Supporting Information

Yang et al. 10.1073/pnas.0914506107

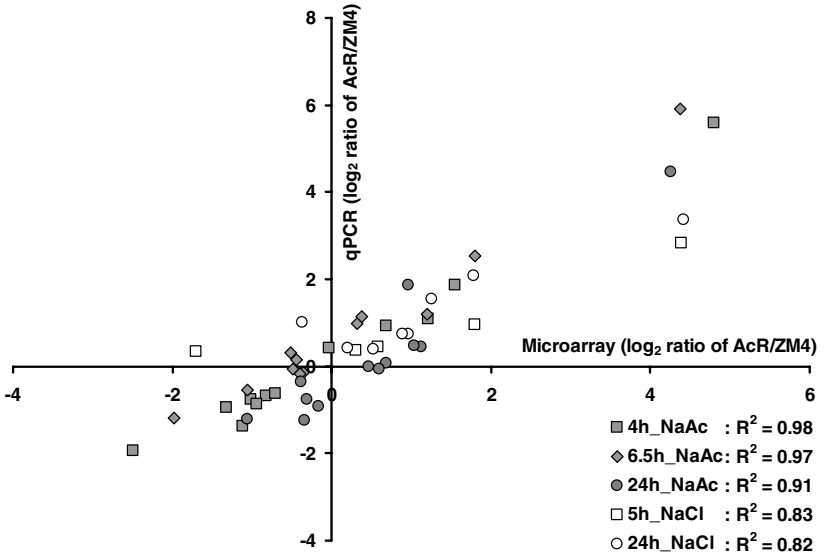

Fig. S1. Supplemental method for real-time quantitative RT-PCR (qRT-PCR) analysis. Microarray data were validated using real-time qRT-PCR as described previously (1) except that the BioRad MyiQ2 Two-Color Real-Time PCR Detection System (BioRad Laboratories) and Roche FastStart SYBR Green Master reagent (Roche Applied Science) were used for this experiment. Twelve genes representing different functional categories and a range of gene expression values based on microarray results over different time point were analyzed using qRT-PCR. Primer pairs were designed as described previously (1) and the oligonucleotide sequences are listed in Table S4.

1 Yang S, et al. (2009) Transcriptomic and metabolomic profiling of *Zymomonas mobilis* during aerobic and anaerobic fermentation. *BMC genomics* 10:34.

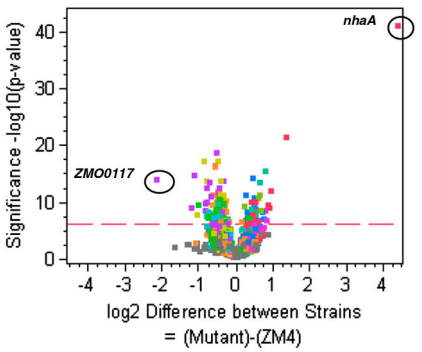

Fig. S2. Volcano plot summary of microarray analysis showing all significantly differentially expressed genes between AcR and the ZM4 wild-type strains for all conditions (sodium acetate and sodium chloride in exponential and stationary phase cells). The X axis shows the difference values (log 2) between AcR and ZM4 expression profiles. The Y axis shows statistical significance values (– log 10 (*p*-values)) for expression differences. The red dashed line shows the statistical significance cut-off used in this study.

