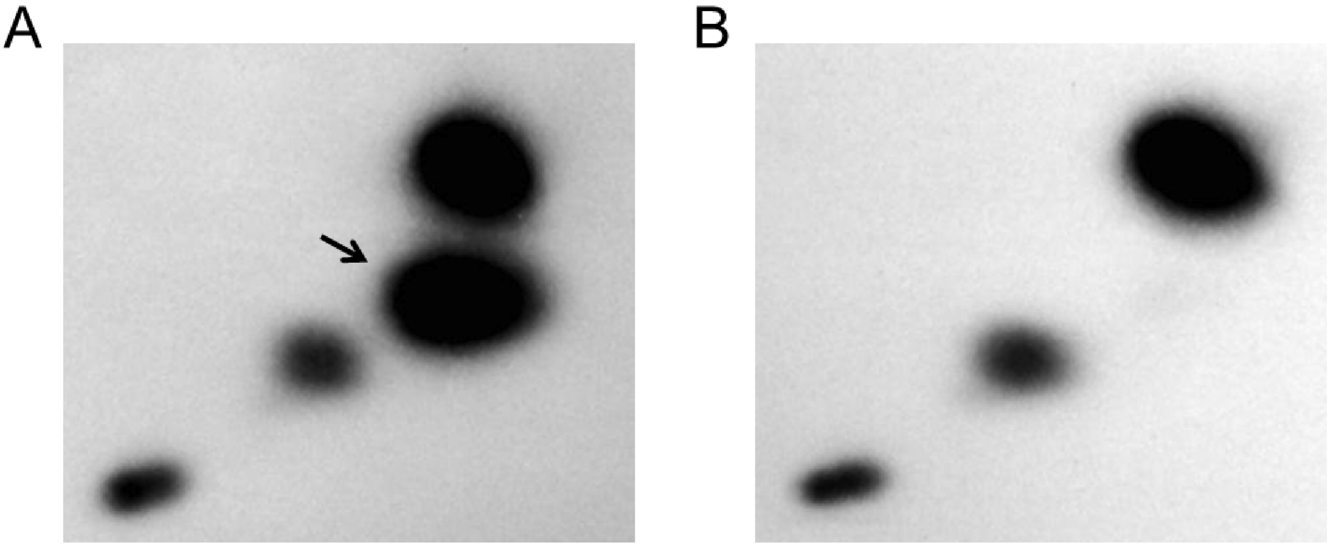

Figure S1. Identification of acetyl-phosphate (AcP) by 2D-TLC. An acid extract of *S. mutans* UA159 was prepared as described in the Methods section. Standard acetyl-[^{32}P]phosphate was synthesized as following: 4.6 µl of acetic anhydride was slowly added in mixture of 32 % [v/v] pyridine, 170 mM K_2HPO_4, and 70 µCi [^{32}P]-orthophosphate, and the mixture was incubated on ice for 2 min. After incubation, 18 µl of 4 M LiOH was added and incubated on ice for 3 min. The synthetic AcP was precipitated by adding 958 µl of ice-cold ethanol. The precipitated AcP was washed twice with 1 ml of ice-cold ethanol by centrifugation and resuspended in 125 µl distilled water. For further purification, 60 µl of ice-cold ethanol was added in the resuspendant, incubated on ice for 15 min, and collected by centrifugation. The purified labeled AcP was dried under vacuum and resuspended in 34 µl of 100 mM Tris-HCl, pH 7.0. The location of AcP was determined by 2D-TLC of either acid extract of *S. mutans* and synthetic acetyl-[^{32}P]phosphate mixture (A) or only *S. mutans* extract (B). For better detection of the synthetic AcP spot, the labeled acid extract of *S. mutans* UA159 was chromatographed five-fold less (20,000 CPM) than in the Figure 2 and 3. An arrow indicates the location of AcP.

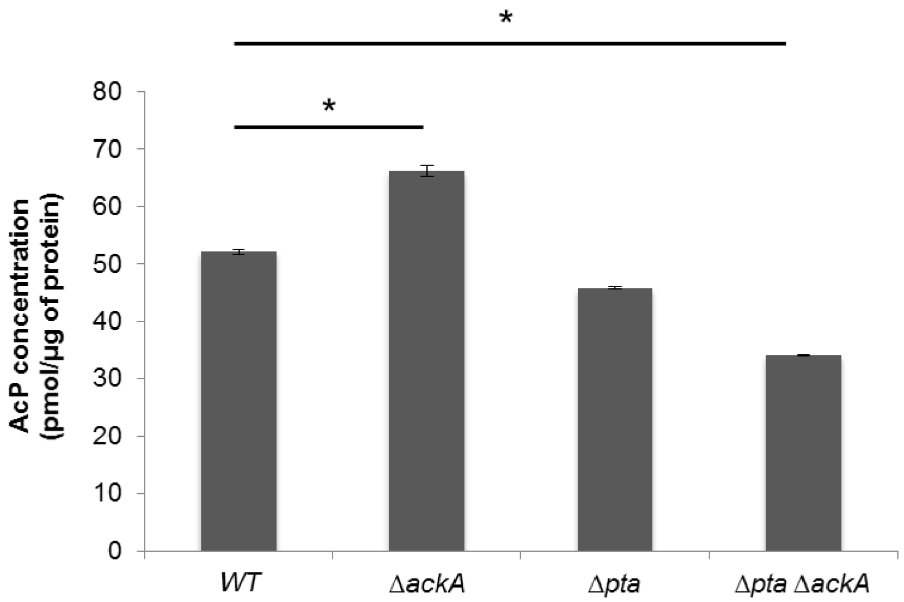

Figure S2. Measurement of intracellular AcP concentration. Exponential cells (OD_{600}= 0.4) aerobically grown in 5 ml of FMC supplemented with 25 mM glucose were washed twice with 250 µl of cold buffer A. Cells were resuspended in 250 µl of fresh cold buffer and mechanically disrupted in a Bead Beater-16 cell disrupter (Biospec Products, Inc., Bartlesville, OK) with glass beads (0.1 mm) at 4°C for 30s, twice. After centrifugation at 10,000 × *g* at 4°C for 5 min, 200 µl of supernatants were mixed with 15 mg of powdered activated charcoal (Sigma) and incubated on ice for 15 min. Aliquots (50 µl) of supernatants were used to determine protein concentration. Charcoal was removed by filtration through 13-mm diameter, 0.22-µm pore size syringe filter (Millipore). For enzymatic conversion of intracellular AcP to ATP, the filtrated extracts of 100 µl were treated with acetate kinase cocktail containing 1 mM $MgCl_2$, 30 µM ADP, and 4 µg purified *E. coli* acetate kinase (Sigma) per ml and incubated at 30°C for 90 min. Triplicate 50 µl samples of the cell lysate were each mixed with 50 µl of CellTiter-Glo reagent (Promega) in a Costar Cell Culture 96-well flat-bottom plate (Corning, Inc.). The mixtures were incubated at room temperature for 10 min and luminescence was measured using a Synergy™ 2 Multi-Mode Microplate Reader (BioTek® Instruments, Inc., USA). *, differs from the wild-type genetic background at $P < 0.01$ (Student's *t*-test).

