Supporting Online Material for

Acetylation of Metabolic Enzymes Coordinates Carbon Source Utilization and Metabolic Flux

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Published 19 February 2010, *Science* **327**, 1004 (2010)
DOI: 10.1126/science.1179687

This PDF file includes:

Materials and methods

Genetic Procedures

Bacterial Strains and Media

Bacteria strains, genes and vectors used in this study are listed in table S2. *Salmonell*a *enterica* serovar typhimurium LT2 *galE* mutant G2466 (*1*) is kindly provided by Prof. Lei Wang of Nankai University, Tianjin, China. Glucose and Citrate growing media used in this study was prepared by using M9 minimal medium supplemented with Biotin (0.1 mM) and VB1 (0.05 mM) with concentrations of glucose or citrate presented as specified in individual study. Antibiotics supplementations in media as required are 100 μg/ml, 17 μg/ml and 50 μg/ml for ampicillin, chloramphenicol and kanamycin, respectively.

Molecular Cloning

Target genes used in this study were cloned from *S. enterica* G2466 genome by amplifying genomic DNA extracted by Easy-DNA kit (Invitrogen, Carlsbad, CA). Each cloned gene was confirmed by DNA sequencing.

Site-directed mutagenesis in this study was carried out by introducing desired mutations by PCR. Primers used are listed below with the introduced nucleotide mutations indicated by asterisks:

gapA K331Q

F-GATTGCTCACATCTCCC*AACTCGAGCACCACC;

R-GGTGGTGCTCGAGTTG*GGAGATGTGAGCAATC.

aceA K308Q

F-GATGCTATCCACGCGC*AATATCCGGGCAAAC;

R-GTTTGCCCGGATATTG*CGCGTGGATAGCATC.

table S2 Bacterial strains and plasmids used in this study

Strains or Plasmids	Genotype and Characteristics	References/Sources
Strains		
S. enterica		
G2466	serovar typhimurium LT2 *galE*	David Hone *et al.*
pat null mutant	G2466 *pat*::*Cm*	This study
cobB null mutant	G2466 *cobB*::*Cm*	This study
cobB/*pat* null mutant	G2466 *cobB*::*Kan, pat*::*Cm*	This study
E. coli		
DH5α	F^- *Ø*80d *lacZ* Δ*M*(*lacZYA-argF*)U169 *deoR recA1 endA1 hsdR17* (r^-_k m^-_k) *supE44*λ^- *thi* $^-$*gyr96 relA1*	Invitrogen
BL21(DE3)	F^-*ompT* r^-_B m^-_B (λDE3)	Novagen
Plasmids		
pET-22b(+)	Expression vector for *E. coli*	Novagen
pET-22-*gapA*	pET-22b(+) based *gapA* expression clone	This study
pET-22-*aceA*	pET-22b(+) based *aceA* expression clone	This study
pET-22-*aceK*	pET-22b(+) based *aceK* expression clone	This study
pET-22-*cobB*	pET-22b(+) based *cobB* expression clone	This study
pET-22-*pat*	pET-22b(+) based *pat* expression clone	This study
pET-22-*icdA*	pET-22b(+) based *icdA* expression clone	This study
pET*tac*	The P_{T7} of pET-22b(+) replaced by P_{tac} for expression in *S. enterica*	This study
pET*tac* -*gapA*	pET*tac* based *gapA* expression clone	This study
pET*tac*-*aceA*	pET*tac* based *aceA* expression clone	This study
pET*tac*-*aceK*	pET*tac* based *aceK* expression clone	This study

pET*tac-gapA* K331Q	pET*tac* based *gapA* K331Q expression clone	This study
pET*tac-aceA* K308Q	pET*tac* based *aceA* K308Q expression clone	This study
pKD46	λ RED recombination helper plasmid	Datsenko, K.A. *et al.*
pKD3	Template plasmid containing *Cm* resistant gene	Datsenko, K.A. *et al.*
pKD4	Template plasmid containing *Kan* resistant gene	Datsenko, K.A. *et al.*

Construction of *S. enterica* Mutants

S. enterica gene deletion mutants were constructed by the method described previously (*2*):

A pair of primers with 50-nt extensions complementary to the regions immediately adjacent to the targeted gene of deletion and 20-nt extensions complementary to the region immediately adjacent to the antibiotic resistant gene flanked by FRT (FLP recognition target) sites was made. The antibiotics resistant gene carrying plasmid pKD3 or pKD4 (table S2) was used as templates for PCR amplification of the knock-out cassette with Invitrogen PCR Kit (Invitrogen, Carlsbad, CA). The PCR products were recovered by using QIAquick Gel Extraction Kit (QIAGen, Frankfurt, Germany) and then transformed into the electrocompetent *S. enterica* G2466 carrying the helper plasmid pKD46 via electroporation by using MicroPulser electroporator (Bio-Rad, Hercules, CA) and 1.0 cm corvette according to the manufacturer's instructions. Pulsed cells were rescued with 0.8 ml SOC medium, incubated at 30 °C for 1 h and 20 h at room temperature before spread onto agar with

incubation at 37 °C to select for *Cm*^{R} or *Kan*^{R} transformants.

Multiple gene disruption was achieved by repeating above procedures consecutively and use different selection markers. *cobB* or/and *pat* deletions were verified by PCR.

The primers for constructing the deletions are as following:

For *cobB* deletion:

F-GCAGGTGGGATGCGCGGTGCTGCTTTTTTACATCTTACCGACTAATCA AGTGTAGGCTGGAGCTGCTTC;

R-AGCCAAAGCGCCTGTCGGGCCTACAGATGAACGTAACGTGAAATGTA GGCCATATGAATATCCTCCTTA.

For *pat* deletion:

F-ACGCGCTATCTGGCAGGAAAAACGCAACATCCGGGAAACGTCACAGC ATAGTGTAGGCTGGAGCTGCTTC;

R-GTCCTCTGTGCTATGCAGACGACATAAGCGAGCAATAATAACATCAGT ACCATATGAATATCCTCCTTA.

Success of deletion of pat and cobB were verified by PCR using following primers:

cobB:F-CATGTTGAGCGCTATCTGGA; R-AGGTCGCCAATGATAAGTCG;

pat: F-TGAACATTTCACCCGTTTCA; R-GAGTTGGCAAGGTGTTGTT.

q-RT-PCR

Cells at various growing phase as indicated were harvested by centrifuging at 8,000 × *g* for 10 min at 4 °C. Total RNA was extracted immediately after harvesting by TRIZOL (GIBCO, USA). Reverse transcription was carried out using the reverse transcription kit (Promega, Madison, WI). The cDNA

samples were diluted between 1:10 ~1:100 to ensure obtaining linearly signaling. Samples were run in triplicate on ABI PRISM 7000 Sequence Detection System (Applied Biosystems), SyberGreen (Applied Biosystems) was used as probe. The primers used to quantify gene expression were:

cobB: F-CCTTTGGGAAGAGCATCG;

R-GCCGCATTGGGTTGTATTT;

pat: F-TCGCACAGGTCACCAAAGA;

R-CGCACCAATACGGCAAA;

16SrRNA: F-TGGAAAGTTCTGTGGATGTCA;

R-AAAGCGTGGGGAGCAAA.

The PCR reactions were carried out under the following conditions: 95 °C for 1 min, 95 °C 15 s, 62 °C 15 s, and 72 °C 30 s for 40 cycles. SyberGreen real time PCR dissociation curves showed that each primer set gave a single and specific product. The efficiency, sensitivity and linearity of RT-PCR reactions were examined. The relationship between threshold cycle (Ct) and the log copy number of cDNA for all genes was linear with an R^2, The amplification efficiency, shown as following, of real time PCR is monitored to ensure validity of quantification results:

	16S rRNA	*cobB*	*pat*
R^2	0.9975	0.9997	0.9978
Amplification efficiency	95%	98%	97%

Statistical analysis was performed using the Statview Statistical package (version 5.0.1). Values of $P<0.05$ were considered statistically significant.

The Growth Properties of *S. enterica*

In all experiments, 17 μg/ml chloramphenicol was supplemented to media that grow *cobB* null or *pat* null mutant strains, 50 μg/ml kanamycin was supplemented to media that grow *cobB*/*pat* double null strain. *S. enterica* G2466 and its derivative strains were first grown overnight in 20 ml LB medium to reach saturation. Cells were harvested by centrifuging at 8,000 × *g* for 10 min and washed with fresh M9 medium for three times. Certain amount of re-suspended (2 ml M9 medium) cells were inoculated into 50 ml fresh M9 medium with indicated carbon source to ensure the initial O.D.600=0.100 in a 250 ml flask. Cells were grown at 37 °C with shaking (220 r.p.m.). Cell growth was measured by taking O.D.600 at time points specified by using Bio-photometer (Eppendorf, Hamburg, Germany). Cuvette chamber was kept at 37 °C while measuring and O.D.600 readings were taken immediately after samples were taken.

Proteomics Screen for Acetylated Proteins

Protein Extraction

S. enterica cells grown under various carbon sources were harvested at mid-log phase by centrifuging at 8,000 × *g* for 15 min. Cells were disrupted by sonication on ice in PBS buffer (pH7.5). Soluble proteins were obtained by collecting supernatant after centrifugation of broken cell suspension at 20,000 × *g* for 40 min at 4 °C. Proteins were precipitated by adding ice-cold acetone to a final concentration of 85%. The protein pellet was washed by 80% ice-cold acetone two times and vacuum dried.

Digestion, Peptide Enrichment and MS Analysis

1. Dried protein pellets were reconstituted in 100 mM ammonium bicarbonate and protein concentrations was determined by Bio-Rad protein assay solution (Bio-Rad, Hercules, CA). Proteins were digested with trypsin overnight with gentle shaking at 37 °C with a trypsin/protein ratio of 1/50. After overnight digestion, additional trypsin was added to the digestion mixture with a trypsin/protein ratio of 1/500 and incubated at 37 °C for another 3-4 hours. The trypsin in the digestion reaction was inactivated by heating at 99 °C for 5 min. The solution was cleared by centrifuging at 10,000 × *g* on desktop centrifuge, solution was vacuum dried two times to remove bicarbonate salts.

2. Anti-acetyllysine antibody was conjugated to protein A beads (Santa Cruz, CA) by mixing 100 μl antiserum (home made) with 50 μl protein A beads in 500 μl pH 7.5 PBS. Conjugated beads were washed by pH 7.5 PBS three times after the conjugation was carried out at 4 °C for 3 hours with gentle shaking.

3. Trypsin digested peptide mixture from step 1 was reconstituted in NETN buffer (0.5% NP40, 1 mM EDTA, 50 mM Tris [pH 8.0], 100 mM NaCl). Conjugated antibody was added to the solution and incubated at 4 °C for 3-5 hours. Supernatant was removed by carefully washing beads by ETN (1 mM EDTA, 50 mM Tris [pH 8.0], 100 mM NaCl) buffer three times.