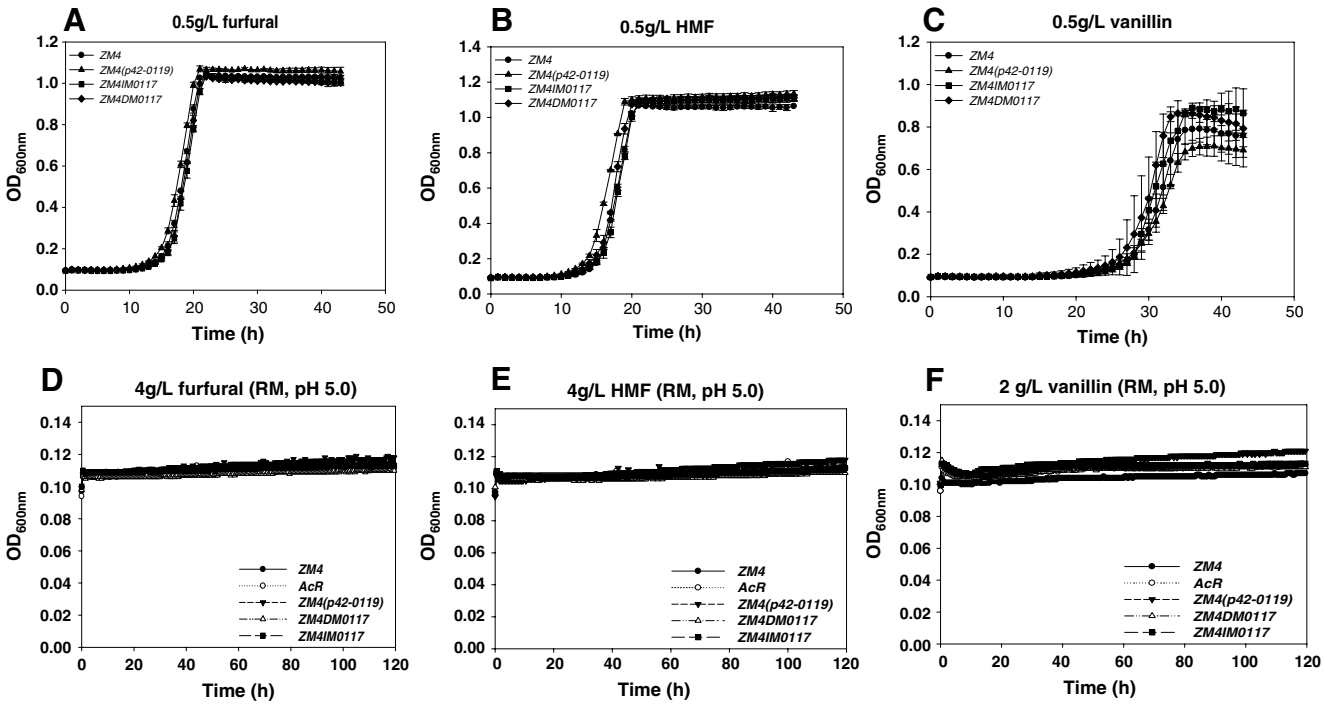

Fig. S3. ZM4 *nhaA* had no effect on inhibitor furfural, HMF, and vanillin tolerance. *Z. mobilis* strains were grown in RM (pH5.0) overnight, and then 5 μL of culture seed was transferred into 250-μL RM media in the Bioscreen plate (Growth Curves USA). The growth differences of different strains were monitored by using a Bioscreen C machine (Growth Curves USA) under anaerobic conditions in RM with 0.5 g/L furfural, pH 5.0 (*A*); RM with 0.5 g/L HMF, pH 5.0 (*B*); RM with 0.5 g/L vanillin, pH 5.0 (*C*); RM with 4 g/L furfural, pH 5.0 (*D*); RM with 4 g/L HMF, pH 5.0 (*E*); RM with 2 g/L vanillin, pH 5.0 (*F*). Strains included in this study were: ZM4: *Zymomonas mobilis* ZM4 wild-type; AcR: previously described ZM4 acetate tolerant mutant; ZM4 (p42-0119): ZM4 containing a gateway plasmid p42-0119 for *nhaA* (*ZMO0119*) expression; ZM4IM0117: ZM4 *hcp* (*ZMO0117*) insertional mutant; ZM4DM0117: deletion mutant affecting *ZMO0117* and part of upstream *nhaA* (Fig. 2 and Table S4). This experiment has been repeated at least two times with similar results. Duplicates were used for each condition.