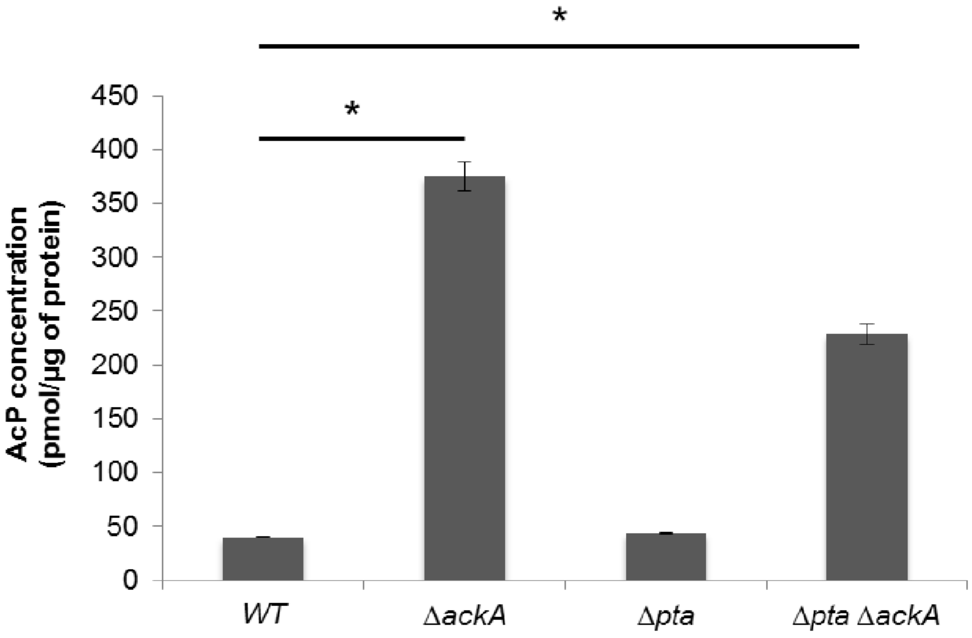

Figure S3. Measurement of intracellular AcP concentration. Experimental procedures were described in the legend of Figure S2. Exponential cells (OD_{600}= 0.4) aerobically grown in 5 ml of FMC supplemented with 25 mM glucose were subjected to 0.003% hydrogen peroxide for 1 hour. *, differs from the wild-type genetic background at $P < 0.01$ (Student's *t*-test).

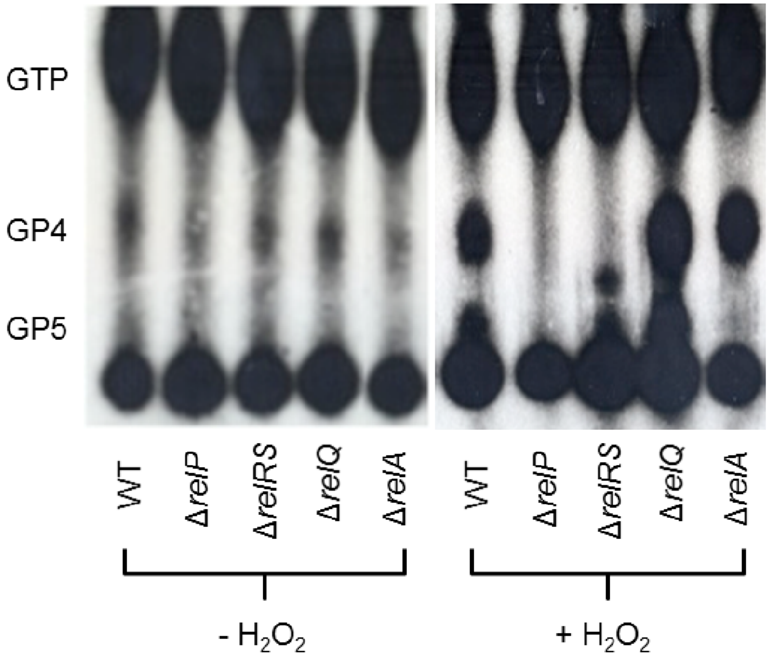

Figure S4. Accumulation of (p)ppGpp in *S. mutans* strains with and without 0.003% hydrogen peroxide. (p)ppGpp accumulation of the wild-type strain was compared with that of the Δ*relP*, Δ*relRS*, Δ*relQ*, and Δ*relA* mutants. Exponentially grown cells were labeled with [^{32}P]-orthophosphate in FMC defined medium and treated with H_2O_2 for 60 min. Nucleotides were extracted by resuspending cells in 10 µl of ice cold 13 M formic acid, followed by three freeze-thaw cycles in a dry ice-ethanol bath. The formic acid extracts were spotted onto PEI-cellulose plates for TLC using 1.5 M KH_2PO_4 buffer.

