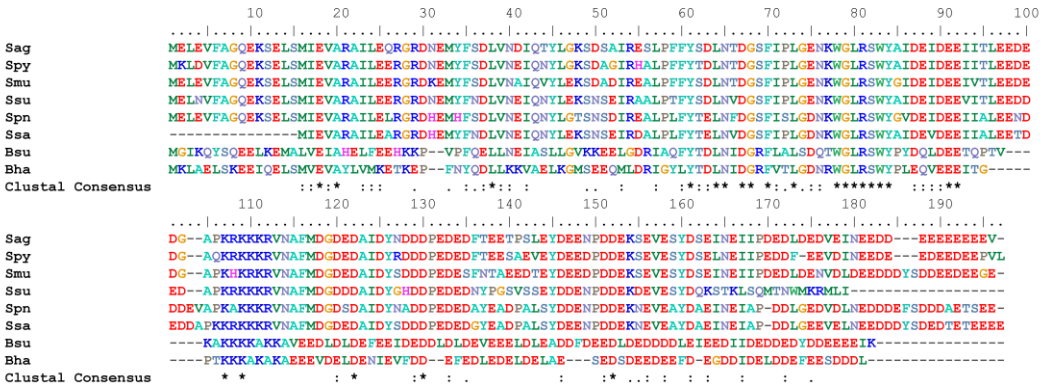
SUPPLEMENTARY

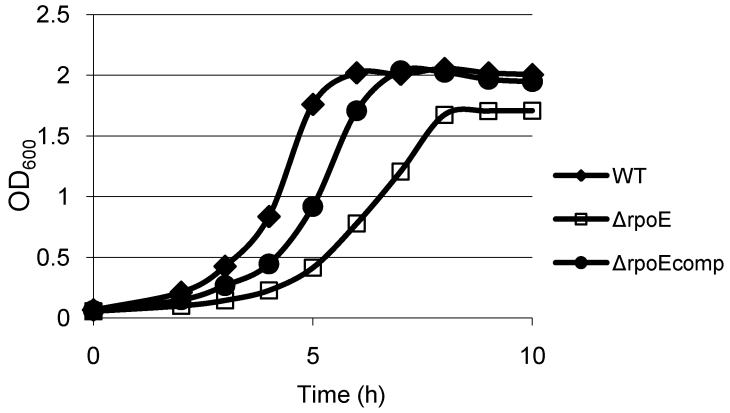
Figure S1



S1. Amino acid sequence alignment of delta proteins using CLUSTAL W 2.0.

Identical amino acid residues are marked with a star, strongly similar with two dots,and weakly similar with one dot. The default colourscheme is used for alignments. Species abbreviations: Sag, *S. agalactiae*; Spy: *S. pyogenes* M1 GAS; Smu, *S. mutans* UA159; Ssu, *S. suis* 98HAH33; Spn, *S. pneumoniae* G54; Ssa, *S. sanguinis* SK36; Bsu: *Bacillus subtilis* ; Bha: *B. halodurans* C-125.

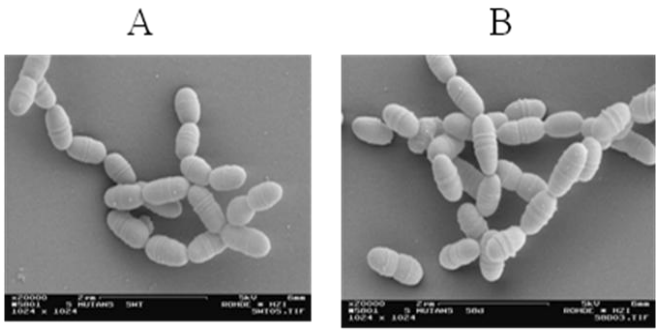
Figure S2



S2. Growth defects of *S. mutans* Δ*rpoE* strain.

The growth of *S. mutans* cultures in THBY medium pH7.5 at 37 ℃ in an atmosphere of 5% CO_2 were recorded every hour by measuring the absorbance (optical density) at 600nm. Strains: WT (wild type), Δ*rpoE*, Δ*rpoE*comp (Δ*rpoE* complementation). The data is a representative result from at least three independent experiments.

