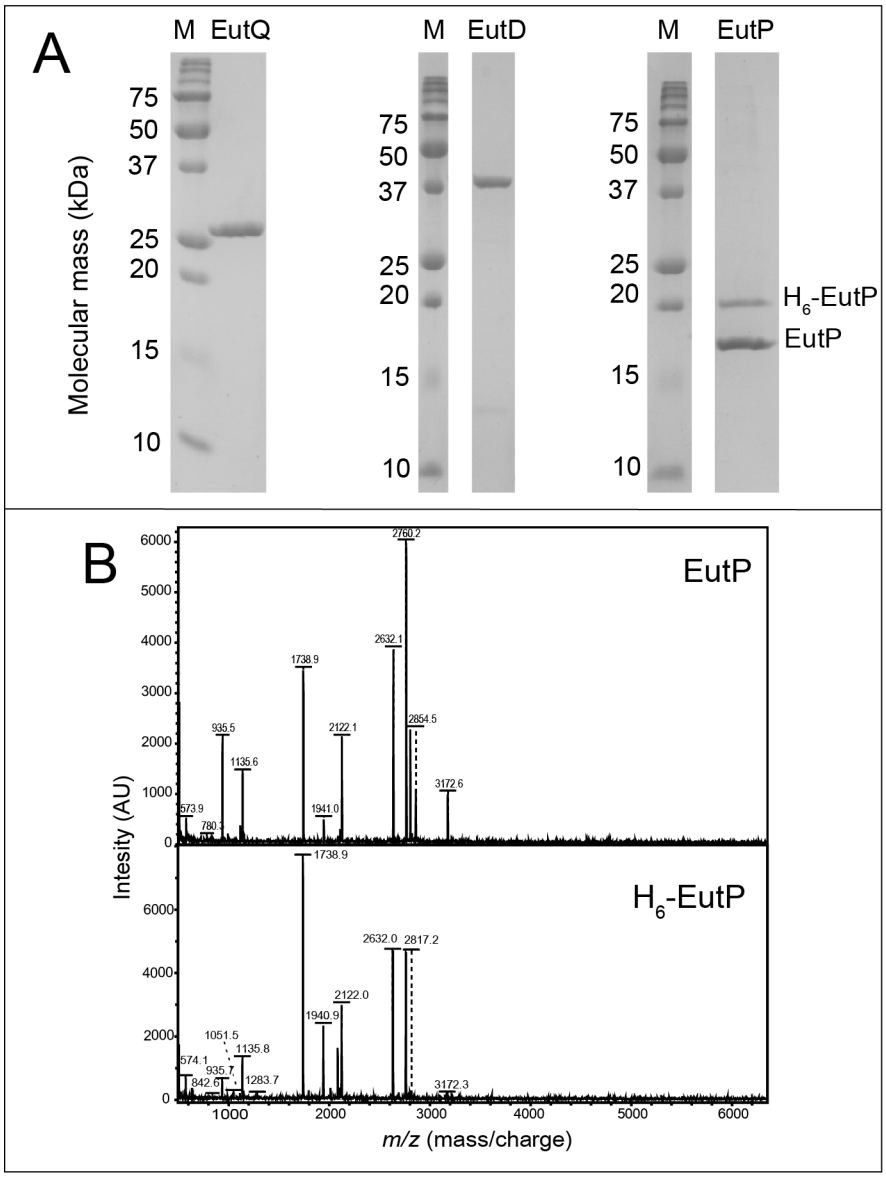

Figure S2. **SDS-PAGE and mass spectroscopy analyses of EutP and EutQ proteins used in these studies**. A. EutQ, >95% homogeneous; EutD, 92% homogeneous; tagless EutP, 76% homogeneous, H_6-EutP, 24% homogeneous. The identity of the proteins in the EutP lane was confirmed by MALDI mass spectrometry fingerprinting analysis using the MASCOT database (Matrix Sciences). B. MALDI-MS spectra were obtained at the Proteomics and Mass Spectrometry Facility of the University of Georgia. Purity was assessed by densitometry using the program ImageJ (version 1.46r, National Institutes of Health); AU, arbitrary units.