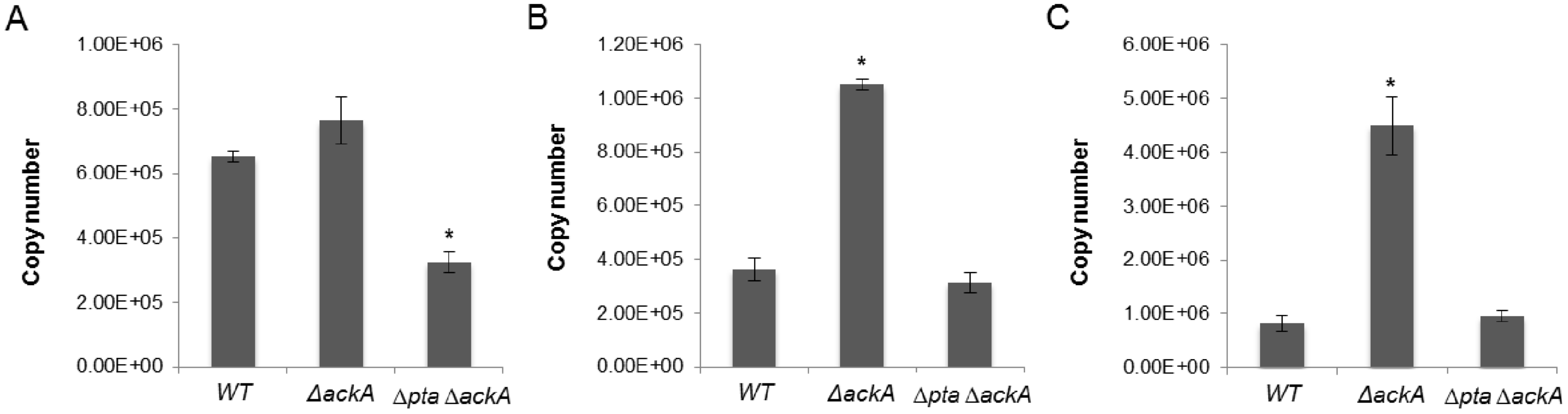

Figure S5. Effects of Pta-Ack pathway in expression of three candidate genes from transcriptome analysis. Cells were grown in triplicate to exponential phase (OD_{600} = 0.4) in defined FMC medium at 37°C in a 5% CO_2 aerobic atmosphere. The copy number of the *luxS* (A), *spxA* (B), *wapA* (C) transcripts was determined using Real-time qRT-PCR. qRT-PCR data are reported as the copies of the target RNA derived from 1 µg of input RNA after normalization to copy number of the 16S rRNA in the same sample. Values shown are the means ± standard deviations for RNAs from three separate cultures. *, differs from the wild-type genetic background at $P < 0.001$ (Student's *t*-test).

Table S1. Summary of AcP-spot density detected in this study.

Strain	**AcP-spot density**[a]		
	Oxygen (Fig. 2A)[b]	**Complementation (Fig. 2B)**	**H_2O_2 (Fig. 3B)**
WT	86.9 ± 17.9[c]	52.3 ± 15.5	70.0 ± 11.9
ΔackA	119.7 ± 9.9, $P \leq 0.05$[d]	103.2 ± 22.8, $P<0.05$	148.9 ± 20.7, $P<0.05$
Δpta	41.2 ± 11.5, $P<0.05$	ND[e]	74.6 ± 20.5
Δpta ΔackA	4.6 ± 7.3, $P<0.05$	ND	91.6 ± 5.8, $P<0.05$
Δpta + com	ND	48.8 ± 17.9	ND
ΔackA + com	ND	43.1 ± 11.2	ND

[a] Sum of pixel in area

[b] Parentheses indicate a figure number, from where the spot density has been calculated

[C] A numerical value that shows the means ± standard deviations of individual spot density

[d] A statistical value, differs from the wild-type genetic background (Student's *t*-test)

[e] ND, not determined

Table S2. Genes differentially expressed by loss of *ackA* gene, compared to the wild-type strain ($P < 0.005$).

Locus	Description	Gene Symbol	Gene Function	Fold change
SMU.1365c	permease		Transport and binding proteins; ABC Superfamily: membrane spanning permease	2.485
SMU.1941	putative membrane lipoprotein	*atmB*	Transport and binding proteins; ABC Superfamily: substrate-binding protein	2.141
SMU.506	putative type II restriction endonuclease		DNA metabolism; DNA replication, recombination, and repair	1.916
SMU.987	cell wall-associated protein precursor WapA	*wapA*	Unassigned	1.914
SMU.549	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	*murG*	Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan	1.901
SMU.557	putative cell division protein DivIVA	*divIVA*	Cellular processes; Cell division	1.879
SMU.21	putative cell shape-determining protein MreD	*mreD*	Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan	1.856
SMU.1089	hypothetical protein		Unknown	1.839
SMU.1206c	hypothetical protein		Unknown	1.815
SMU.454	putative cell division protein	*ftsL*	Cellular processes; Cell division	1.808
SMU.1050	ribose-phosphate pyrophosphokinase	*krpS*	Purines, pyrimidines, nucleosides, and nucleotides; Purine ribonucleotide biosynthesis || Cellular processes; Chaperones	1.802
SMU.1732c	hypothetical protein		Unknown	1.784
SMU.1091	cell wall protein, WapE	*wapE*	Hypothetical	1.755
SMU.1923c	transcriptional regulator NrdR	*nrdR*	Unknown	1.720
SMU.1090	hypothetical protein		Unknown	1.720
SMU.712	phosphoenolpyruvate carboxylase	*capP*	Central intermediary metabolism; Other metabolism	1.710
SMU.701c	hypothetical protein		Unassigned	1.678
SMU.1706	hypothetical protein		Unknown	1.675
SMU.1366c	putative ABC transporter, ATP-binding protein		Transport and binding proteins; ABC Superfamily: ATP-binding protein	1.661
SMU.2138	replicative DNA helicase	*dnaC*	DNA metabolism; DNA replication, recombination, and repair	1.659
SMU.716	putative peptidoglycan branched peptide synthesis protein, alanine adding enzyme, beta-lactam resistance factor MurN	*murN*	Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan || Cellular processes; Toxin production and resistance	1.655
SMU.475	hypothetical protein		Unknown	1.638
SMU.2104a	50S ribosomal protein L32	*rpmF*	Transport and binding proteins; Amino acids, peptides, and amines	1.637
SMU.864	putative ABC transporter, permease		Transport and binding proteins; ABC Superfamily: membrane	1.607