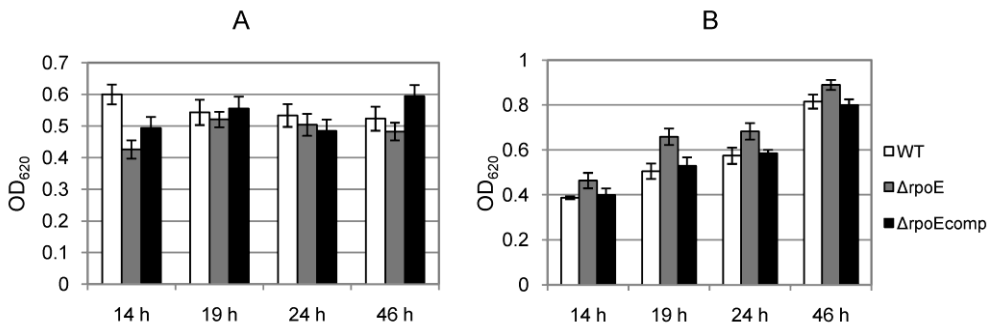
Figure S3



S3. *S. mutans* wild type and Δ*rpoE* strains cell phenotype under scanning electron microscopy.

A: wild type strain; B: Δ*rpoE* mutant.

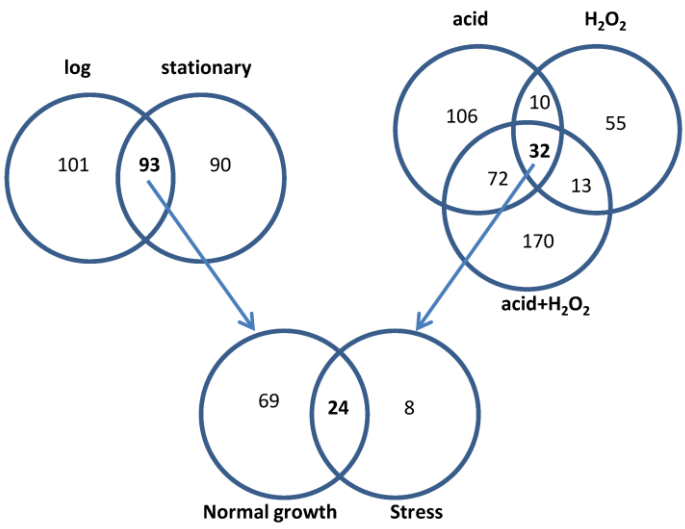
Figure S4



S4. Biofilm quantification by crystal violet staining.

Biofilm of *S. mutans* wild-type (blue columns), Δ*rpoE* (red columns), and Δ*rpoE*comp (green columns) grown in 96-well microtiter plates in THBYS (A) and BMS (B) medium were stained at different time points with 0.1% crystal violet and the absorbance of the extracted stain was measured at 620 nm. Data presented above is representative result from at least three independent experiments. Mean and standard deviation of twelve replicas of each sample are given.

Figure S5



S5. Venn diagram showing the number of differentially expressed genes in the Δ*rpoE* mutant compared to the wild type strain under 5 experimental conditions.

Sample collection was as following: Log, M0/W0; stationary, M1/W1; acid, M2/W2; H_2O_2, M3/W3; acid + H_2O_2, M4/W4.

Figure S6

smu-r1

A

>chromosome Streptococcus mutans UA159 (Nucleotides 530356 - 530591 Reverse Strand)