4. The bound peptides were eluted by 0.1% trifluoroacetic acid.

5. The resulting peptides were assayed by continuously separated by SCX followed by C18 columns (Dionex, Sunnyvale, CA) before being subjected to MS/MS analysis in an LTQ-Orbitrap mass spectrometer (Thermo Electron,

Bremen, Germany).

6. The MS data were initially searched against the NCBI database with the aid of the Sequest search engine. Searches for acetylated peptides were done against *S. enterica* proteins database by first allowing only singly charged fragments followed by allowing both singly charged and multiply charged fragments. To collect positive hits, the following selection thresholds were applied: mass accuracy: 5 ppm, delta cn: 0.1, primary score: 200, rsp: 5, those peptides having scores (XC) above 1.5, 2.0 and 2.5 for ion charges at +1, +2 and +3 and higher were automatically selected as positives identification. Peptides with marginal scores were under manual spectra examination to be included in the positive list. The data from three experiments were combined. Positive hits from both searches were then combined but marked differently.

SILAC Quantification. ^{15}N labeled S*. enterica* cells were obtained by growing cells in $^{15}NH_4Cl$ containing minimal medium without adding other nitrogen source. The ^{15}N labeling efficiency reached 99.6% by MS analysis after three rounds of inoculation. Proteins of ^{15}N labeled cells and unlabeled cells were obtained by sonication, followed by acetone precipitation and vacuum drying. Equal amount (0.5mg) of labeled and unlabeled proteins were mixed and subject to trypsin digestion for 20 hours at protein/trypsin ratio of 50/1, resulted peptides were vacuum dried and re-dissolved in 100 μl MS buffer (10% acetonitril v/v, 0.5% formic acid w/v). 50μl of the peptides sample was subject to nano-LC/LTQ-Orbitrap MS/MS Analysis. Quantification of acetylated peptides was achieved by adapting published methods (*3, 4*): the resulted MS/MS spectra was searched against NCBI S*. enterica* library by

Sequest searching engine, with presettings of either only ^{14}N or ^{15}N present in the peptides. The acetylated peptides were extracted and those were previously identified by enrichment procedures were used to quantify the acetylation level changes of corresponding proteins. The chromatographic mass peaks of the identified both ^{14}N and ^{15}N acetylated peptides were extracted, and the peak areas were calculated using the PepQuan module in Bioworks (Thermo Electron, San Jose, CA). Chromatographic peak extraction and area integration were performed using a mass tolerance of 1.5 amu, minimum threshold of 1,000, and peak smoothing point of 5. The peak area ratio of each pair of ^{14}N and ^{15}N acetylated peptide was calculated normalized to the ratio of one none acetylated ribosomal protein 50S L6 peptide: DGYADGWAQAGTAR. Average of ratios in 3 different runs of the same pair of acetylated peptides was reported.

Metabolic Flux Measurements.

^{13}C labeling experiment

All *Salmonella enterica* LT2 strains were pre-cultured on LB medium to late-exponential growth phase and washed twice with carbon source free M9 minimal medium. The labeling cultures were started at O.D.600~0.1 and grown at 37°C in triplicates in 20 ml of M9 minimal medium supplemented with 20% (w/w) mixture of both labeled and unlabeled glucose or citrate as carbon source. 20% (w/w) uniformly ^{13}C-labeled glucose (U-^{13}C, >99%; Cambridge

Isotope Laboratories, Andover, MA) and 80% (w/w) unlabeled glucose, or 20% of $^{13}C_6$-labeled citrate (SIGMA) and 80% (w/w) unlabeled citrate was added. Cell growth was monitored spectrophotometrically at 600nm. Cell samples were taken at mid-exponential-growth phase (O.D.600 of ~0.4) for either glucose depletion assay or GC-MS analysis.

GC-MS sample preparation and analysis.

Cells growing at mid-exponential-growth phase from 5 ml culture were harvested by centrifugation. The cell pellet was washed with 1 ml 0.9% (w/vol) NaCl and then hydrolyzed in 200 µl of 6 M HCl at 105°C for 24 h. The filtrate of hydrolysate was dried in a heating block at 60°C, and proteinogenic amino acids were derivatized at 85°C for 1 h in 75 µl tetrahydrofuran (Sigma) and 75 µl *N*-methyl-*N*-[tert-butyldimethylsilyl] trifluoroacetamide (Sigma). After filtration, 2 µl of derivatized sample was assayed in an Agilent 6890-5973 GC/MS. The mass spectral data were obtained for fragments of most derivatized amino acids. Prior to analysis of cellular metabolites, the raw mass isotopomer distribution vector (MDV) was corrected for the natural isotope abundances of carbon atoms introduced from the derivatization reagent and all non-carbon atoms involved in the whole fragment (*5*). Citrate was quantified by high-pressure liquid chromatography with an Agilent model 1100 instrument (Agilent, Santa Clara, CA) equipped with Shodex RSpak KC-811 (8 x 300 mm; Shodex Inc., Tokyo, Japan) and an Agilent DAD detector. A mobile phase

consisting of 6 mM $HClO_4$ at a flow rate of 1.0 ml/min was used, and the column was operated at 50°C.

Glucose depletion (glycolytic) assay.

Cells growing at mid-exponential-growth phase (O.D.600 around 0.4) from 5 ml culture, started at the same glucose concentrations and cell densities, were harvested by centrifugation. Culture medium was saved and the glucose level remained in culture medium was determined using commercial enzymatic assay kits (R-Biopharm AG, Darmstadt, Germany). The depletion of glucose was determined by comparing difference of initial medium glucose concentration and final concentration in the culture medium.

Analysis of flux ratios and flux distributions.

The GC-MS-derived mass isotope distributions were analyzed as previously described (*5*). In brief, from the mass distributions of the amino acids the ^{13}C labeling patterns of their respective precursor molecules in central metabolism were inferred. A set of probabilistic equations and the mass distributions of selected metabolite fragments were then combined to calculate the relative contribution of individual pathways to the formation of a given metabolite.

Intracellular fluxes were estimated by fitting a flux distribution to the above flux ratios and quantitative physiological data within a stoichiometric model described previously for *E. coli* (*6*). Precursor requirements for biomass formation were taken from previous described (*6*). The sum of the weighed

square residuals of the constraints from both metabolite balances and flux ratios was calculated and the residuals were weighed by dividing through the experimental error (χ^2 value). Through an iterative process of data fitting, a flux solution corresponding to the minimal χ^2 value was sought. To ensure the global error minimum of the flux solution, multiple calculations were performed from different random starting points, and the best solution that was reproducibly attained was presented as the estimated result of flux distribution.

Biochemical Procedures

Protein Expression and Purification

To express proteins in E. Coli, pET-22b(+) system was employed. To express proteins in the *S. enterica* G2466 and its derived mutant strains, the vector pET-22b(+) was engineered by changing T7 promoter to *tac* promoter without changing the vector backbone (pET*tac*, table S2). The protein was expressed in and purified from *S. enterica* according to the protocol in *E. coli*. All the constructed expression plasmids were introduced individually into *E. coli* BL21(DE3) or into *S. enterica* strains and the transformed strains were grown in LB medium containing 100 μg/ml ampicillin at 37 °C. IPTG was added to a final concentration of 0.5 mM when O.D.600 reading reached 0.6-0.8. The induction was carried out at 20 °C for 3-4 h. The cells were harvested by centrifugation and cell pellet was suspended in ice-cold PBS buffer (0.1 M Sodium Phosphate, 0.15 M NaCl, pH 7.0) containing 1.0 mM

pheylmethylsulfonyl fluoride (PMSF) and 0.5 mM DTT. Cells were disrupted by using the Cell Ultrasonic System (Sonics & Materials INC. Newtown, CT. USA) with short bursts of 10 s followed by intervals of 30 s for cooling until cell suspension becomes clear. Unbroken cells and cell debris were removed by centrifuging at 21,000 × *g* for 40 min at 4 °C. The supernatant was supplemented with 20 mM imidazole, loaded onto Nickel resin column (GE Healthcare) and purified with ÄKTA™ FPLC™ System (GE Healthcare, Piscataway, NJ). After washed with 10 volume binding buffer A (20 mM Sodium Phosphate, 500 mM NaCl and 20 mM Imidzaole, pH 7.5), proteins were eluted by elution buffer (20 mM Sodium Phosphate, 500 mM NaCl and 500 mM imidazole, pH 7.5). The purity of proteins was checked by SDS-PAGE before shock-frozen storing at -80 °C.

Pat-mediated *in vitro* acetylation was modified from published method (*7*). The *in vitro* acetylation reaction was performed in the buffer containing 50 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 10% glycerol, 1 mM dithiothreitol and 10 mM sodium butyrate. The acetylation was carried out by adding 10 μg of Pat, 0.2 mM acetyl-CoA in a volume of 100 μl. Reaction mixtures were completely mixed and incubated at 37 °C for two hours. The acetylated protein was used directly for assay or precipitated out for re-deacetylation.

CobB-mediated *in vitro* deacetylation was modified from the method described elsewhere (*8*). The *in vitro* deacetylation reaction was performed in buffer contains 40.0 mM HEPES (pH 7.0), 6.0 mM $MgCl_2$, 1.0 mM NAD^+, 1.0

mM dithiothreitol and 10 % glycerol. Substrate proteins (10 µg), CobB (10 µg) and reaction buffer were incubated at 37 °C in a total volume of 100 µl. The de-acetylated protein was used directly for assays or precipitated out for re-acetylation.

AceA assay was followed by publicized method (*9*). All procedures were carried out at 30 °C and the reaction mixture for the LDH-coupled assay contains, in a final volume of 1 ml of 40 mM HEPES (pH 7), 6.0 mM $MgC1_2$, 4.0 mM threo-D,L isocitrate, 0.28 mM NADH and 45 units lactate dehydrogenase (Sigma, St. Louis, MO). NADH oxidation is monitored spectrophotometrically at 340 nm (F-4600, Hitachi Corp., Japan).

GapA glycolytic assay was carried out by following method described elsewhere (*10*). The reaction was carried out at 30 °C by adding of sample to substrate solution containing 100 mM Tris/HCl (pH 8.5), 1.5 mM NAD^+, 5.0 mM sodium phosphate and 3.2 mM D-glyceraldehydes-3-phosphate, absorbance change at 340 nm was monitored.

GapA gluconeogenic assay: The formation of GapA's gluconeogensis substrate, 1, 3-diphosphoglycerate, was generated by published method (*11*). The reaction mixture composed of 80mM triethanolamine (pH 8.5), 8.0 mM $MgSO_4$, 0.25 mM NADH, 2.4 mM ATP, 12 mM 3-phosphoglycerate and 200 pg/ml glyceraldehyde-3-phosphate dehydrogenase. The reaction was stopped by heating at 99^0C for 5 min after the reaction was allowed to proceed at 37^0C for half hour. To initiate gluconeogene sis reaction, 0.1 mM NADH

and 10 ng GapA protein were added to the solution and the decrease in NADH was monitored spectrophotometrically at 340 nm (F-4600, Hitachi Corp., Japan) at 30^0C.