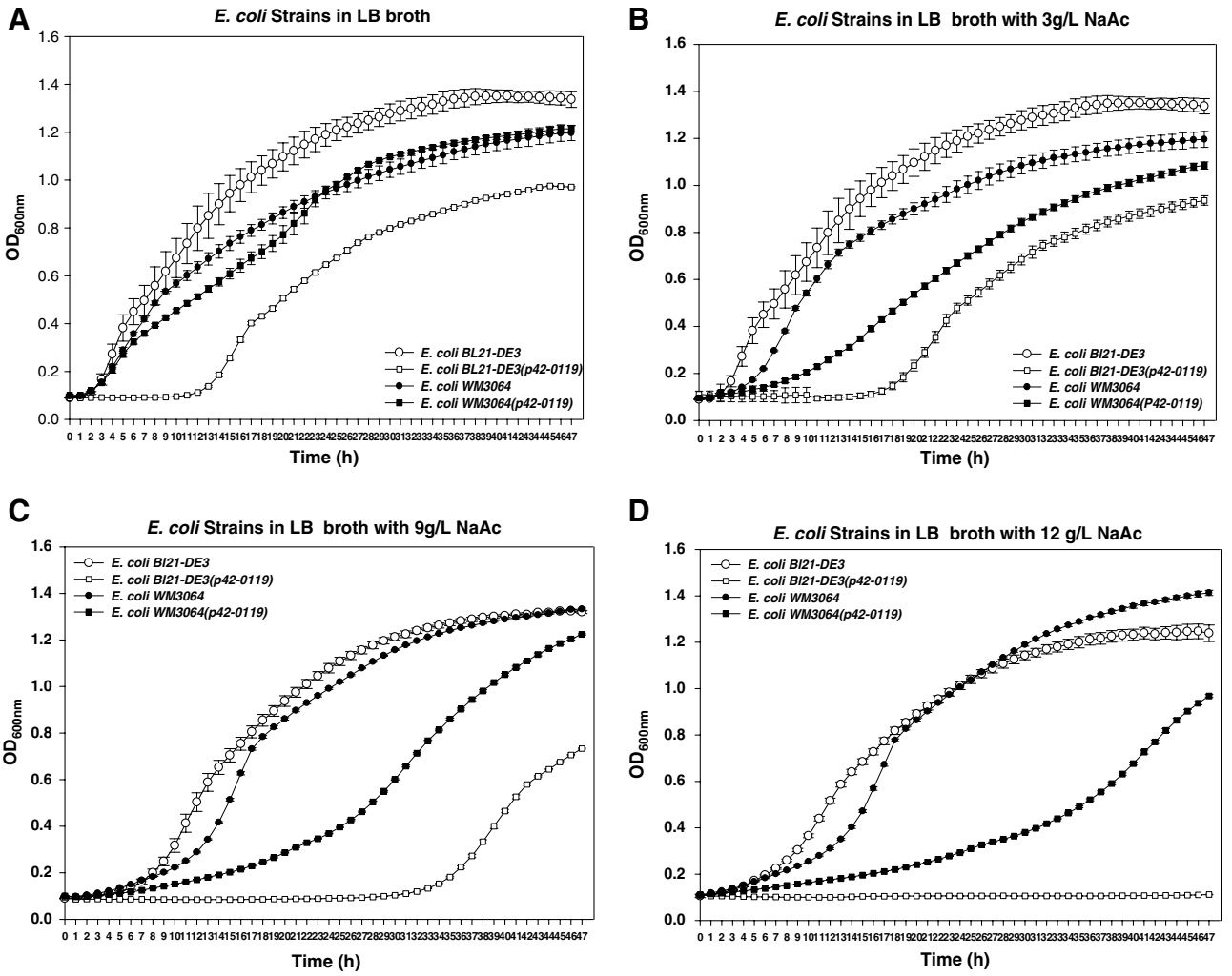

Fig. S4. Overexpression of ZM4 *nhaA* in *E. coli* had a negative effect on sodium acetate tolerance. Two *E. coli* strains (WM3064 and BL21-DE3, the latter is used for protein overexpression) were grown in LB overnight, and then 5 μL of culture seed was transferred into 250-μL LB broth in the Bioscreen plate (Growth Curves USA). The growth differences of different strains were monitored by Bioscreen C (Growth Curves USA) in LB broth, pH 7.0 (*A*); LB with 3 g/L NaAc, pH 7.0 (*B*); LB with 6 g/L NaAc, pH 7.0 (*C*), LB with 12 g/L NaAc, pH 7.0 (*D*). Strains included in this study were: *E. coli* BL21-DE3 (p42-0119): *E. coli* BL21-DE3 containing a gateway plasmid p42-0119 for *nhaA* (ZMO0119) expression; *E. coli* WM3064 (p42-0119): *E. coli* BL21-DE3 containing a gateway plasmid p42-0119 for *nhaA* (ZMO0119) expression. Duplicates were used for each condition.