	component		spanning permease	
SMU.2084c	transcriptional regulator Spx	*spxA*	Unknown	1.575
SMU.353	hypothetical protein		Biosynthesis of cofactors, prosthetic groups, and carriers; Thiamine	1.562
SMU.917c	putative 6-pyruvoyl tetrahydropterin synthase		Biosynthesis of cofactors, prosthetic groups, and carriers; Other	1.552
SMU.1209c	hypothetical protein		Hypothetical	1.531
SMU.2074	anaerobic ribonucleoside triphosphate reductase	*nrdD*	Purines, pyrimidines, nucleosides, and nucleotides; 2'-Deoyribonucleotide metabolism	1.527
SMU.844	hypothetical protein		Unknown	1.521
SMU.1688	putative extramembranal protein, DltD protein	*dltD*	Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan	1.519
SMU.06	GTP-dependent nucleic acid-binding protein EngD		Unassigned	1.512
SMU.1302	putative surface adhesin, AdcA protein homolog, putative Zn-binding lipoprotein	*adcA*	Transport and binding proteins; ABC Superfamily: substrate-binding protein	1.510
SMU.1276c	septation ring formation regulator EzrA		Cellular processes; Cell division	1.502
SMU.697	translation initiation factor IF-3	*infC*	Protein synthesis; Translation factors	1.501
SMU.938	putative phosphomevalonate kinase		Central intermediary metabolism; Other metabolism	1.479
SMU.1060	signal recognition particle protein subunit, Ffh	*ffh*	Cellular processes; Adaptations to atypical conditions	1.465
SMU.53	hypothetical protein		Unknown	1.462
SMU.337	hypothetical protein		Unassigned	1.455
SMU.1635	putative UDP-N-acetylglucosamine pyrophosphorylase	*glmU*	Central intermediary metabolism; Other metabolism	1.452
SMU.1412c	putative ABC transporter, membrane protein subunit and ATP-binding protein		Transport and binding proteins; ABC Superfamily: membrane spanning permease || Transport and binding proteins; ABC Superfamily: ATP-binding protein	1.410
SMU.2086	competence damage-inducible protein A	*cinA*	Cellular processes; DNA transformation || DNA metabolism; DNA replication, recombination, and repair	1.405
SMU.1728	transcription elongation factor GreA	*greA*	Transcription; Transcription factors	1.394
SMU.1083c	hypothetical protein		Unknown	1.388
SMU.521	hypothetical protein		Unknown	1.359
SMU.1460	putative dTDP-4-keto-L-rhamnose reductase	*rmlC*	Cell envelope; Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides	1.350
SMU.418	transcription elongation factor NusA	*nusA*	Transcription; Transcription factors	1.344
SMU.1063	putative ABC transporter, ATP-binding protein, proline/glycine betaine transport system	*opuAa*	Transport and binding proteins; ABC Superfamily: ATP-binding protein	1.341
SMU.1122	cytidine deaminase	*cdd*	Purines, pyrimidines, nucleosides, and nucleotides; Nucleotide	1.320

			and nucleoside interconversions	
SMU.469	Holliday junction-specific endonuclease	*recU*	DNA metabolism; DNA replication, recombination, and repair	1.316
SMU.331	putative transcriptional regulator		Transcription; Transcription factors	1.314
SMU.1653	putative D-3-phosphoglycerate dehydrogenase	*serA*	Amino acid biosynthesis; Serine family	1.310
SMU.518	hypothetical protein		Unknown	1.273
SMU.1660c	DNA replication intiation control protein YabA		Unknown	1.269
SMU.1663	thymidylate kinase	*tmk*	Purines, pyrimidines, nucleosides, and nucleotides; Pyrimidine ribonucleotide biosynthesis	1.237
SMU.972	UDP-N-acetylenolpyruvoylglucosamine reductase	*murB*	Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan	1.230
SMU.1913c	putative immunity protein, BLpL-like		Hypothetical	1.219
SMU.1312	aspartate aminotransferase	*aspB*	Amino acid biosynthesis; Aspartate family	1.212
SMU.1624	ribosome recycling factor	*frr*	Protein synthesis; Translation factors	1.187
SMU.1838	preprotein translocase subunit SecA	*secA*	Protein fate; Protein and peptide secretion and trafficking	1.183
SMU.1054	putative glutamine amidotransferase		Purines, pyrimidines, nucleosides, and nucleotides; Purine ribonucleotide biosynthesis	1.165
SMU.1659c	hypothetical protein		Unknown	1.133
SMU.2165	putative SpoJ		Cellular processes; Cell division	1.126
SMU.86	hypothetical protein		Unknown	0.915
SMU.413	putative ABC transporter, ATP-binding protein		Transport and binding proteins; ABC Superfamily: ATP-binding protein	0.883
SMU.1172c	hypothetical protein		Unknown	0.874
SMU.234	threonine dehydratase	*ilvA*	Amino acid biosynthesis; Pyruvate family	0.865
SMU.1511c	putative acetyltransferase		Unassigned	0.861
SMU.1429	putative UDP-N-acetylmuramyl tripeptide synthetase MurC	*murC2*	Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan	0.818
SMU.343	hypothetical protein		Unknown	0.817
SMU.1808c	putative integrase fragment		DNA metabolism; DNA replication, recombination, and repair	0.816
SMU.1592	putative dipeptidase PepQ	*pepQ*	Protein fate; Degradation of proteins, peptides, and glycopeptides	0.806
SMU.1423	putative pyruvate dehydrogenase, TPP-dependent E1 component alpha-subunit	*pdhA*	Energy metabolism; Fermentation	0.798
SMU.857	putative uracil permease		Transport and binding proteins; Nucleosides, purines and pyrimidines	0.795
SMU.872	putative PTS system, fructose-specific enzyme IIABC component		Transport and binding proteins; Carbohydrates, organic alcohols, and acids || Signal transduction; PTS	0.795
SMU.16	putative amino acid permease		Transport and binding proteins; Amino acids, peptides, and	0.794