AceK assay was carried by coupling AceK activity to ICDH activity as described elsewhere (*12*). The phosphorylation reaction system contains 40.0 mM HEPES (pH7.0), 1.0 mM ATP, 2.0 mM $MgCl_2$, 10.0 µg ICDH and AceK, respectively. After incubating at 37 °C for one hour, the activity of ICDH was detected spectrophotometrically by monitoring the reduction of NADH at 340 nm with 40.0 mM HEPES (pH7.0), 2.0 mM threo-D,L isocitrate, 0.5 mM NAD^+, 2.0 mM $MnCl_2$, 0.5 µg ICDH in the reaction system.

Protein concentrations were determined by using the Bradford protein assay reagent (Bio-Rad, Hercules, CA) with known concentration of bovine serum albumin (BSA) as control per the manufacturer's instructions.

Western blotting: For normal western blotting, standard western blotting procedures were followed. For acetylation western blotting, 50 mM Tris (pH 7.5) with 10% (V/V) Tween-20 and 1% peptone (Amresco, Solon, Ohio) was used for blocking and 50 mM Tris (pH 7.5) with 0.1% peptone was used to prepare primary and secondary antibodies.

S1A

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Pat  809 EFAVLVRSDLKGLGLGRRLMEKLIAYTRDHGLKRLNGITMPNNRGMVALARKLGFQVDIQL 869
         E +V V    +G GLGR+L+ +LI   R  G   +       N   + L   LGF V  Q+
YncA  82  EHSVYVHPAHQGKGLGRKLLSRLIDEARRCGKHVMVAGIESQNAASIRLHHSLGFTVTAQM 142

Pat  810 FAVLVRSDLKGLGLGRRLMEKLIAYTRDHGLKRLNGITMPNNRGMVALARKLGFQ 864
         F + V  D +  GLGR L+E LI      G+  L       +N   +AL   LGF
RimI  67  FNIAVDPDFQRRGLGRMLLEHLIDELETRGVVTLWLEVRASNAAAIALYESLGFN 121

Pat  818 LKGLGLGRRLMEKLIAYTRDHGLKRLNGITMPNNRGMVALARKLGFQ 864
         ++G GL ++L    + + R+ G KR    T    R  +AL  +LGF+
YjgM  99  IRGQGLAKKLALMALDHAREQGFKRCYLETTAFLREAIALYERLGFE 145

Pat  808 AEFAVLVRSDLKGLGLGRRLMEKLIAYTRDHGLKRLNG-----ITMPNNRGMVALARKLGFQVDIQL 869
         AEF +++  + +G GL  R  +       D+G  LN      I   N   + + RKLGF+V+ +L
SpeG  84  AEFQIIISPEYQGKGLASRAAK----LAMDYGFTVLNLYKLYLIVDKENEKAIHIYRKLGFRVEGEL 146
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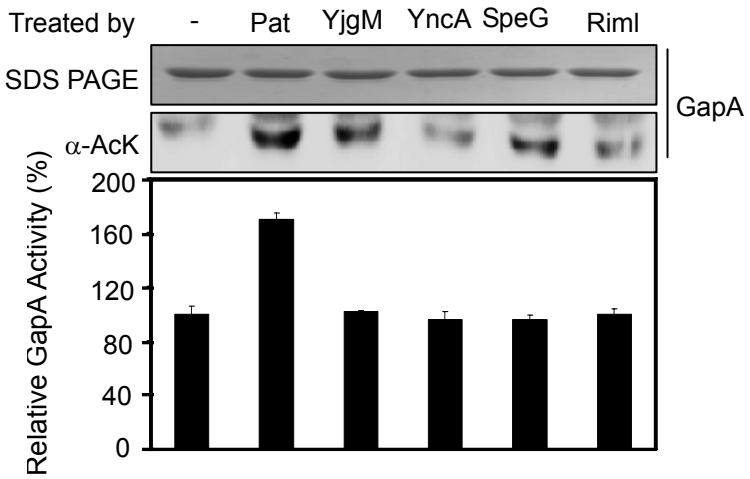
S1B



Wang et al, fig. S1

fig. S1. Pat and CobB are major acetyltransferase/ deacetylase in *S. enterica* that catalyze the reversible lysine acetylation for metabolic enzymes. (S1A) Pat homologous proteins identified from the genome of *S. enterica*. Amino acids sequences of bacteria acetyltransferase Pat, human acetyltransferases (GCN5, PCAF, CBP/P300, Elp3, Hat1, Tip 60), bacteria deacetylase CobB and human NAD^+ dependent deacetylases (Sirt1-7) were subject to Blast search in *S. enterica* genome in NCBI. No homology was found for Sirts and CobB, however, NP_460549.1 (putative acyltransferase, YncA), NP_463414.1 (ribosomal protein alanine acetyltransferase, Riml), NP_46333.1 (putative acetyltransferase, YjgM) and NP_460462.1 (spermidine N1-acetyltransferase, SpeG) showed similarities to Pat catalytic domain. **(S1B) Candidate acetyltransferases from *S. enterica* other than Pat do not affect the GapA activity.** Proteins share homology to Pat catalytic domain were cloned, expressed and purified to homogeneity. Their acetyltransferase catalytic activity toward metabolic enzymes was tested *in vitro* by using purified GapA , AceA and AceK as substrates. Similar results were obtained for all three proteins, but only the representative results of GapA are shown. SpeG and YgjM showed weak acetyltransferase activity. Unlike acetylated by Pat, GapA acetylated by SpeG, YcnA and YgjM did not cause detectable changes in its catalytic activity.

Wang et al, fig. S1

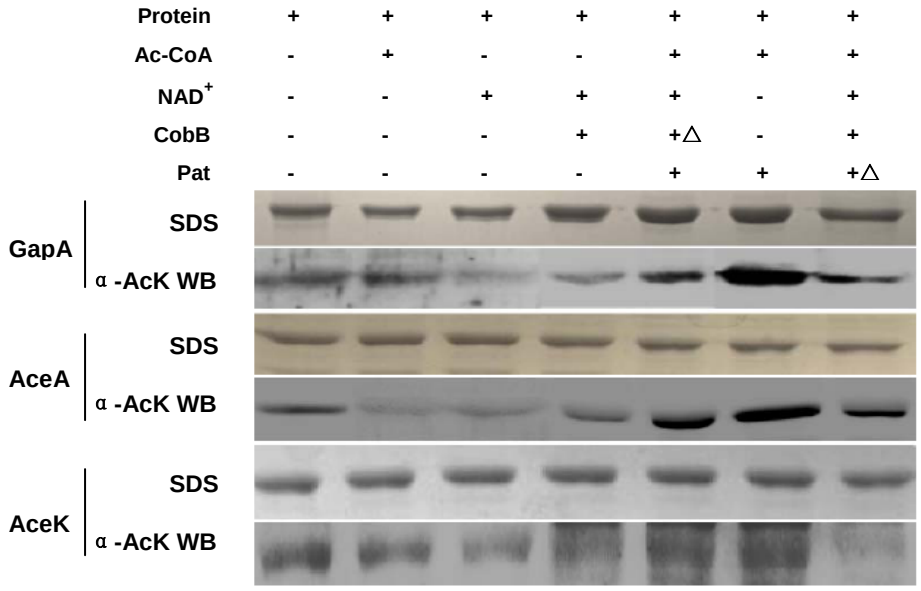


fig. S2. Pat acetylates and CobB deacetylates metabolic enzymes *in vitro*. Prototype strain expressed and purified GapA, AceA and AceK proteins were subject to *in vitro* acetylation by Pat and *in vitro* deacetylation by CobB. Resulted protein acetylation levels were probed by anti-acetyllysine Western blotting. △ indicates either re-acetylation after deacetylation or re-deacetylation after acetylation.

Wang et al, fig. S2

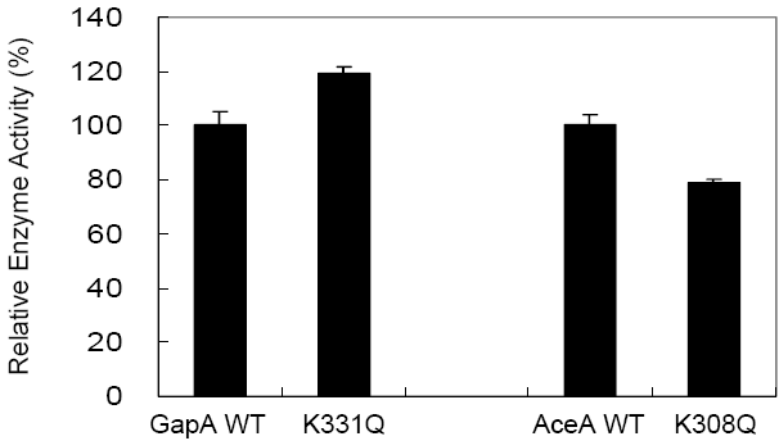


fig. S3. **GapA and AceA acetylation mimetic mutations made at the identified acetylation sites changed their catalytic activities.** *S. enterica* GapA K331Q mutant, AceA K308Q mutant were expressed and purified to homogeneity. The specific activity of each enzyme was determined and compared to their wild type counter parts expressed and purified by the same protocol. Mean values of triplicate assays with SD are shown. Specific activities for wild type enzymes are arbitrarily set as 100%.