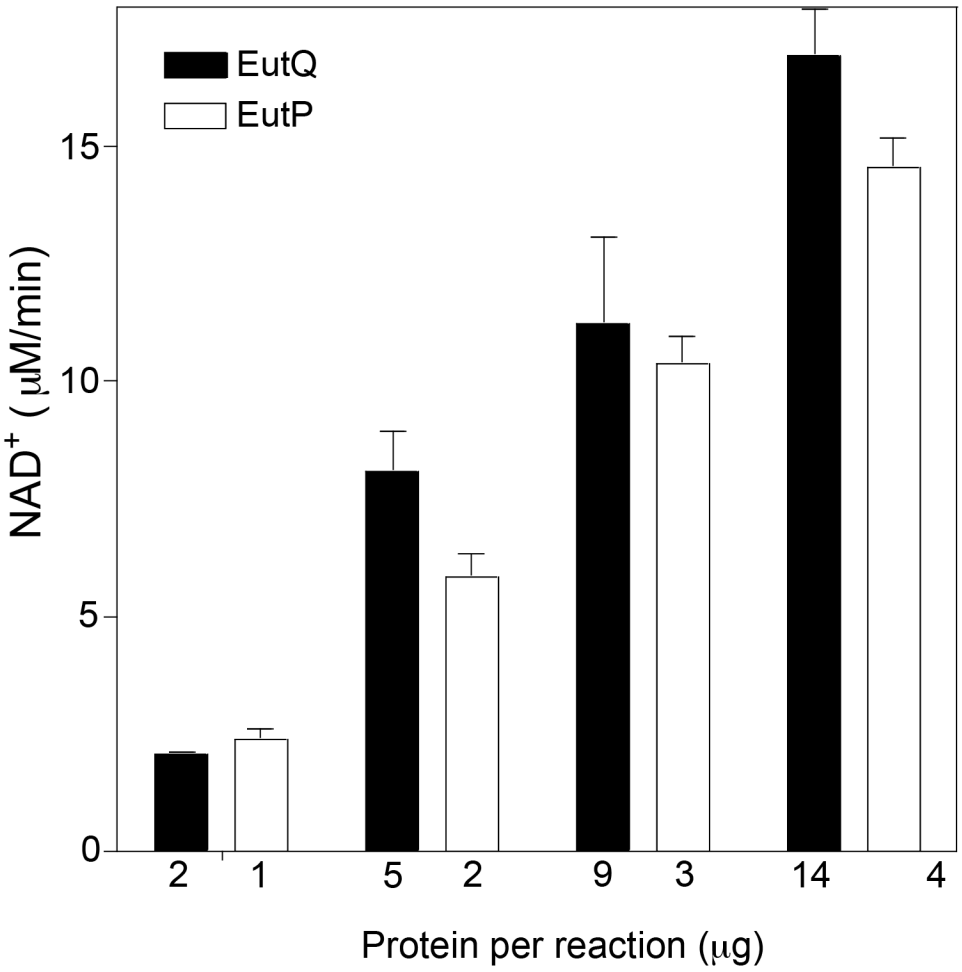

Figure S3. Enzyme-dependent acetate kinase activity associated with EutQ and EutP. Activities were determined by coupling the EutQ or EutP reaction to NAD^+-consuming reduction of pyruvate to lactate. Pyruvate was derived from PEP, which was consumed by pyruvate kinase to re-generate ATP from ADP produced by EutP or EutQ *in situ*. Pyruvate was in turn reduced by the NADH-consuming lactate dehydrogenase. NAD^+ formation was monitored by the decrease in absorbance at 340 nm. Concentration of substrates and enzymes for the coupling system are described under *Experimental Procedures.* For the EutQ and EutP reaction acetate was provided at 30 mM (42 fold over the K_m of EutP and EutQ) and ATP at 6 mM (12-fold over the K_m of EutP and EutQ).