-35

GAGTTACTCCTCTAAAATGATATGACATATTATATTACAATTTGAATAAAAATACAAGTT

-10 Transcription start

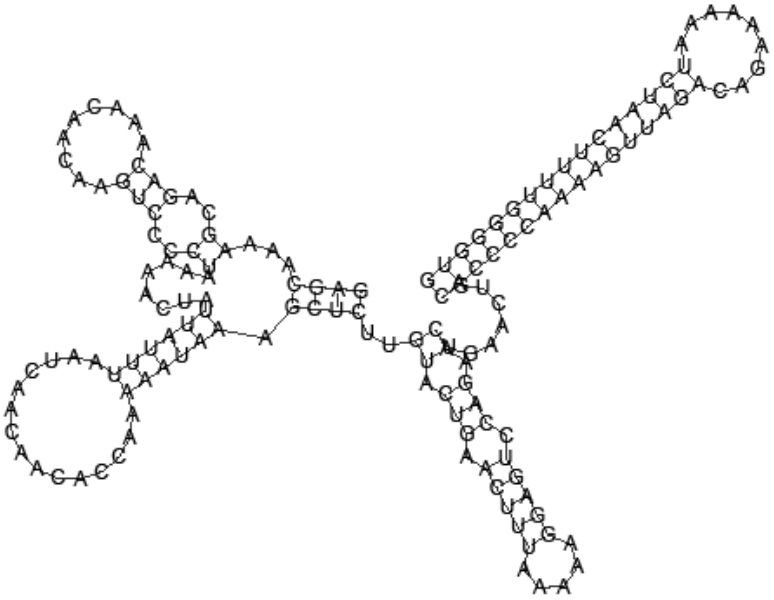
ACCTTTTAACAATTACAA**G**AGCAAAAGCAGACAAACAACAAGTCCCCTAAAAACTATTA

TTTAATCAACAACACCAAAAATAAAGCTCTTGTACTGAACTTTAAAAAGGAGTCCAGAAC

TGAACTGCACCCCAAAAGTTAGACAGAAAAAATCTAACTTTTGGGGTGTTTTTATT

Terminator

B



C

r1.1 **SMU.948** hypothetical protein

```
sRNA              2   AGCAAAAGCAGACAAACAACAAGUCCCC--UAAAAACUAUUAUUUAAUCAACAACACCAAAAAUA  64
                      ||  ||  ||||  |  |||  ||||:||  |   ||||||  | :|  |  |||     ||||||||||||
mRNA (SMU.948)   69   UCCUUGUCGUGUAUUUUAUGUUUAGUGUUUAUUUUU--UCGUCAUUUA-----UGUGGUUUUUAU  12
```

r1.2 **SMU.1025** putative transcriptional regulator

```
sRNA                    100     ACUGAACUGCACCCCAAA      117
                                ||||||||||||||||||
mRNA (SMU.1025)         -89     UGACUUGACGUGGGGUUU      -106
```

r1.3 **SMU.2059c** putative integral membrane protein

```
sRNA                    1       GAGCAAA-AGCAGACAAACAACAAGUCCCCUAAAAACUAUU---AUUUAAUCAACA        52
                                ||||||  |: |:||| | |||||||||  | | || | ||   |||||| | |||
mRNA (SMU.2059c)        55      CUCGUUAAUUCUUUGUCUCUUGUUCAGG--ACUAUUAAAAACUUUAAAUUCGCUGU        2
```

r1.4 **SMU.327** DNA repair protein RadA

```
sRNA                    48      CAACAACACCAA-AAAUA--AAGCUCU-UGUACUGAACUUU       84
                                || |||||||||| ||||   ||:|||| ||:||:|  ||||
mRNA (SMU.327)          -119    GUAGUUGUGGUUAUUUAAGAUUUGAGAGACGUGGC--GAAA      -157
```

r1.5 **SMU.1507c** hypothetical protein

```
sRNA                    28      CCUAAAAACU-AUU----AUUUAAUCAACAACA       55
                                |||| ||||| ||:    |||| ||||||||||
mRNA (SMU.1507c)        71      GGAUAUUUGAGUAGCAGUUAAAAUAGUUGUUGU       39
```

S6. Predicted smu-r1 promoter and terminator (A), secondary structure (B), and interaction with target mRNAs (C). A: The putative -35 and -10 regions were marked by green colour. The sequence of smu-r1 was underlined. The predicted transcription start was indicated by bold, while the putative terminator was marked by red colour.