Wang et al, fig. S3

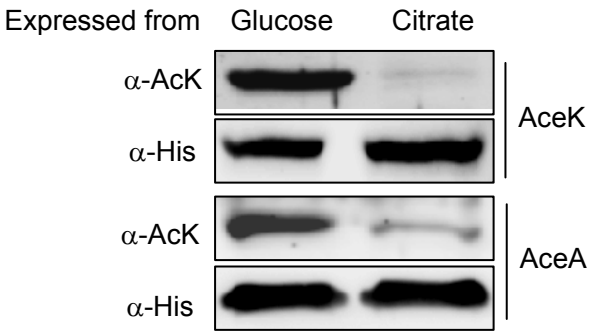
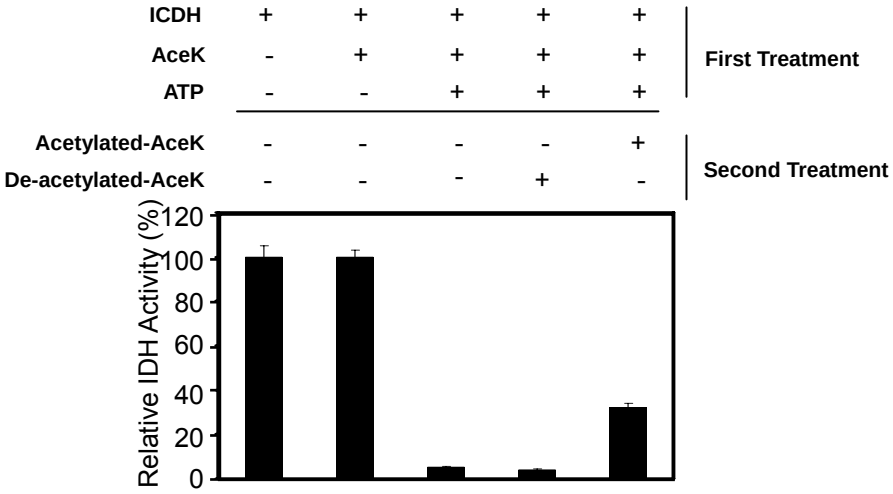


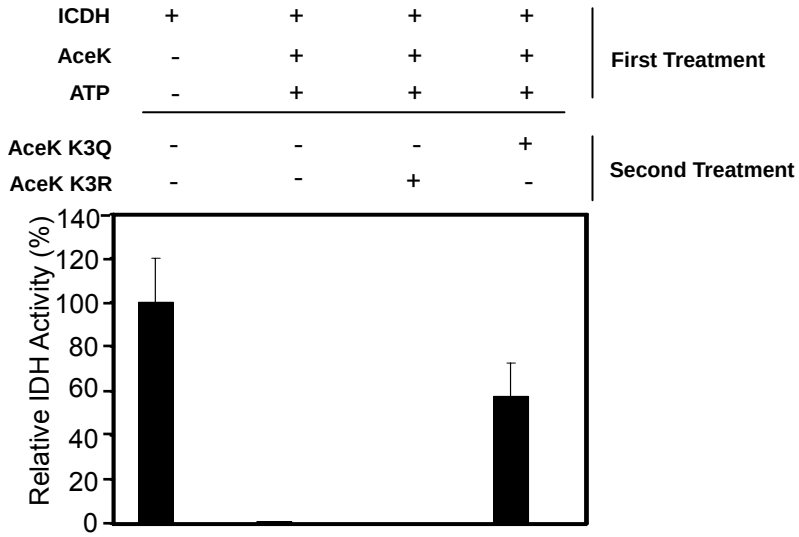
fig. S4. **AceA and AceK expressed from cells grow on glucose has higher acetylation level than those grow on citrate.** His tagged AceA and AceK proteins were expressed from *S. enterica* prototype strain grow in M9 minimal medium with either 50 mM glucose or 50 mM citrate as carbon source. Proteins were each purified by nickel column and the acetylation level of each purified protein was probed by anti-acetyllysine antibody.

Wang et al, fig. S4

S5A



S5B



S5C

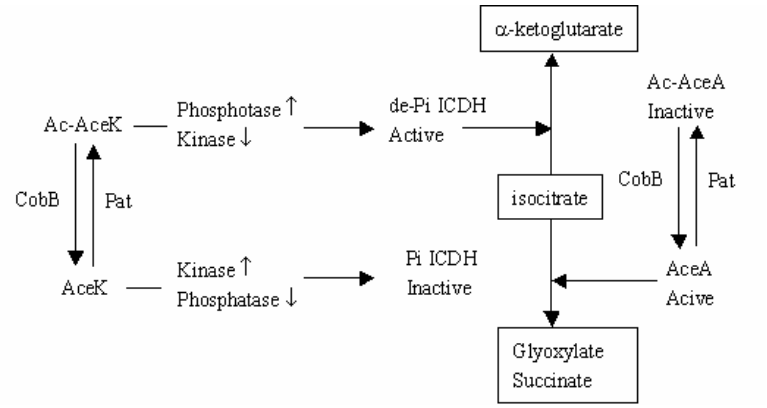


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fig. S5. Acetylated AceK has activated phosphatase activity. (S5A) Acetylated AceK activates phosphorylated ICDH. Purified *S. enterica* ICDH (encoded by *icdA*) protein was first phosphorylated by AceK for 30 min., the resulted protein, precipitated by ammonium sulphate, was then incubated with either CobB de-acetylated AceK or Pat acetylated AceK at 37 ^{o}C for 1 hour. Relative specific ICDH activities after each treatment were then determined. Untreated ICDH activity was set as 100% and the average value of triplicate assays with SD were shown. **(S5B) Acetylation mimetic AceK mutation activates phosphorylated ICDH.** Purified ICDH protein was first co-incubated with ATP and AceK to inactivate ICDH, acetylation mimetic triple mutation (lysine72, 83 and 553 all changed to glutamine, designated as AceK K3Q) and none acetylation mimetic triple mutation (lysine72, 83 and 553 all changed to arginine, designated as AceK K3R) was then added and incubate for 30 minutes before each specific ICDH activity was measured, respectively. AceK untreated activity was set as 100% arbitrarily and the average value of triplicate assays with SD were shown. These results indicates that acetylation of AceK may have activated phosphatase activity. **(S5C) Schematic rationale for assaying the biochemical effect of reversible acetylation of AceK against its activity towards inhibition/activation of activity of ICDH via reversible phosphorylation.** The effect of reversible acetylation of AceA against its isocitrate lyase activity is also shown.

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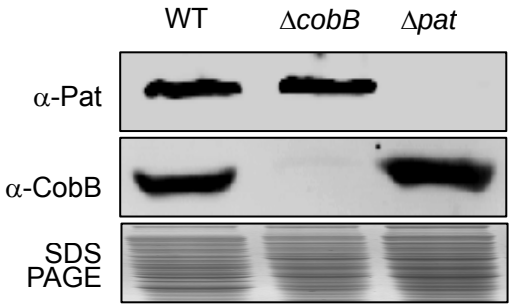

fig. S6. **Specificity of Pat and CobB antibodies.** Prototype, Δ*pat* and Δ*cobB S. enterica* strains were grown to mid-log phase in LB and harvested by centrifugation. Cells were lysed and protein levels was determined by SDS PAGE, specificity of anti-Pat and anti-CobB antibodies were determined by detecting presence of Pat and CobB proteins in each cell lysate.

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table S1

Access	Refrence	Acetylated peptides	Z+1 fragments only	All fragments allowed
ACEA_SALTY	Isocitrate lyase	R.PYSAEEVVK*LRGSVNPECTLAQLGAAK.M	√	
ACEK_SALTY	Isocitrate dehydrogenase kinase/phosphatase	R.NK*AAWLVGK.L	√	
ACSA_SALTY	Acetyl-coenzyme A synthetase	K.VKNTSFAPGNVSIK*WYEDGTLNLAANCLDR.H	√	
ARAB_SALTY	Ribulokinase	K.VIGTSTCDILIADK*QSVGDRAVK.G	√	
ARNB_SALTY	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotrans	K.NITCAEGGIVVTDNPQFADK*LRSLK.F	√	
AROK_SALTY	Shikimate kinase 1	R.GVVVYLETTIEK*QLARTQRDKKR.P	√	
APSL_SALTY	Anaerobic ribonucleoside-triphosphate reductase	R.IAGLGK*NR.K	√	
CAPP_SALTY	Phosphoenolpyruvate carboxylase	K.LK*NQPDLNDATIK.K	√	
CARB_SALTY	Carbamoyl-phosphate synthase large chain	R.KGFADARLAK*LAGVREAEIR.K	√	
CBIK_SALTY	Sirohydrochlorin cobaltochelatase	-.MK*K*ALLVVSFGTSYHDTCEK.N	√	
CDH_SALTY	CDP-diacylglycerol pyrophosphatase	K.K*YGHDIPDSAVSLAINSR.L	√	
CHBG_SALTY	UPF0249 protein chbG	R.ELPALGVGMHFVLTLGK*PVSEMPGLTR.D	√	
CHEY_SALTY	Chemotaxis protein cheY	R.RIVRNLLK*ELGFNNVEEAEDGVDALNK.L	√	
CISY_SALTY	Citrate synthase	R.PRQLYTGYDK*RDFK*SALKR	√	
CRL_SALTY	Sigma factor-binding protein crl	K.K*FTALGPYIR.E	√	
CYSG_SALTY	Siroheme synthase	R.EAQK*GKR.V	√	
CYSJ_SALTY	Sulfite reductase [NADPH] flavoprotein alpha-component	K.DAPLIATLSVNQK*ITGRNSEK.D	√	
DCUP_SALTY	Uroporphyrinogen decarboxylase	K.KMMYADPQALHLLLDKLAK*SVTLYLNAQIK.A	√	
DGAL_SALTY	D-galactose-binding periplasmic protein precursor (GBP)	R.KALDSYDK*AYYVGTDSKESGVIQGDLIAK.H	√	
DHAS_SALTY	Aspartate-semialdehyde dehydrogenase	K.LKK*EVSIPTVEELLAAHNPWAK.V	√	
DHSB_SALTY	Succinate dehydrogenase iron-sulfur subunit	R.SCREGVCGSDGLNMNGK*NGLACITPISALTQPGK.K	√	
DTI_SALTY	DNA topoisomerase 3	K.CVIELEIAK*GK.F	√	
DNAA_SALTY	Chromosomal replication initiator protein dnaA	R.DK*YLNNINGLLNTFCGADAPQLR.F	√	
DNAB_SALTY	Replicative DNA helicase	R.VFQIAENRANK*DEGPK*SIDQILDATVAR.I	√	
DUSA_SALTY	tRNA-dihydrouridine synthase A	R.YLSENAHK*AGADVAVLEQALK.L	√	
EFG_SALTY	Elongation factor G	K.NK*GVQAMLDAVIDYLPSPVDVPAINGILDDGK.D	√	
ENO_SALTY	Enolase	R.VAK*YNQLIRIEEALGEKAPYNGRK*EIK.G	√	
FADJ_SALTY	Fatty acid oxidation complex subunit alpha	K.LAK*K*QGKTPIVVSDKAGFYVNR.I	√	
FLGM_SALTY	Negative regulator of flagellin synthesis (Anti-sigma-28 factor	K.TRQEK*TSAATSASVTLSDAQAK.L	√	
FLIE_SALTY	Flagellar hook-basal body complex protein fliE	K.FTLGEPGIALNDVMADMQK*ASVSMQMGIQVR.N	√	
FUMC_SALTY	Fumarate hydratase class II	R.K*VHPNDDVNK.S	√	
G3P1_SALTY	Glyceraldehyde-3-phosphate dehydrogenase	K.LVSWYDNETGYSNK*VLDLIAHISK.-	√	
G6PI_SALTY	Glucose-6-phosphate isomerase	R.SNTPIIVDGK*DVMPEVNAVLEK.M	√	
GIDA_SALTY	tRNA uridine 5-carboxymethylaminomethyl modification enzy	K.FRAK*AVVLTVGTFLDGK.I	√	
GLGB_SALTY	1,4-alpha-glucan-branching enzyme	K.TGRK*VGKLECLDARGFFCGVLPR.R	√	
GLK_SALTY	Glucokinase	R.AIVKSDNRLPENLRPK*DITER.A	√	
GLNA_SALTY	Glutamine synthetase	R.FGASISGSHVAIDDIEGAWNSSTK*YEGGNK.G	√	
GLNE_SALTY	Glutamate-ammonia-ligase adenylyltransferase	R.ARLAWGMK*AENWPQLVGELTDHMANVR.R	√	
GPMB_SALTY	Probable phosphoglycerate mutase gpmB	R.HYGALQGLNK*AETAEK.Y	√	