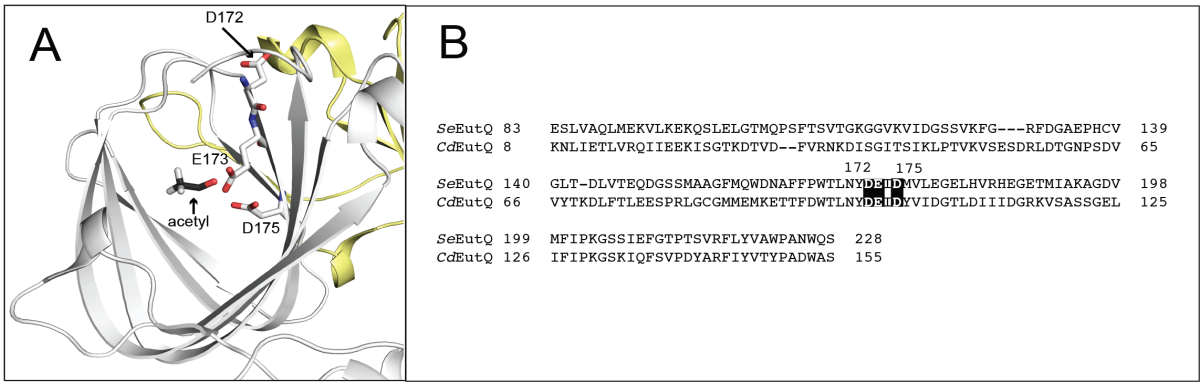

Figure S4. The putative EutQ active site. A. Modeling an acetyl moiety into the structure of the *S.* Typhimurium EutQ protein. In this panel one can visualize where acetate might bind in *Se*EutQ The acetyl moiety was modeled into the structure at the approximate location shown in the homologous *Clostridium difficile* EutQ (2) (PDB: 2PYT, 4AXO). B. BLAST alignment of *Cd*EutQ and *Se*EutQ featuring the conserved acidic residues proposed to form the EutQ active site (E value: 7e-30, identities: 36%).