			amines	
SMU.441	putative transcriptional regulator		Transcription; Transcription factors	0.780
SMU.764	alkyl hydroperoxide reductase	*ahpC*	Cellular processes; Detoxification	0.774
SMU.1621c	hypothetical protein		Unknown	0.768
SMU.385	putative glycoprotein endopeptidase		Protein fate; Degradation of proteins, peptides, and glycopeptides	0.767
SMU.595	dihydroorotate dehydrogenase 1A	*pyrD*	Purines, pyrimidines, nucleosides, and nucleotides; Pyrimidine ribonucleotide biosynthesis	0.762
SMU.1611c	putative permease, multi-drug resistance efflux pump		Cellular processes; Detoxification || Transport and binding proteins; Other	0.755
SMU.1970c	putative phenylalanyl-tRNA synthetase, beta subunit		Protein synthesis; tRNA aminoacylation	0.752
SMU.1389	hypothetical protein	*pckA*	Energy metabolism; Glycolysis/gluconeogenesis	0.749
SMU.1789c	hypothetical protein		Unknown	0.748
SMU.181	putative mevalonate kinase		Biosynthesis of cofactors, prosthetic groups, and carriers; Other	0.743
SMU.1267c	hypothetical protein		Hypothetical	0.735
SMU.1578	biotin--protein ligase	*birA*	Protein fate; Protein modification and repair	0.729
SMU.1548c	putative histidine kinase		Signal transduction; Two-component systems	0.728
SMU.391c	hypothetical protein		Unknown	0.724
SMU.568	putative amino acid ABC transporter, ATP-binding protein		Transport and binding proteins; ABC Superfamily: ATP-binding protein	0.722
SMU.387	putative DNA-binding/iron metalloprotein/AP endonuclease		Protein fate; Degradation of proteins, peptides, and glycopeptides	0.722
SMU.638	putative 16S pseudouridylate synthase		Protein synthesis; tRNA and rRNA base modification	0.719
SMU.2036	putative peptidase	*pepO*	Protein fate; Degradation of proteins, peptides, and glycopeptides	0.718
SMU.260	hypothetical protein		Unknown	0.714
SMU.634	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	*queA*	Protein synthesis; tRNA and rRNA base modification	0.700
SMU.952	homocysteine methyltransferase	*mmuM*	Energy metabolism; Amino acids and amines	0.697
SMU.1073	formate--tetrahydrofolate ligase	*fthS*	Purines, pyrimidines, nucleosides, and nucleotides; Purine ribonucleotide biosynthesis || Amino acid biosynthesis; Aspartate family || Amino acid biosynthesis; Histidine family	0.692
SMU.435	putative N-acetylglucosamine-6-phosphate deacetylase		Energy metabolism; Other	0.691
SMU.572	putative tetrahydrofolate dehydrogenase/cyclohydrolase	*folD*	Biosynthesis of cofactors, prosthetic groups, and carriers; Folic acid || Amino acid biosynthesis; Aspartate family	0.687
SMU.318	putative hippurate hydrolase		Central intermediary metabolism; Other metabolism || Protein synthesis; Degradation of proteins, peptides, and	0.685

			glycopeptides	
SMU.1646c	hemolysis inducing protein		Unknown	0.683
SMU.2127	putative succinate semialdehyde dehydrogenase		Central intermediary metabolism; Other metabolism	0.675
SMU.1931	16S rRNA methyltransferase GidB	*gidB*	Cellular processes; Cell division	0.668
SMU.151	hypothetical protein		Unassigned	0.657
SMU.1109c	putative integral membrane protein, permease		Unknown	0.650
SMU.349	dimethyladenosine transferase	*ksgA*	Cellular processes; Toxin production and resistance	0.642
SMU.1573	S-adenosylmethionine synthetase	*metK*	Central intermediary metabolism; Other metabolism	0.641
SMU.913	glutamate dehydrogenase		Amino acid biosynthesis; Glutamate family	0.638
SMU.1869	putative thioredoxin	*trxA*	Energy metabolism; Electron transport	0.626
SMU.18	hypothetical protein		Hypothetical	0.625
SMU.390	hypothetical protein		Hypothetical	0.618
SMU.1867c	putative alcohol dehydrogenase		Energy metabolism; Fermentation	0.603
SMU.951	putative amino acid permease		Transport and binding proteins; Amino acids, peptides, and amines	0.590
SMU.493	formate acetyltransferase (pyruvate formate-lyase 2)	*pfl2*	Energy metabolism; Fermentation	0.589
SMU.530c	hypothetical protein		Unknown	0.580
SMU.125	hypothetical protein		Unknown	0.567
SMU.1399	hypothetical protein		Hypothetical	0.560
SMU.925	hypothetical protein		Hypothetical	0.557
SMU.1298	50S ribosomal protein L31 type B	*rpmE2*	Protein synthesis; Ribosomal proteins: synthesis and modification	0.553
SMU.1203	branched-chain amino acid aminotransferase	*ilvE*	Amino acid biosynthesis; Pyruvate family	0.541
SMU.564	hypothetical protein		Unknown	0.529
SMU.1702c	putative phosphatase		Unassigned	0.505
SMU.924	thiol peroxidase	*tpx*	Cellular processes; Adaptations to atypical conditions	0.442
SMU.929c	hypothetical protein		Unknown	0.430
SMU.1978	putative acetate kinase	*ackA*	Energy metabolism; Anaerobic	0.036

Table S3. Genes differentially expressed by loss of both *pta* and *ackA* genes, compared to the wild-type strain ($P < 0.005$).

Locus	Description	Gene Symbol	Gene Function	Fold-change
SMU.1013c	putative Mg2+/citrate transporter		Transport and binding proteins; Carbohydrates, organic alcohols, and acids	5.127
SMU.1023	oxaloacetate decarboxylase	*pycB*	Amino acid biosynthesis; Pyruvate family	4.962
SMU.1016	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	*bcc*	Fatty acid and phospholipid metabolism; Other	4.896
SMU.1022	2'-(5''-triphosphoribosyl)-3'-dephospho-CoA:apo- citrate lyase	*citX*	Unknown	4.723
SMU.1018	hypothetical protein		Hypothetical	4.561
SMU.1365c	permease		Transport and binding proteins; ABC Superfamily: membrane spanning permease	3.989
SMU.1366c	putative ABC transporter, ATP-binding protein		Transport and binding proteins; ABC Superfamily: ATP-binding protein	3.522
SMU.1020	putative citrate lyase CilB, cytryl-CoA lyase, beta subunit	*cilB*	Energy metabolism; Fermentation || Energy metabolism; TCA cycle	3.504
SMU.1360c	hypothetical protein		Hypothetical	3.302
SMU.1012c	putative transcriptional regulator		Transcription; Transcription factors	2.686
SMU.1342	putative bacitracin synthetase 1, BacA	*bacA1*	Cellular processes; Toxin production and resistance	2.349
SMU.53	hypothetical protein		Unknown	2.009
SMU.671	citrate synthase	*citZ*	Energy metabolism; TCA cycle	1.895
SMU.58	hypothetical protein		Hypothetical	1.863
SMU.2005	adenylate kinase	*adk*	Purines, pyrimidines, nucleosides, and nucleotides; Nucleotide and nucleoside interconversions	1.687
SMU.131	putative lipoate-protein ligase	*lplA*	Protein fate; Protein modification and repair	1.656
SMU.129	branched-chain alpha-keto acid dehydrogenase subunit E2	*adhC*	Energy metabolism; Glycolysis/gluconeogenesis	1.631
SMU.52	hypothetical protein		Unknown	1.604
SMU.287	putative ComB, accessory factor for ComA		Transport and binding proteins; ABC Superfamily: substrate-binding protein || Cellular processes; Competence	1.505
SMU.1081c	hypothetical protein		Unknown	1.436
SMU.1910c	hypothetical protein		Hypothetical	1.426
SMU.521	hypothetical protein		Unknown	1.391
SMU.2033c	hypothetical protein		Unknown	1.362
SMU.419	hypothetical protein		Unknown	1.353
SMU.1392c	putative acetyltransferase		Unknown	1.328