HILD_SALTY	Transcriptional regulator hilD	K.YFK*TTPSTFIK*MANH	√
HNS_SALTY	DNA-binding protein H-NS (Histone-like protein HLP-II)	R.TRK*LQQYREMLIADGIDPNELLNSMAAAK.S	√
HSLU_SALTY	ATP-dependent hsl protease ATP-binding subunit hslU	K.NILMIGPTGVGK*TEIARR.L	√
IPYR_SALTY	Inorganic pyrophosphatase	K.AQITHFFEHYK*DLEKGK.W	√
K6PF_SALTY	6-phosphofructokinase II	K.PNLK*ELSALVNRDLTQPDDVRK.A	√
KPRS_SALTY	Ribose-phosphate pyrophosphokinase	-.MPDMK*LFAGNATPELAQR.I	√
KPYK1_SALTY	Pyruvate kinase I	K.GVVSQLVK*EINSTDDFYR.L	√
KPYK2_SALTY	Pyruvate kinase II	K.FLLDANLGK*GEGDK*EK.V	√
LAMB_SALTY	Maltoporin precursor (Maltose-inducible porin)	K.LADGASK*DGWMFTAEHTQSMLK*GYNK.F	√
LDCI_SALTY	Lysine decarboxylase, inducible	R.QADGRYTVK*VLK.E	√
LEP_SALTY	Signal peptidase I	R.RARQAAAQTASGDALDNATLNK*VAPK.P	√
LEPA_SALTY	GTP-binding protein lepA	R.CSAK*TGVGVTDVLER.L	√
LEU21_SALTY	3-isopropylmalate dehydratase large subunit 1	K.TLYEK*LFDAHVVFEAPNETPLLYIDR.H	√
LGUL_SALTY	Lactoylglutathione lyase	R.EAGPVK*GGSTIIAFVEDPDGYK.I	√
LUXS_SALTY	S-ribosylhomocysteine lyase	R.MQAPAVRVAK*TMNTPHGDAITVFDLRFCIPNK.E	√
MDH_SALTY	Malate dehydrogenase	R.K*PGMDRSDLFNVNAGIVK.N	√
METN1_SALTY	Methionine import ATP-binding protein metN 1	R.VTELLDLVGLGDKHDSYPANLSGGQK*QR.V	√
MSGA_SALTY	Virulence protein msgA	R.EIILNELTK*RVHQLFPDAQVK.V	√
MURB_SALTY	UDP-N-acetylenolpyruvoylglucosamine reductase	K.VGEK*FNVWLEPEVRFIGR.S	√
MURE_SALTY	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopi	R.AEAVTNAIMQAK*DNDVVLIAGK.G	√
NANH_SALTY	Sialidase	K.AEGEHFTDQKGNTIVGSGSGGTTK*YFR.I	√
NRDR_SALTY	Transcriptional repressor nrdR	R.ATGEREVPSKMIGNLVMEQLK*K*LDK.V	√
NUSA_SALTY	Transcription elongation protein nusA (N utilization substanc	R.EK*IFEALESALATATK*K.K	√
OMPD_SALTY	Outer membrane porin protein ompD precursor	R.TESQGADK*DK*TRLAFAGLK.F	√
PAGD_SALTY	Virulence protein pagD precursor	K.K*QARQISSPSCPTTK.P	√
PEPQ_SALTY	Xaa-Pro dipeptidase	K.AWVPVTQVPNCWLLVDGVNK*PK.L	√
PGK_SALTY	Phosphoglycerate kinase	R.FNK*GEKK*DDEALSK*K.Y	√
PGM_SALTY	Phosphoglyceromutase	R.HYGALQGLNK*AETAEK.Y	√
PGTB_SALTY	Phosphoglycerate transport system sensor protein pgtB	R.QTIDELLEIGMVK*NK.M	√
PHSA_SALTY	Thiosulfate reductase precursor	K.VAPTLAK*LNIKNMPKPTAQR.I	√
PHSC_SALTY	Thiosulfate reductase cytochrome B subunit	R.CIRQTRFYLFGIMK*GEAHPFVATEQNK.F	√
PMRD_SALTY	Signal transduction protein pmrD	-.MEWLVKKSHYVK*K.R	√
POTD_SALTY	Spermidine/putrescine-binding periplasmic protein precursor	K.IDK*SKLTNFHNLDPEMLNK.P	√
PPCK_SALTY	Phosphoenolpyruvate carboxykinase [ATP]	R.DTLWWSDK*GK.G	√
PRC_SALTY	Tail-specific protease precursor	K.FDELSLKLTGK*SDK.E	√
PSD_SALTY	Phosphatidylserine decarboxylase proenzyme	K.ILQAKGHNYSLEALLAGNYLMADK*FR.N	√
PTGCB_SALTY	PTS system glucose-specific EIICB component (EIICB-Glc) (	R.VLIKALDLK*TPGREDTTDDAK.A	√
Q7CPQ7_SALTY	fructose/tagatose biphosphate aldolase	R.HYMQPAK*QAMK*EVVR.K	√
Q8ZK67_SALTY	Fructose-bisphosphatase	K.AALK*ARDIVAGIASEEEDEIVVFEGCEHAK.Y	√
Q8ZKL4_SALTY	Malate synthase	R.FTPK*RNK*LLAAR.I	√
Q8ZRT1_SALTY	Pyruvate dehydrogenase	K.AEGK*SEFAENDAYVHATPLIRRLAR.E	√
QSEB_SALTY	Transcriptional regulatory protein qseB	K.PK*EFALLELLLRNK*GRVLPRK.L	√
QSEC_SALTY	Sensor protein qseC	R.DK*ALTQLHAGIDR.A	√

RAPA_SALTY	RNA polymerase-associated protein rapA	R.IGQAHDIQIHVPYLEK*TAQSVLVR.W	√
RBFA_SALTY	Ribosome-binding factor A	R.DLAYAKVFVTFLNDK*DEDAVK*AGIK.A	√
RDGB_SALTY	Nucleoside-triphosphatase rdgB	K.VVLATGNAGK*VR.E	√
RECJ_SALTY	Single-stranded-DNA-specific exonuclease recJ	R.KEIEQGMQAEALILCEK*LER.S	√
RL11_SALTY	50S ribosomal protein L11	K.AAGIK*SGSGK*PNK.D	√
RL14_SALTY	50S ribosomal protein L14	K.GDVLK*AVVVRTK.K	√
RL16_SALTY	50S ribosomal protein L16	K.VLYEMDGVPEELAREAFK*LAAAK.L	√
RL2_SALTY	50S ribosomal protein L2	K.GKPFAPLVEK*NSK.S	√
RL22_SALTY	50S ribosomal protein L22	-.METIAK*HRHAR.S	√
RL23_SALTY	50S ribosomal protein L23	K.LFEVEVEVVNTLVVK*GK*VK.R	√
RL29_SALTY	50S ribosomal protein L29	R.MQAASGQLQQSHLLK*QVRR.D	√
RL3_SALTY	50S ribosomal protein L3	K.KVDVTGTSK*GK.G	√
RL35_SALTY	50S ribosomal protein L35	K.ATK*RK*RHLRPK.A	√
RL4_SALTY	50S ribosomal protein L4	R.DATGIDPVSLIAFDK*VVMTADAVK.Q	√
RLUB_SALTY	Ribosomal large subunit pseudouridine synthase B	R.EVEREYAVRVFGQVDESK*LRDLSR.G	√
RLUD_SALTY	Ribosomal large subunit pseudouridine synthase D	K.EWILNQRVLVNGQLCDK*PK.E	√
RLUF_SALTY	Ribosomal large subunit pseudouridine synthase F	K.SNEK*ARPTSSGK*RFTSPGRK*K*K.G	√
RP5M_SALTY	Probable sigma(54) modulation protein (ORF95)	K.FAK*LEQYFER.I	√
RPOD_SALTY	RNA polymerase sigma factor rpoD (Sigma-70)	K.AKGRSHAAAQEEILK*LSEVFK.Q	√
RRF_SALTY	Ribosome recycling factor (Ribosome-releasing factor) (RRF	R.DANDK*VKALLKDK.A	√
RS19_SALTY	30S ribosomal protein S19	K.AVESGDKK*PLR.T	√
RS3_SALTY	30S ribosomal protein S3	K.PELDAK*LVADSITSQLER.R	√
SELD_SALTY	Selenide, water dikinase	R.VKK*NSTAQAGCK*LFLTK.P	√
SILR_SALTY	Probable transcriptional regulatory protein silR	K.RLRAK*IDNDYGTKLNQTVR.G	√
SIPC_SALTY	Cell invasion protein sipC (Effector protein sipC)	K.LEYK*GLQNERGALK.H	√
SIRC_SALTY	Transcriptional regulator sirC	K.VYNIIISDLTRK*WSQAEVAGK.L	√
SODF_SALTY	Superoxide dismutase [Fe]	K.HHQTYVTNLNNLIK*GTAFEGK.S	√
SPAN_SALTY	Surface presentation of antigens protein spaN	K.K*AVEKHKTEYSGDK*KDR.D	√
SPAS_SALTY	Surface presentation of antigens protein spaS	K.EEVKREMKEQEGNPEVK*SKR.R	√
SSRP_SALTY	SsrA-binding protein	K.K*K*AHK*PGSATIALNKR.A	√
SUCC_SALTY	Succinyl-CoA synthetase beta chain	R.GK*AGGVK*VVK*SK*EEIRAFAENWLGK.R	√
SYC_SALTY	Cysteinyl-tRNA synthetase	R.NITDIDDK*IIK.R	√
SYP_SALTY	Prolyl-tRNA synthetase	K.AVK*DSK*SPLVALLVRGDHELNEVK.A	√
SYS_SALTY	Seryl-tRNA synthetase	R.RGFK*LDVDK*LRALEERR.K	√
SYV_SALTY	Valyl-tRNA synthetase	R.LAK*EVAKIEGEIARIEGK.L	√
SYW_SALTY	Tryptophanyl-tRNA synthetase	K.VMK*DGAEK*ASARAAETLKAVYEAIGFVAK.P	√
SYY_SALTY	Tyrosyl-tRNA synthetase	R.RLHQNQVFGLTVPLITKADGTK*FGK.T	√
T1M_SALTY	Type I restriction enzyme StySJI M protein	K.FSREWIRSAKSDSLDISWLK*DK.D	√
T1S_SALTY	Type I restriction enzyme StySJI specificity protein (S proteir	R.LPK*GESLIAENTGFPYIR.A	√
T200_SALTY	Transposase for insertion sequence element IS200	R.K*LCEWK*NVRILEAECCADHIHMLLEIPPK.M	√
T3RE_SALTY	Type III restriction-modification system StyLTI enzyme	K.GIDIYYPNLPEEQANNRYIVDSVTAKK*LILR.R	√
TALA_SALTY	Transaldolase A	R.SPLEPYVVEEDPGVK*SVR.N	√
TATE_SALTY	Sec-independent protein translocase protein tatE	K.LRTLGGDLGTAIK*GFK*K.A	√