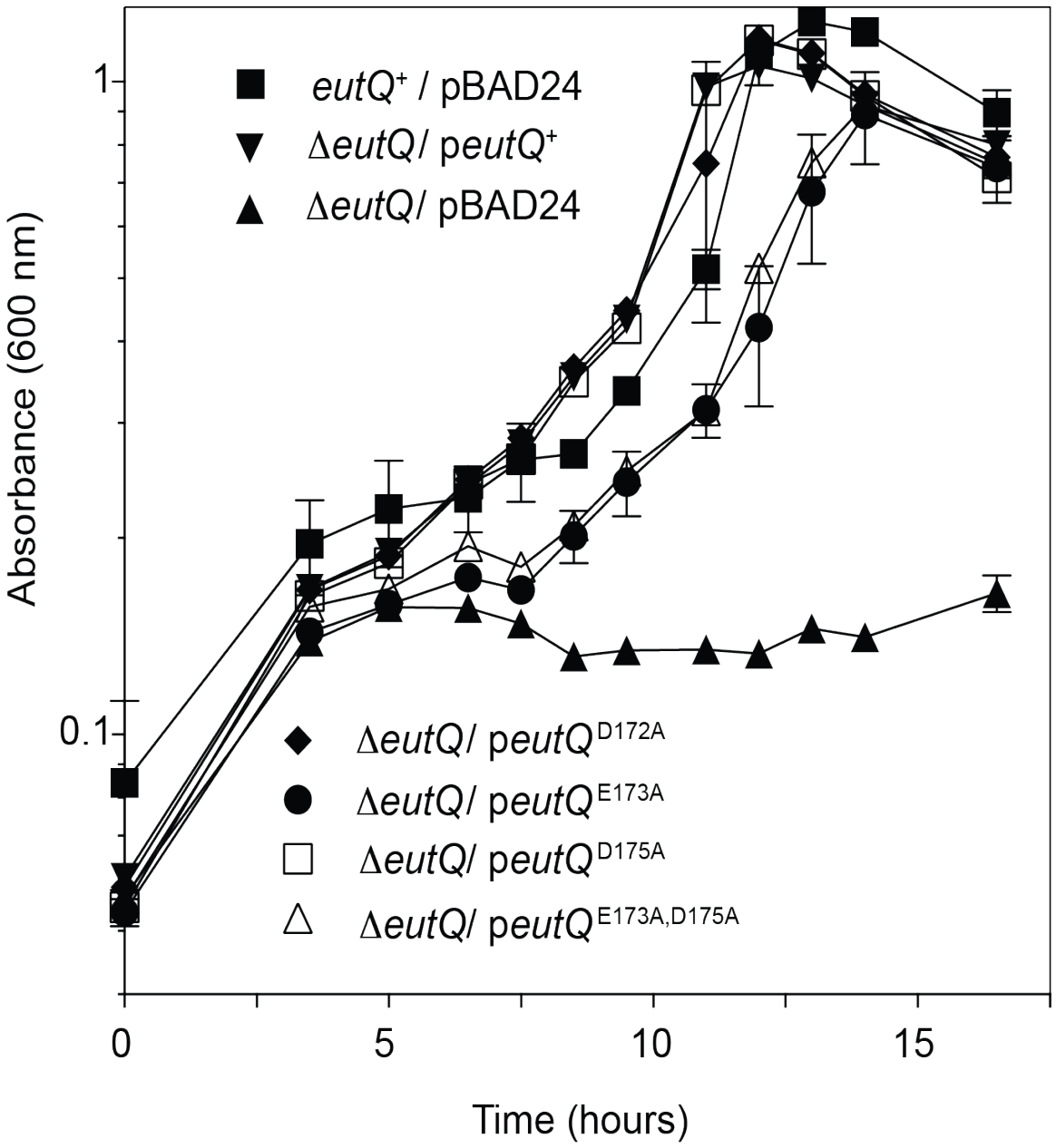

Figure S5. Complementation of Δ*eutQ* strains carrying plasmids encoding EutQ active-site variants. Generation times based on optical desities are: *eutQ*+ / *p*EutQ^{WT} (3.1 h), Δ*eutQ* / *p*EutQ^{WT} (3.4 h),), Δ*eutQ* / *p*EutQ^{D172A} (3.4 h),), Δ*eutQ* / *p*EutQ^{E173A} (3.7 h),), Δ*eutQ* / *p*EutQ^{E173A,D175A} (3.7 h),), Δ*eutQ* / *p*BAD24 (NG).