SMU.915c	7-cyano-7-deazaguanine reductase		Unknown	1.319
SMU.782	hypothetical protein		Unknown	1.309
SMU.1096	putative ABC transporter, ATP-binding protein, choline transporter	*opuBa*	Transport and binding proteins; ABC Superfamily: ATP-binding protein	1.307
SMU.365	glutamate synthase (large subunit)	*gltA*	Amino acid biosynthesis; Glutamate family	1.297
SMU.1063	putative ABC transporter, ATP-binding protein, proline/glycine betaine transport system	*opuAa*	Transport and binding proteins; ABC Superfamily: ATP-binding protein	1.249
SMU.691	peptidase T	*pepT*	Protein fate; Degradation of proteins, peptides, and glycopeptides	1.226
SMU.2142	hypothetical protein	*rpiB*	Energy metabolism; Pentose phosphate pathway	1.218
SMU.109	permease (efflux protein)		Transport and binding proteins; Other	1.186
SMU.982	putative BglB fragment	*bglB2*	Energy metabolism; Sugars	1.097
SMU.1850	putative aminopeptidase P	*pepP*	Protein fate; Degradation of proteins, peptides, and glycopeptides	1.077
SMU.1936c	hypothetical protein		Unknown	0.868
SMU.613	hypothetical protein		Hypothetical	0.868
SMU.73	hypothetical protein		Unknown	0.857
SMU.1612c	hypothetical protein		Unknown	0.852
SMU.841	putative aminotransferase		Biosynthesis of cofactors, prosthetic groups, and carriers; Other	0.844
SMU.1802c	hypothetical protein		Unknown	0.831
SMU.413	putative ABC transporter, ATP-binding protein		Transport and binding proteins; ABC Superfamily: ATP-binding protein	0.828
SMU.1957	putative PTS system, mannose-specific IID component		Signal transduction; PTS	0.820
SMU.1292c	hypothetical protein		Unknown	0.810
SMU.973	putative spermidine/putrescine ABC transporter, ATP-binding protein	*potA*	Transport and binding proteins; ABC Superfamily: ATP-binding protein	0.800
SMU.1655c	hypothetical protein		Hypothetical	0.800
SMU.996	ferrichrome ABC transporter permease protein		Transport and binding proteins; ABC Superfamily: membrane spanning permease	0.799
SMU.761	putative protease		Unassigned	0.796
SMU.1621c	hypothetical protein		Unknown	0.779
SMU.1223	dihydroorotate dehydrogenase 1B	*pyrDB*	Purines, pyrimidines, nucleosides, and nucleotides; Pyrimidine ribonucleotide biosynthesis	0.772
SMU.67	putative acyltransferase		Unassigned	0.765

SMU.1467	adenine phosphoribosyltransferase	*apt*	Purines, pyrimidines, nucleosides, and nucleotides; Salvage of nucleosides and nucleotides	0.762
SMU.1676c	hypothetical protein		Transport and binding proteins; Carbohydrates, organic alcohols, and acids	0.757
SMU.942	putative hydroxymethylglutaryl-CoA reductase	*mvaA*	Energy metabolism; Other	0.745
SMU.2126c	putative purine-nucleoside phosphorylase		Purines, pyrimidines, nucleosides, and nucleotides; Salvage of nucleosides and nucleotides	0.738
SMU.1288	50S ribosomal protein L19	*rplS*	Protein synthesis; Ribosomal proteins: synthesis and modification	0.736
SMU.1446c	putative ABC transporter, permease protein		Transport and binding proteins; ABC Superfamily: membrane spanning permease	0.734
SMU.880	multiple sugar-binding ABC transporter, permease protein MsmG	*msmG*	Transport and binding proteins; Carbohydrates, organic alcohols, and acids || Energy metabolism; Sugars || Transport and binding proteins; ABC Superfamily: membrane spanning permease	0.715
SMU.725c	hypothetical protein		Unknown	0.713
SMU.929c	hypothetical protein		Unknown	0.711
SMU.857	putative uracil permease		Transport and binding proteins; Nucleosides, purines and pyrimidines	0.705
SMU.1119c	putative sugar ABC transporter, permease protein		Transport and binding proteins; ABC Superfamily: membrane spanning permease	0.704
SMU.133c	putative MDR permease, transmembrane efflux protein		Transport and binding proteins; Carbohydrates, organic alcohols, and acids	0.701
SMU.1593c	putative CDP-diglyceride synthetase		Unknown	0.696
SMU.838	glutathione reductase	*gshR*	Biosynthesis of cofactors, prosthetic groups, and carriers; Glutathione and analogs || Cellular processes; Detoxification	0.686
SMU.1324	putative cell-division protein FtsX	*ftsX*	Cellular processes; Cell division || Transport and binding proteins; ABC Superfamily: membrane spanning permease	0.681
SMU.1389	hypothetical protein	*pckA*	Energy metabolism; Glycolysis/gluconeogenesis	0.678
SMU.1950	putative pseudouridylate synthase		Protein synthesis; tRNA and rRNA base modification	0.673
SMU.1587c	hypothetical protein		Hypothetical	0.668
SMU.387	putative DNA-binding/iron metalloprotein/AP endonuclease		Protein fate; Degradation of proteins, peptides, and glycopeptides	0.664
SMU.1203	branched-chain amino acid aminotransferase	*ilvE*	Amino acid biosynthesis; Pyruvate family	0.660
SMU.2146c	hypothetical protein		Unknown	0.657
SMU.1570	putative maltose/maltodextrin ABC transporter, MalG permease	*malG*	Transport and binding proteins; ABC Superfamily: membrane spanning permease	0.657
SMU.1958c	putative PTS system, mannose-		Signal transduction; PTS	0.656