THIH_SALTY	Thiazole biosynthesis protein thiH	R.K*LGFEHLLLVTGEHQAK.V	√
TILS_SALTY	tRNA(Ile)-lysidine synthase	R.FK*APGVLHIVGRNGGRK.L	√
TPIS_SALTY	Triosephosphate isomerase	K.ESDELIAK*K*FAVLK.E	√
TRAT_SALTY	TraT complement resistance protein precursor	K.K*LMMVTLVSSTLALSGCGAMSTAIK*K.R	√
TRMD_SALTY	tRNA (guanine-N(1)-)-methyltransferase	K.AAAGEGAK*VIYLSPQGRK.L	√
TRMJ_SALTY	tRNA (cytidine/uridine-2'-O-)-methyltransferase trmJ	R.LFTRARPESQELNILRGILASIEQQNK*GK	√
TRMU_SALTY	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransfera	R.KIAEDLGLVTAKK*K.D	√
TRPB_SALTY	Tryptophan synthase beta chain	R.GDK*DIFTVHDILKAR.G	√
TRPR_SALTY	Trp operon repressor	R.ELK*TELGAGIATITR.G	√
TRUD_SALTY	tRNA pseudouridine synthase D	R.ILK*NGCNTRFVADALAKFLKIHAR.E	√
TYPH_SALTY	Thymidine phosphorylase	K.YLPTAMLSK*AVYADTEGFISAMDTR.A	√
UHPA_SALTY	Transcriptional regulatory protein uhpA	R.ANLLEK*LGVSNDVELAHR.M	√
UPP_SALTY	Uracil phosphoribosyltransferase	K.HPLVKHK*LGLMRENDISTK.R	√
UPPS_SALTY	Undecaprenyl pyrophosphate synthetase	K.KQGK*IRAFGHK*AGAK.S	√
URK_SALTY	Uridine kinase	R.VK*TNYDHPNAMDHSLLFQHLQALK.R	√
UVRC_SALTY	UvrABC system protein C	K.K*RLSSYFRSNLASR.K	√
VSDE_SALTY	Virulence protein vsdE	R.INSKNINNNDSNEVK*RIK.D	√
WZC_SALTY	Tyrosine-protein kinase wzc	K.ALEDEK*AK*LNGRVTAMPK.T	√
YAAI_SALTY	UPF0412 protein yaaI precursor	K.EGQTTGWININSDNDNK*R.C	√
YACG_SALTY	UPF0243 zinc-binding protein yacG	K.RCQLIDLGEWAAEEK*R.I	√
YAEH_SALTY	UPF0325 protein yaeH	K.SVKFK*YPRQRK*TVVADGIGQGYK.E	√
YBEA_SALTY	UPF0247 protein ybeA	K.RGK*NADIK*RILDK.E	√
YCAD_SALTY	Uncharacterized MFS-type transporter ycaD	R.HSSSISAMLK*LRQAR.L	√
YCEB_SALTY	Uncharacterized lipoprotein yceB precursor	R.EEPNK*VTLTGDARLDMNSLFGSQK*ATMK.L	√
YCIA_SALTY	Acyl-CoA thioester hydrolase yciA	R.CVK*RGTTSISINIEVWVK.K	√
YEAH_SALTY	UPF0229 protein yeaH	R.YK*AQIKQSISEAINK*R.S	√
YEDQ_SALTY	Cellulose synthesis regulatory protein	R.QRINDKEILVTK*STTLR.I	√
YEEX_SALTY	UPF0265 protein yeeX	-.METTK*PSFQDVLEFVR.L	√
YFCN_SALTY	UPF0115 protein yfcN	K.KK*TSLSEEDQALFRQLMVGTRK.I	√
YFEK_SALTY	Uncharacterized protein yfeK precursor	R.INAMLNALAQK*K.D	√
YFEW_SALTY	UPF0214 protein yfeW precursor	K.DMPGDK*IK*GKNK*LR.I	√
YFHR_SALTY	Uncharacterized protein yfhR	R.EPKQK*IFIPDGDHIDAFSGR.Y	√
YGIQ_SALTY	UPF0313 protein ygiQ	R.RQNRNTRPALTK*HTPVEHQRQGLAANK*K.R	√
YICS_SALTY	Uncharacterized protein yicS	K.DLRK*MCTPK*GALTDEAWEK*K.I	√
YIFB_SALTY	Uncharacterized protein yifB	K.K*RVIAAHERQYRRQK.K	√
YIGF_SALTY	Uncharacterized protein yigF	K.DYINDGSLSEK*WK.Y	√
YIHI_SALTY	UPF0241 protein yihI	R.RK*TREELNQEARDRK*R.L	√
YIIZ_SALTY	Uncharacterized protein yiiZ precursor	K.PFQEAVQPIYDGLK*NKPRLYGLYQRIQTAK.N	√
YJGA_SALTY	UPF0307 protein yjgA	K.MLRQRDVEPIRQALDK*LK.N	√
YNCE_SALTY	Uncharacterized protein yncE precursor	R.LYTTNADGEFITIDTASNK*ILSRKK.L	√
YNFC_SALTY	UPF0257 lipoprotein ynfC precursor	R.DAQTLEK*KVQLQGK.C	√
YQFB_SALTY	UPF0267 protein yqfB	R.K*TITIRDASESHFK.A	√
YSCR_SALTY	Virulence protein yscR	R.QFLQK*NSEEK*EANYFRNLIK.R	√

ZRAR_SALTY	Transcriptional regulatory protein zraR	K.EVILAALEK*TGGNK*TEAARQLGITRK*TLLAK.L	√	
YJDB_SALTY	UPF0141 membrane protein yjdB	K.HYDEELAHHQEGLLDIIQRAGINVLWNDNDGGCK*GACDR.V		√
ARNC_SALTY	Undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose	R.LVAK*ADEGFDVVGTVRQNRQDSLFRK.S		√
CYSD_SALTY	Sulfate adenylyltransferase subunit 2	R.QGRMIDRDQAGSMELK*K*R.Q		√
IF2_SALTY	Translation initiation factor IF-2	K.AEREAAEQAK*REAAEKAK*REAAEK.D		√
IF3_SALTY	Translation initiation factor IF-3	R.FLEEGDK*AK*ITLR.F		√
MREB_SALTY	Rod shape-determining protein mreB	R.IK*HEIGSAYPGDEVREIEVRGRNLAEGVPR.G		√
PDXH_SALTY	Pyridoxamine 5'-phosphate oxidase	R.LADPTAMVVATVDDK*GQPYQRIVLLK*HYDEK.G		√
SPED_SALTY	S-adenosylmethionine decarboxylase proenzyme	-.MKKLKLHGFNNLTK*SLSFCIYDICYAK.T		√
SRA_SALTY	Stationary-phase-induced ribosome-associated protein (SRA	R.K*VVTEGDTSSVVNNPTGRKRRADSQK		√
SYT_SALTY	Threonyl-tRNA synthetase	R.MHELAEKNYDVIK*K*KVSWHDAR.E		√
TATA_SALTY	Sec-independent protein translocase protein tatA	K.K*AMSDDDAKQDKTSQDADFTAK*SIADKQGEAK*K.E		√
TIG_SALTY	Trigger factor (TF)	R.VK*GLIEEMASAYEDPKEVIEFYSKNK*ELMDNMR.N		√
XERC_SALTY	Tyrosine recombinase xerC	R.LSELVGLDIK*HLDLDTGEVWVMGK.G		√
XGPT_SALTY	Xanthine phosphoribosyltransferase	R.LMPSEQWK*GIIAVSR.G		√
XNI_SALTY	Uncharacterized exonuclease xni	R.K*KLETHK*EMAFLCRDIARLQTDLHIDGNLQQLR.L		√
XYLA_SALTY	Xylose isomerase	R.MVEDGELDK*RVAK*RYAGWNGELGQQILK.G		√
YAJQ_SALTY	UPF0234 protein yajQ	R.AK*LLK*RGIEGASLDVPDEFVHSGK*TWYVEAK*LK.Q		√
YBAK_SALTY	Uncharacterized protein ybaK	K.KVAK*ALGAKKVDMADPMVAQRTTGYLVGGISPLGQKKR.L		√
YCFP_SALTY	UPF0227 protein ycfP	R.PEEYADIATKCVTNFREK*NR.D		√
YCHJ_SALTY	UPF0225 protein ychJ	K.ENGQWYYIDGTRPQLGRNDPCPCGSGK*K*FK.K		√
YCIH_SALTY	Uncharacterized protein yciH	R.QTSGRK*GK*GVCLITGIEMNDAELTK*LAAELK*K*K.C		√
YJBB_SALTY	Uncharacterized protein yjbB	R.EVLRIGDAMEQMMEGLKKVMHGEPREEK*ALRK.L		√

table S1. Proteomic identification of acetylated proteins in *S. enterica*.

Proteins with acetylated peptide(s) qualified by MS analysis with representative acetylated peptide are shown. Positive hits from singly charged fragments search and positive hits of allow both singly charged and mutiply charged fragments search are marked differently. Perspective acetylated lysine residues within the identified peptides are marked by asterisks. Green colored rows are proteins involved in metabolic pathways.