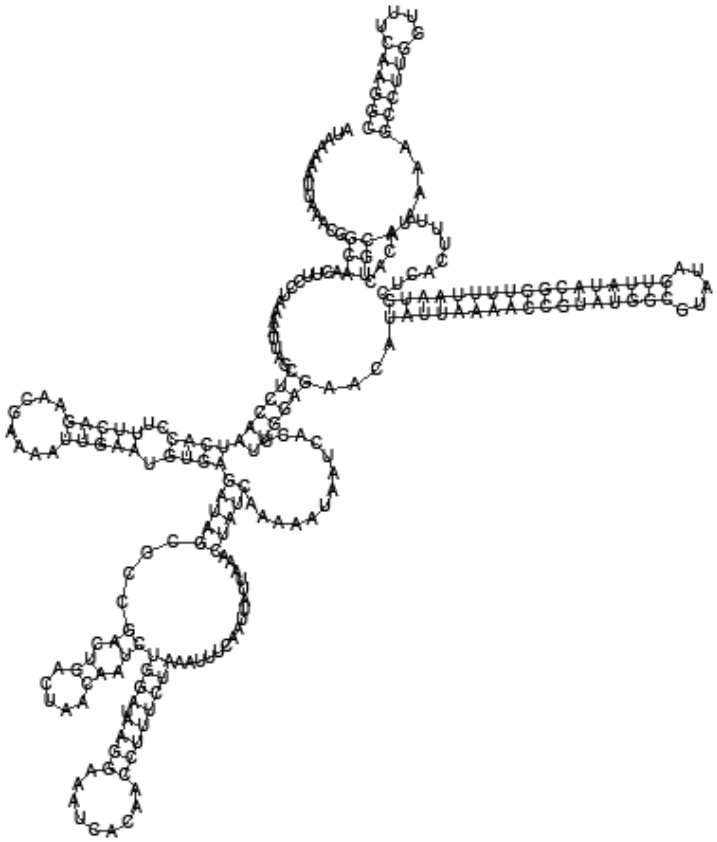
Figure S7

smu-r2

A

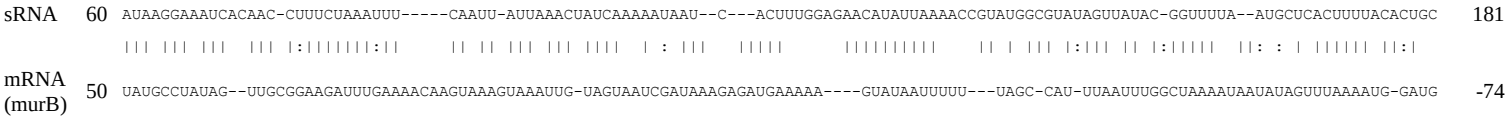
>chromosome Streptococcus mutans UA159 (Nucleotides 739911 - 740429 Reverse Strand)
TTCATATCCTCCCCTAACATTCAATAATAATTTAAAGCAAACCAGATAATCTTATTTTAC
AGACAATCATACCTTATTACATTTTACTTCTCACTTCAAAGAATAGCTGCCAAAACAACA
GACTAATCACTCCGCTTATATTATAGCATAAAATATTATGCAGAAGTTAGGATTATATAA
TGTAGCGAAAGTCAAAGCTCAGCAATATTGCAGCATTCTATACTAACAACATAACTTGAG
-35 -10 Transcription start
AGTGACATTAACAATAAAAATTAAACGGCAACTTCCTAAAATTAGCTCCAATCACCTTTC
AGAACGAAAATTGAATGTGAGATAGCGCCGACTGACTAACAATCTGGATAAGGAAATCAC
AACCTTTCTAAATTTCAATTATTAAACTATCAAAAATAATCACTTTGGAGAACATATTAA
AACCGTATGGCGTATAGTTATACGGTTTTAATGCTCACTTTTACACTGCAAAAAGCCTTG
GTTTCAAGGCTTTTCATTGCTACGATTTGTCAAAGGTTT
Terminator