	specific IIC component			
SMU.621c	hypothetical protein		Unknown	0.653
SMU.119	putative alcohol dehydrogenase class III	*adh*	Energy metabolism; Fermentation	0.653
SMU.1109c	putative integral membrane protein, permease		Unknown	0.651
SMU.2056	recombination factor protein RarA		Cellular processes; Cell division	0.647
SMU.1323	hydrolase		Unknown	0.645
SMU.523	hypothetical protein		Unknown	0.644
SMU.1722c	putative integral membrane protein		Unknown	0.644
SMU.1266	imidazole glycerol phosphate synthase subunit HisH	*hisH*	Amino acid biosynthesis; Histidine family	0.639
SMU.636	putative N-acetylglucosamine-6-phosphate isomerase		Central intermediary metabolism; Amino sugars	0.632
SMU.570	putative ferrous ion transport protein B	*feoB*	Transport and binding proteins; Cations and iron carrying compounds	0.631
SMU.856	bifunctional pyrimidine regulatory protein PyrR uracil phosphoribosyltransferase	*pyrR*	Regulatory functions; DNA interactions || Purines, pyrimidines, nucleosides, and nucleotides; Pyrimidine ribonucleotide biosynthesis	0.623
SMU.2055	putative acetyltransferase		Unassigned	0.622
SMU.1828	hypothetical protein		Unknown	0.617
SMU.2075c	hypothetical protein		Unknown	0.606
SMU.771c	hypothetical protein		Hypothetical	0.604
SMU.1902c	hypothetical protein		Hypothetical	0.603
SMU.474	S-ribosylhomocysteinase	*luxS*	Cellular processes; Other	0.601
SMU.321	hypothetical protein		Unknown	0.600
SMU.495	glycerol dehydrogenase	*gldA*	Energy metabolism; Sugars	0.599
SMU.1807c	putative integral membrane protein, permease		Unassigned	0.592
SMU.388	putative integral membrane protein, branched-chain amino acid permease		Transport and binding proteins; Amino acids, peptides, and amines	0.592
SMU.291	transketolase	*tkt*	Energy metabolism; Pentose phosphate pathway	0.587
SMU.1399	hypothetical protein		Hypothetical	0.582
SMU.911c	hypothetical protein		Hypothetical	0.582
SMU.1115	L-lactate dehydrogenase	*ldh*	Energy metabolism; Glycolysis/gluconeogenesis	0.580

SMU.1649	putative exodeoxyribonuclease III	*exoA*	DNA metabolism; DNA replication, recombination, and repair	0.572
SMU.303	hypothetical protein		Unknown	0.561
SMU.564	hypothetical protein		Unknown	0.544
SMU.1450	putative amino acid permease		Unassigned	0.491
SMU.1545c	hypothetical protein		Unknown	0.474
SMU.493	formate acetyltransferase (pyruvate formate-lyase 2)	*pfl2*	Energy metabolism; Fermentation	0.454
SMU.1043c	phosphotransacetylase	*pta*	Energy metabolism; Fermentation	0.243
SMU.1978	putative acetate kinase	*ackA*	Energy metabolism; Anaerobic	0.040

Table S4. Genes differentially expressed by loss of *ackA* versus both *pta* and *ackA* genes (*P* < 0.005).

Locus	Description	*Gene symbol*	Gene Function	Fold-change
SMU.1043c	phosphotransacetylase	*pta*	Energy metabolism; Fermentation	4.88
SMU.1419	putative transcriptional regulator		Transcription; Transcription factors	2.23
SMU.935	putative amino acid ABC transporter, permease protein		Transport and binding proteins; ABC Superfamily: membrane spanning permease	2.21
SMU.81	heat shock protein GrpE (HSP-70 cofactor)	*grpE*	Protein fate; Protein folding and stabilization	2.18
SMU.80	heat-inducible transcription repressor	*hrcA*	Regulatory functions; DNA interactions	2.15
SMU.1941	putative membrane lipoprotein	*atmB*	Transport and binding proteins; ABC Superfamily: substrate-binding protein	1.99
SMU.932	hypothetical protein		Unknown	1.94
SMU.496	putative cysteine synthetase A, O-acetylserine lyase	*cysK*	Amino acid biosynthesis; Serine family	1.91
SMU.880	multiple sugar-binding ABC transporter, permease protein MsmG	*msmG*	Transport and binding proteins; Carbohydrates, organic alcohols, and acids || Energy metabolism; Sugars || Transport and binding proteins; ABC Superfamily: membrane spanning permease	1.9
SMU.01	chromosomal replication initiation protein	*dnaA*	DNA metabolism; DNA replication, recombination, and repair	1.89
SMU.987	cell wall-associated protein precursor WapA	*wapA*	Unassigned	1.85
SMU.1412c	putative ABC transporter, membrane protein subunit and ATP-binding protein		Transport and binding proteins; ABC Superfamily: membrane spanning permease || Transport and binding proteins; ABC Superfamily: ATP-binding protein	1.77
SMU.459	putative ABC transporter, amino acid binding protein		Transport and binding proteins; ABC Superfamily: substrate-binding protein	1.76
SMU.1206c	hypothetical protein		Unknown	1.67
SMU.2084c	transcriptional regulator Spx	*spxA*	Unknown	1.62
SMU.1050	ribose-phosphate pyrophosphokinase	*krpS*	Purines, pyrimidines, nucleosides, and nucleotides; Purine ribonucleotide biosynthesis || Cellular processes; Chaperones	1.6
SMU.864	putative ABC transporter, permease component		Transport and binding proteins; ABC Superfamily: membrane spanning permease	1.6
SMU.1938c	putative ABC transporter, permease protein		Transport and binding proteins; ABC Superfamily: membrane spanning permease	1.6
SMU.1659c	hypothetical protein		Unknown	1.59