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Pyruvate kinase

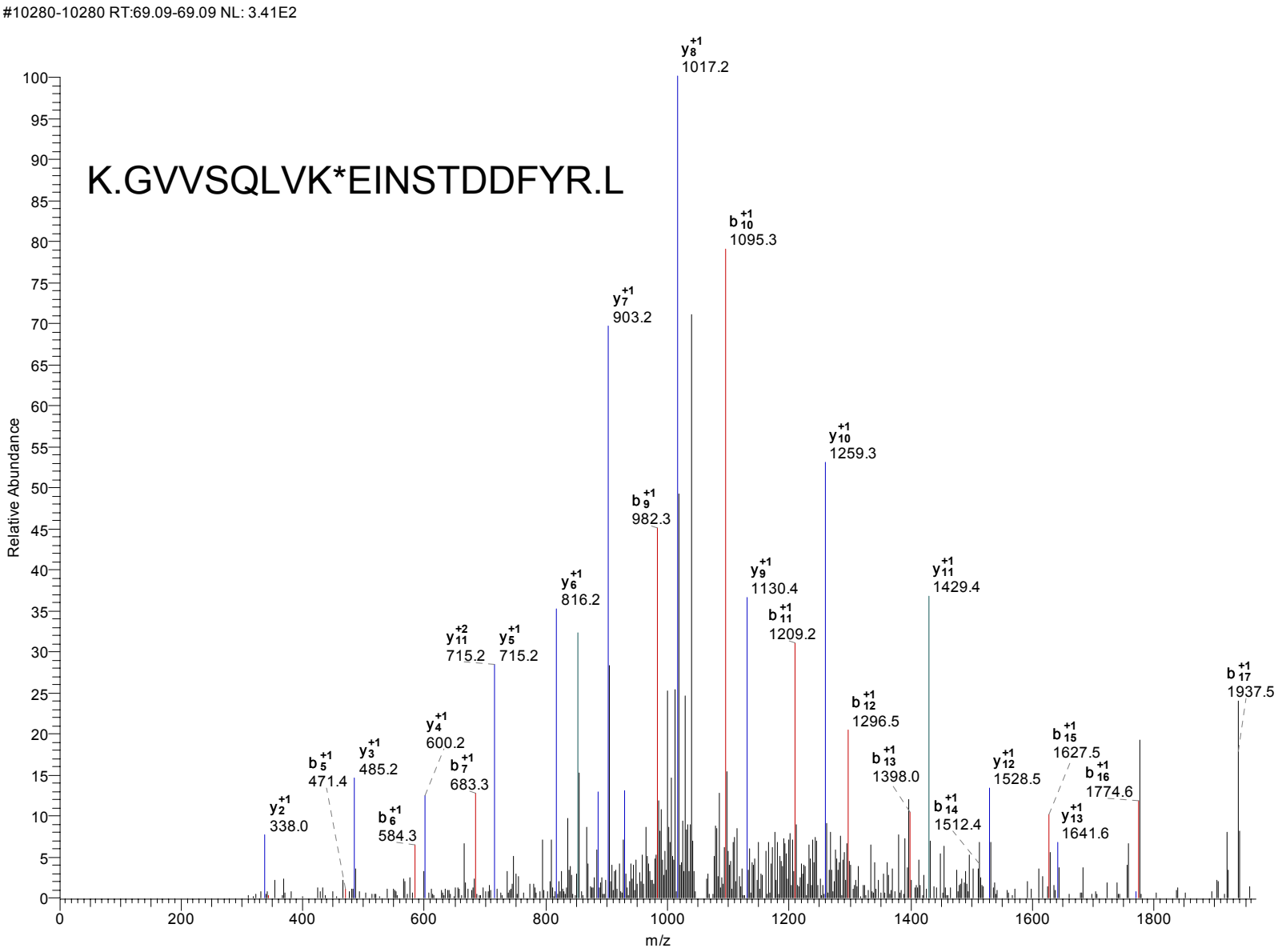

table S2

Anaerobic ribonucleoside-triphosphate reductase

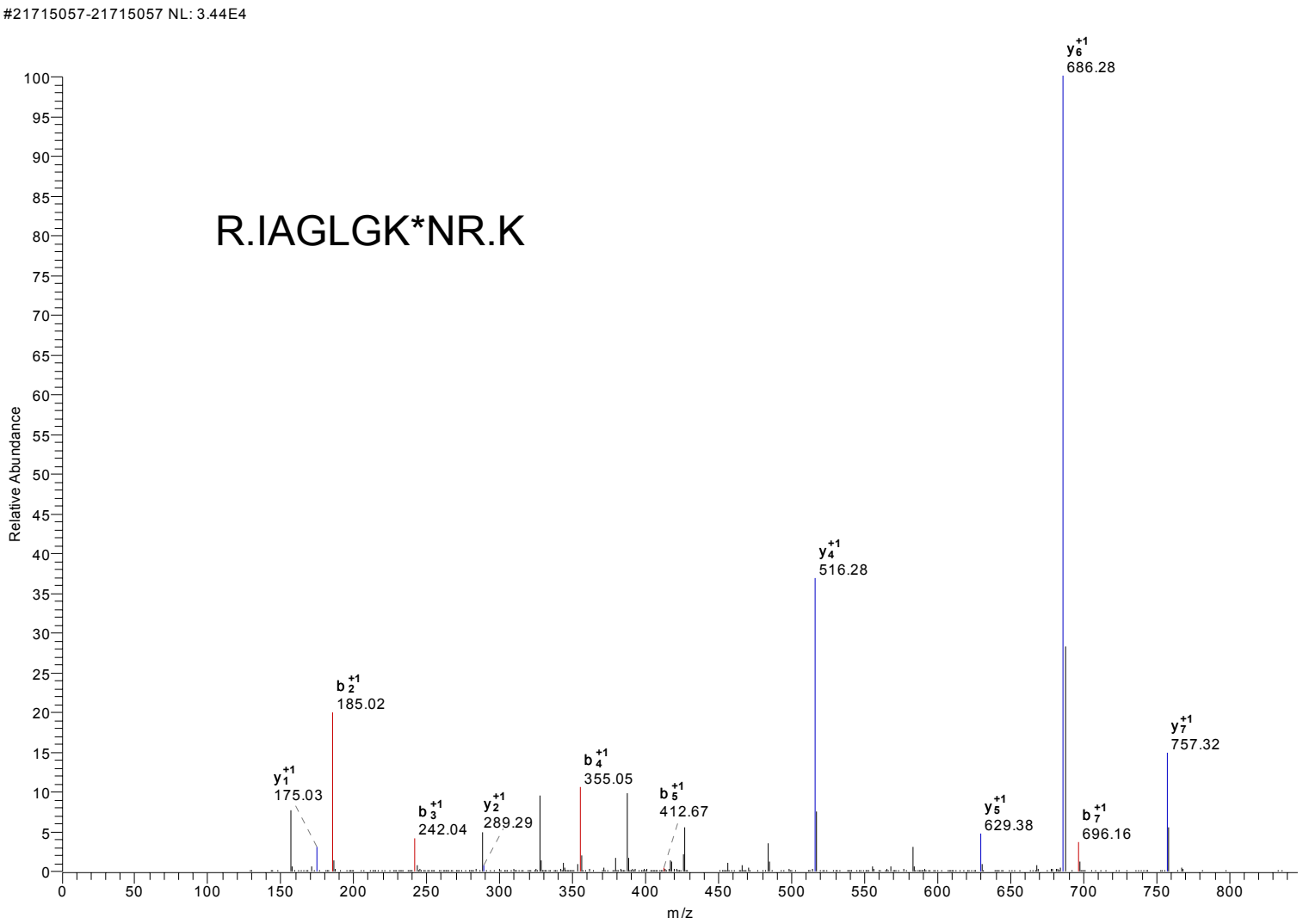

table S2

Phosphoglyceromutase

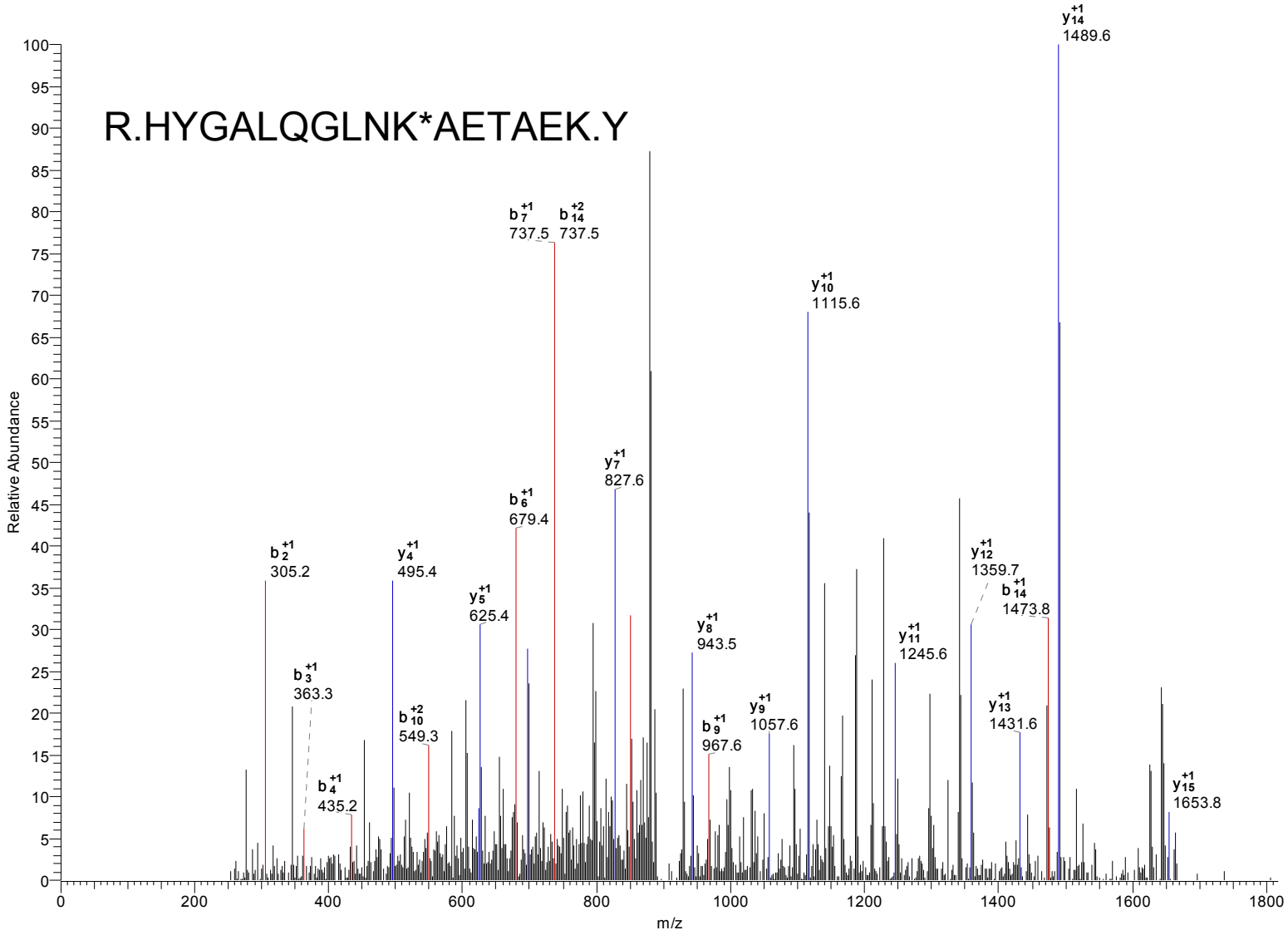

table S2

Cell invasion protein sipC

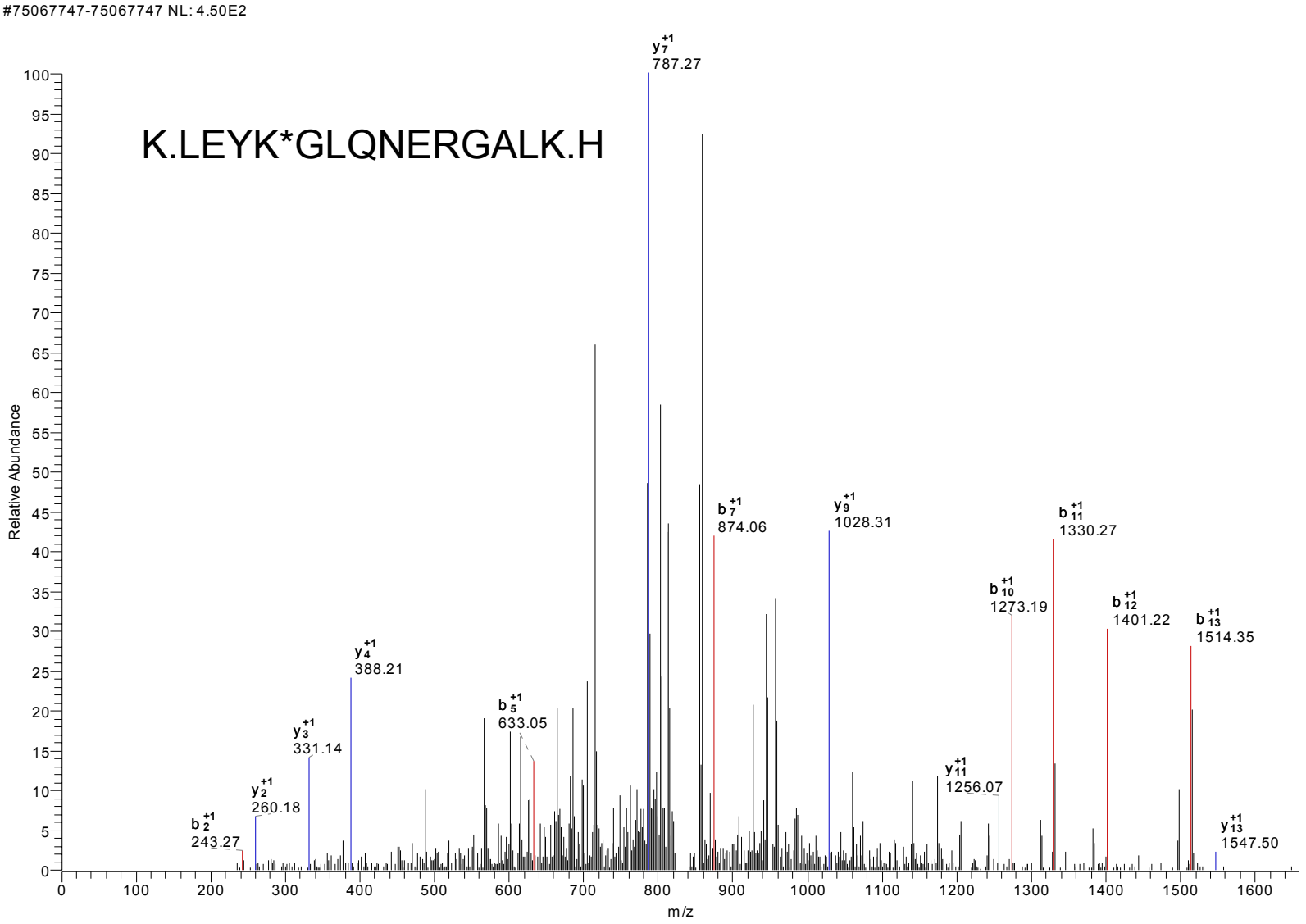

table S2

DNA topoisomerase 3

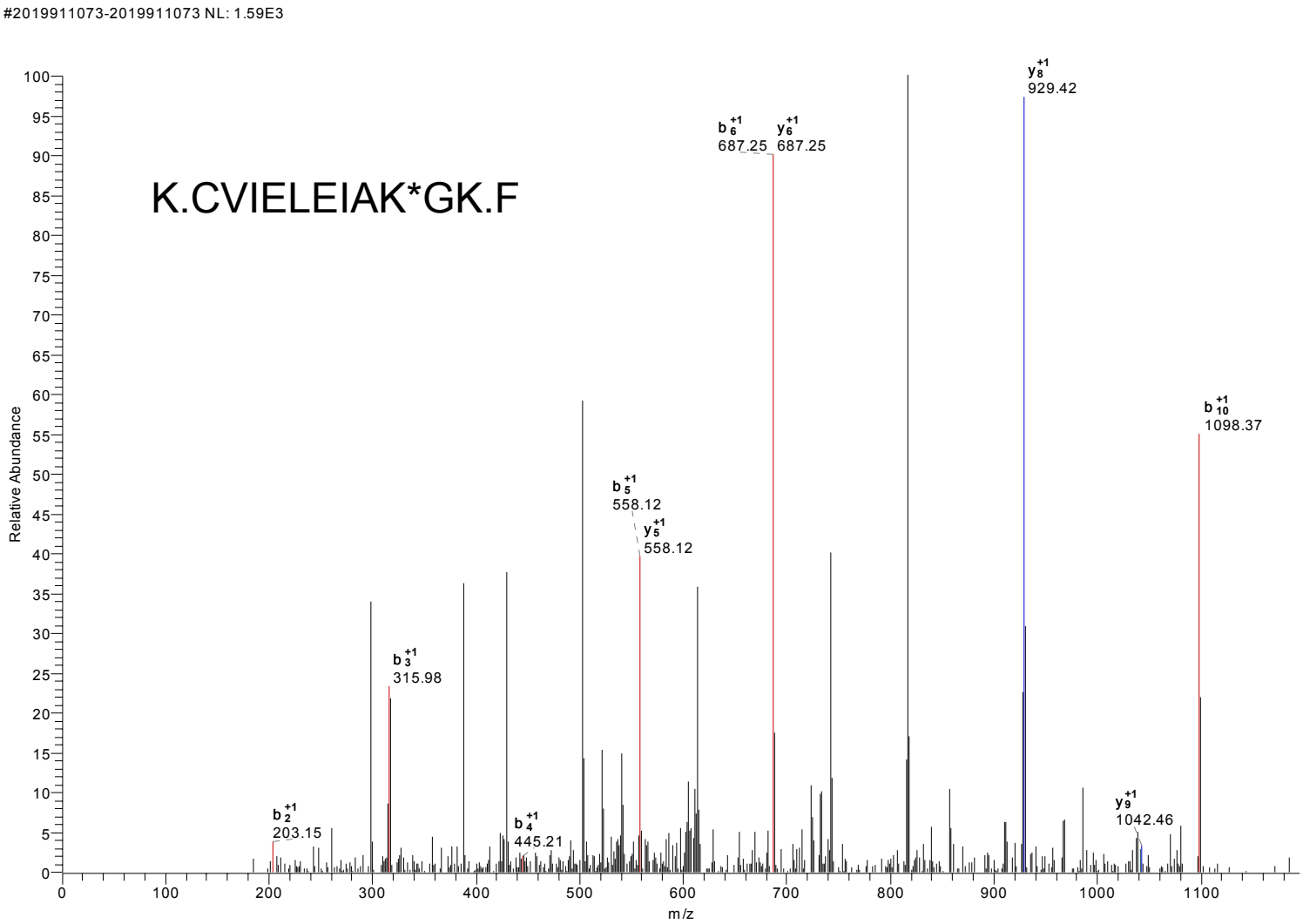

table S2

table. S2. Examples of MS identification of acetylated peptides from *S. enterica*. Mass spectra of 5 acetylated peptides from 5 different proteins of *S. enterica* are presented. Perspective acetylated lysine residues are marked.

Wang et al, table. S2

Abbreviations for metabolic enzymes shown in Figure 1:

AceA: Isocitrate Lyase; AceK: Isocitrate Dehydrogenase Kinase/Phosphatase; ACO: Aconitase; ACS: Acetyl-CoA Synthetase; AE: Aldose 1-epimerase; ALD: Fructose-bisphosphate Aldolase; APase: Acylphosphatase; CS: Citrate Synthase; ENL: Enolase; FBPase: Fructose-1,6-bisphosphatase; FH: Fumarate Hydratase; GapA: Glyceraldehyde-3-Phosphate Dehydrogenase; GK: Glucose Kinase; GPI: Phosphohexose Isomerase; ICDH: Isocitrate Dehydrogenase; MDH: Malate Dehyrogenase; MS: Malate Synthase; PDH: Pyruvate Dehydrogenase; PEPCase: Phosphoenolpyruvate