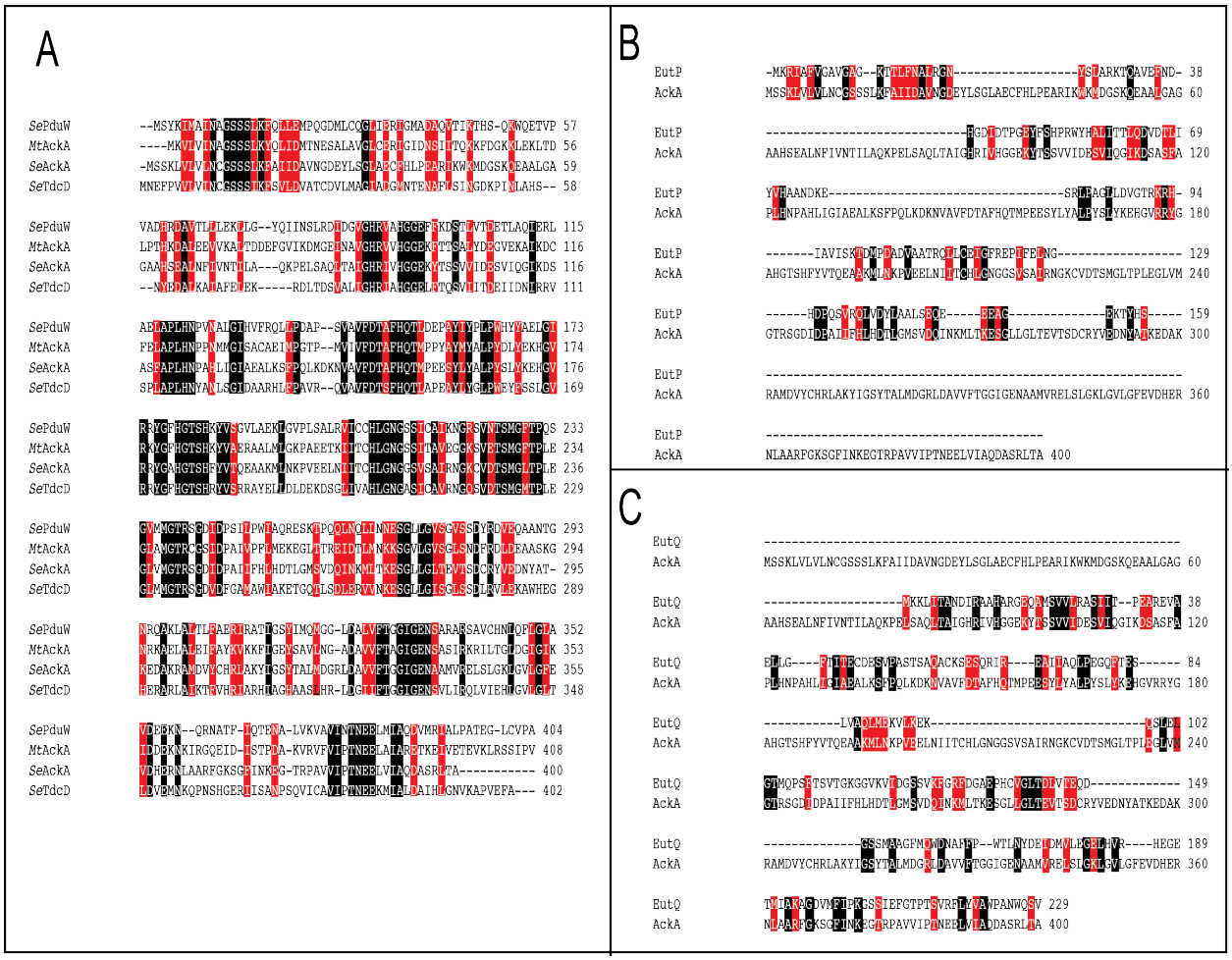

Figure S6. Multiple sequence alignments of acetate kinases and comparisons to EutP and EutQ. Conserved residues are highlighted in black. Highly similar residues are highlighted in grey. A) Aligment of the known acetate kinases used in this study (*Se* = *S.* Typhimurium) and the AckA of *Methanosarcina thermophila* (*Mt*AckA), of which there are several crystal structures binding inhibitors and substrate analogues (PDB: 1TUU, 1TUY, 1G99) *Mt*AckA is 44% identical to *Se*AckA and 42% to *Se*PduW and *Se*TdcD. *Se*AckA is 39% identical to *Se*TdcD and 46% to *Se* PduW. *Se*TdcD is 43% identical to *Se*PduW. B) Alignment of *S.* Typhimurium EutP with the housekeeping acetate kinase AckA (19.9% identity),, of the same bacterium. C) Aligment of *S.* Typhimurium EutQ with the housekeeping acetate kinase AckA (24.2% identity), of the same bacterium. Predicted *Se*AckA ADP-binding residues D285 and N337 align with EutQ E147 and N170. Predicted *Se*AckA acetate-binding residue H94 aligns with EutQ H14. All alignments were performed using ClustalW2 (EMBL-EBI,Cambridgeshire, UK).

Supplemental references

1. Berkowitz, D., Hushon, J. M., Whitfield, H. J., Jr., Roth, J., and Ames, B. N. (1968) Procedure for identifying nonsense mutations. *J. Bacteriol.* **96**, 215-220
2. Pitts, A. C., Tuck, L. R., Faulds-Pain, A., Lewis, R. J., and Marles-Wright, J. (2012) Structural insight into the *Clostridium difficile* ethanolamine utilisation microcompartment. *PLOS One* **7**, e48360