SMU.1727	OxaA-like protein precursor		Cell envelope; Other	1.57
SMU.353	hypothetical protein		Biosynthesis of cofactors, prosthetic groups, and carriers; Thiamine	1.51
SMU.712	phosphoenolpyruvate carboxylase	*capP*	Central intermediary metabolism; Other metabolism	1.5
SMU.1958c	putative PTS system, mannose-specific IIC component		Signal transduction; PTS	1.47
SMU.244	undecaprenyl pyrophosphate phosphatase	*uppP*	Cellular processes; Detoxification || Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan	1.44
SMU.2086	competence damage-inducible protein A	*cinA*	Cellular processes; DNA transformation || DNA metabolism; DNA replication, recombination, and repair	1.42
SMU.551	cell division protein FtsA	*ftsA*	Cellular processes; Cell division	1.42
SMU.1457	putative dTDP-glucose-4,6-dehydratase	*rmlB*	Cell envelope; Surface polysaccharides, lipopolysaccharides and antigens	1.41
SMU.1570	putative maltose/maltodextrin ABC transporter, MalG permease	*malG*	Transport and binding proteins; ABC Superfamily: membrane spanning permease	1.4
SMU.586	hypothetical protein		Unknown	1.39
SMU.1324	putative cell-division protein FtsX	*ftsX*	Cellular processes; Cell division || Transport and binding proteins; ABC Superfamily: membrane spanning permease	1.39
SMU.1204	DNA topoisomerase IV subunit A	*parC*	DNA metabolism; DNA replication, recombination, and repair	1.39
SMU.1195	permease		Transport and binding proteins; ABC Superfamily: membrane spanning permease	1.35
SMU.1957	putative PTS system, mannose-specific IID component		Signal transduction; PTS	1.33
SMU.1663	thymidylate kinase	*tmk*	Purines, pyrimidines, nucleosides, and nucleotides; Pyrimidine ribonucleotide biosynthesis	1.32
SMU.1936c	hypothetical protein		Unknown	1.31
SMU.1935c	hypothetical protein		Unknown	1.31
SMU.469	Holliday junction-specific endonuclease	*recU*	DNA metabolism; DNA replication, recombination, and repair	1.29
SMU.1460	putative dTDP-4-keto-L-rhamnose reductase	*rmlC*	Cell envelope; Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides	1.26
SMU.1653	putative D-3-phosphoglycerate dehydrogenase	*serA*	Amino acid biosynthesis; Serine family	1.26
SMU.1655c	hypothetical protein		Hypothetical	1.25
SMU.858	aspartate carbamoyltransferase catalytic subunit	*pyrB*	Purines, pyrimidines, nucleosides, and nucleotides; Pyrimidine ribonucleotide biosynthesis	1.25
SMU.1054	putative glutamine amidotransferase		Purines, pyrimidines, nucleosides, and nucleotides; Purine	1.25

			ribonucleotide biosynthesis	
SMU.2074	anaerobic ribonucleoside triphosphate reductase	*nrdD*	Purines, pyrimidines, nucleosides, and nucleotides; 2'-Deoyribonucleotide metabolism	1.25
SMU.1801c	GTP-binding protein YqeH	*yqeH*	Unknown	1.21
SMU.841	putative aminotransferase		Biosynthesis of cofactors, prosthetic groups, and carriers; Other	1.19
SMU.972	UDP-N-acetylenolpyruvoylglucosamine reductase	*murB*	Cell envelope; Biosynthesis and degradation of murein sacculus and peptidoglycan	1.16
SMU.328	putative carbonic anhydrase		Unknown	0.87
SMU.1573	S-adenosylmethionine synthetase	*metK*	Central intermediary metabolism; Other metabolism	0.75
SMU.53	hypothetical protein		Unknown	0.73
SMU.59	adenylosuccinate lyase	*purB*	Purines, pyrimidines, nucleosides, and nucleotides; Purine ribonucleotide biosynthesis	0.73
SMU.764	alkyl hydroperoxide reductase	*ahpC*	Cellular processes; Detoxification	0.72
SMU.952	homocysteine methyltransferase	*mmuM*	Energy metabolism; Amino acids and amines	0.72
SMU.349	dimethyladenosine transferase	*ksgA*	Cellular processes; Toxin production and resistance	0.71
SMU.1808c	putative integrase fragment		DNA metabolism; DNA replication, recombination, and repair	0.7
SMU.530c	hypothetical protein		Unknown	0.67
SMU.1702c	putative phosphatase		Unassigned	0.65
SMU.125	hypothetical protein		Unknown	0.64
SMU.365	glutamate synthase (large subunit)	*gltA*	Amino acid biosynthesis; Glutamate family	0.64
SMU.367	hypothetical protein		Unassigned	0.64
SMU.1073	formate--tetrahydrofolate ligase	*fthS*	Purines, pyrimidines, nucleosides, and nucleotides; Purine ribonucleotide biosynthesis || Amino acid biosynthesis; Aspartate family || Amino acid biosynthesis; Histidine family	0.63
SMU.925	hypothetical protein		Hypothetical	0.61
SMU.36	hypothetical protein		Unknown	0.61
SMU.128	putative acetoin dehydrogenase (TPP-dependent), E1 component beta subunit	*adhB*	Energy metabolism; Fermentation	0.57
SMU.924	thiol peroxidase	*tpx*	Cellular processes; Adaptations to atypical conditions	0.52
SMU.671	citrate synthase	*citZ*	Energy metabolism; TCA cycle	0.47
SMU.1366c	putative ABC transporter, ATP-binding protein		Transport and binding proteins; ABC Superfamily: ATP-binding protein	0.47

SMU.129	branched-chain alpha-keto acid dehydrogenase subunit E2	*adhC*	Energy metabolism; Glycolysis/gluconeogenesis	0.46
SMU.1012c	putative transcriptional regulator		Transcription; Transcription factors	0.42
SMU.1017	putative oxaloacetate decarboxylase, sodium ion pump subunit	*oadB*	Amino acid biosynthesis; Pyruvate family	0.37
SMU.1360c	hypothetical protein		Hypothetical	0.35
SMU.1020	putative citrate lyase CilB, citryl-CoA lyase, beta subunit	*cilB*	Energy metabolism; Fermentation || Energy metabolism; TCA cycle	0.29
SMU.1018	hypothetical protein		Hypothetical	0.24
SMU.1023	oxaloacetate decarboxylase	*pycB*	Amino acid biosynthesis; Pyruvate family	0.23
SMU.1022	2'-(5''-triphosphoribosyl)-3'-dephospho-CoA:apo- citrate lyase	*citX*	Unknown	0.21
SMU.1013c	putative Mg2+/citrate transporter		Transport and binding proteins; Carbohydrates, organic alcohols, and acids	0.21
SMU.1016	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	*bcc*	Fatty acid and phospholipid metabolism; Other	0.15