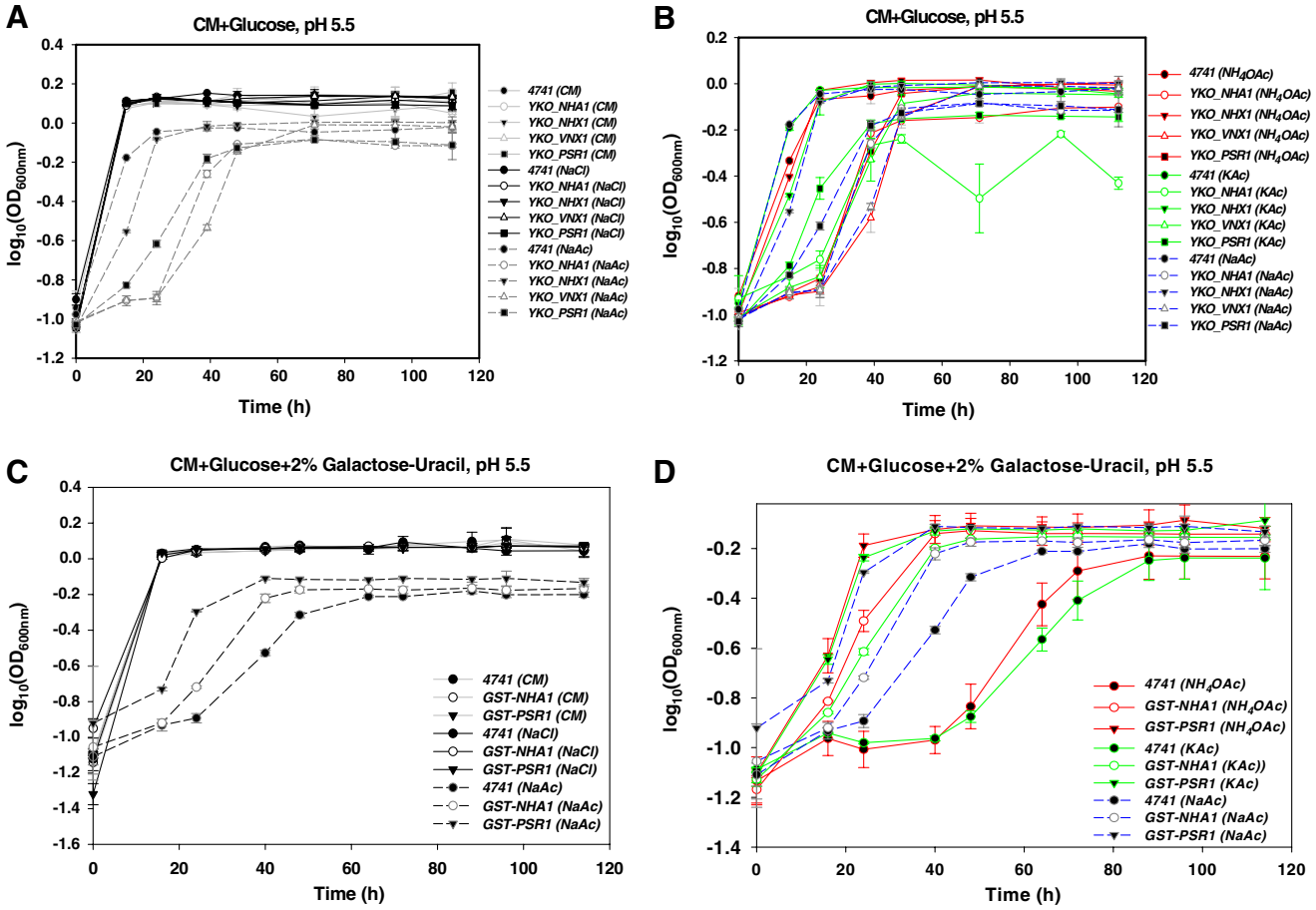

Fig. S5. *S. cerevisiae* Na/H antiporter genes play a role in sodium acetate tolerance. Growth was monitored using an aerobic Bioscreen C (Growth Curves USA) in CM + glucose medium at pH 5.5, and either CM + glucose (pH 5.5) with 244 mM NaCl, or 244 mM NaAc for wild-type BY4741 and deletion mutants strains (*A*); CM + glucose with 244 mM NaAc, or 244 mM KAc, or 244 mM NH4OAc at pH 5.5 for wild-type BY4741 and deletion mutants strains (*B*); CM + glucose + 2% galactose_uracil at pH 5.5, CM + glucose + 2% galactose_uracil with 244 mM NaCl, or 244 mM NaAc at pH 5.5 for wild-type BY4741 and GST overexpression strains (*C*); CM + glucose + 2% galactose_uracil with 244 mM NaAc, or 244 mM KAc, or 244 mM NH_4OAc at pH 5.5 for wild-type BY4741 and GST overexpression strains (*D*). This experiment has been repeated at least three times with similar result. PSR1 is responsible for plasma stress response and used as a control.

Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)
Table S3 (DOC)
Table S4 (DOC)
Table S5 (DOC)