Carboxylase; PEPCK: Phosphoenolpyruvate Carboxykinase; PFK: Phosphofructokinase; PGK: Phosphoglycerate Kinase; PGM: Phosphoglycerate Mutase; PK: Pyruvate Kinase; PTS: Glucose-Specific Phosphotransferase Enzyme IIA Component; SCS: Succinyl-CoA Synthetase; SDH: Succinate Dehydrogenase; TPI: Triose Phosphate Isomerase; 50S L4: ribosomal protein 50S L4; 50S L11: ribosomal protein 50S L11; 50S L14: ribosomal protein 50S L146; 50S35: ribosomal protein 50S L35.

Supplementary References:

1. Hone, D. *et al*, *J. Infect. Dis.* **156**, 167 (1987).
2. Datsenko, K.A. & Wanner, B.L., *Proc. Natl. Acad. Sci. U S A* **97**, 6640 (2000).
3. Andreev, V.P. *et al*, *J. Proteome Res.* **5**: 2039 (2006).
4. Wu, C.C. *et al*, *Anal. Chem.* **76**: 4951(2004).
5. Zamboni, N., *et al.*, *BCM Bioinformatics*. **6**:209 (2005).
6. Hua, Q. *et al*. *J Bacteriol* **185:**7053 (2003).
7. Chenguang Wang *et al*, *Methods in Molecular Biology* **241**, 207 (2004).
8. Hallows, W.C., *et al*, *Proc Natl. Acad. Sci. U S A* **103**, 10230 (2006).
9. Giachetti *et al*, E. *Experientia* **40** (1984).
10. Kim, J.Y. *et al*, *J. Basic Microbiol.* **42**, 181 (2002).
11. Wolfgang K.G. and Bucher, T., *Eur. **J.** Biochem.* **17** 568 (1970).
12. Stueland, C.S. *et al*, *J. Biol. Chem.* **263**, 19475 (1988).