B



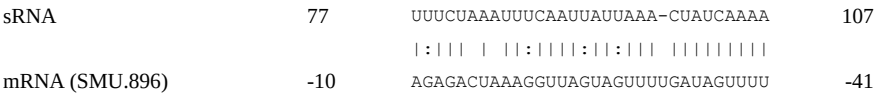
C

r2.1 **murB** UDP-N-acetylenolpyruvoylglucosamine reductase



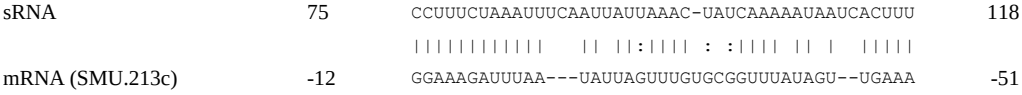
r2.2 **SMU.896** hypothetical protein

Score: -97 Pvalue: 0.000684607

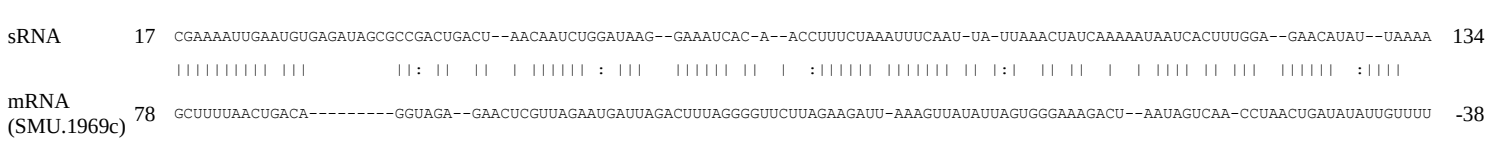


r2.3 **SMU.213c** hypothetical protein

Score: -88 Pvalue: 0.00236569

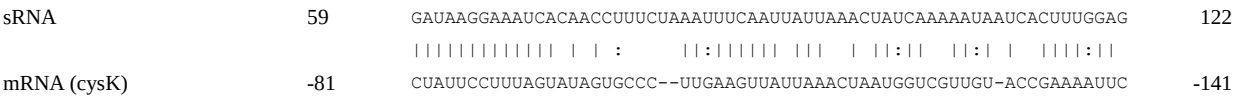


r2.4 **SMU.1969c** putative transcriptional regulator



r2.5 **cysK** putative cysteine synthetase A; O-acetylserine lyase

Score: -83 Pvalue: 0.00470788



r2.6 **SMU.375** hypothetical protein

Score: -82 Pvalue: 0.00540194

```
                                  ||| | :|:|||| | |   ||||||||| |||  |  ||    |||||| |||:|||
mRNA (SMU.375)          -79       CUUCCUUUUUAUCACAG---ACUGAUUGU-AGAAGUUGUC----AGUGUUUGAAGGAU      -128
```

r2.7 **SMU.1392c** putative acetyltransferase

Score: -80 Pvalue: 0.00711096

```
sRNA                 8   CUUUCAGAAC--GAAAAUUGAAUGUGAGAUAGCGCCGACUGACUAACAAUCUGGAUAAGGAAAUCACAACCUUU--CUAAAUUUCAAUUAUU--AAACU----AUCAAAAA   108
                         ||||||||||  ||| ||:   |||:|  | | |||:| |  ||||     |:||:| :||| ||    |||||  ||   |||  ||: ||  |||||    ||||||||
mRNA (SMU.1392c)    46   GAAAGUCUUGUCCUUAUAG---ACAUUGAA-CACGGUU-A--GAUU-----AUCUGUAUCUUCAGA---GGAAAAAGA---AAAUAUAGAAAACUUUGAAUCAUAGUUUUU   -47
```

r2.8 **SMU.874** bifunctional homocysteine S-methyltransferase/5,10-methylenetetrahydrofolate reductase protein

Score: -80 Pvalue: 0.00711096

```
sRNA  48  CUAACAAUCUGGAUAAGGAAA-UCACAACCUUUCUAAA-UUUC--AAUUAUUAAACUAUCAAAAAUAAUCACUUUGGAGAACAUAUUAAAACCGU-AUGGC-GUA-UAGUUAUACGGUU-UUAAUGC-UCACUUUUACACUGCAA  183
          |:| |||||||||:| ||  | || |  ||||:||  | ||||  |||| || |     |||  |  | | || | ||  || |:|||||| | | :|| |:| | ||| |  |||||| |:|| |   ||||| ||::|||
mRNA
(SMU.  16  GGUCGUUAGACCUGUACCAGUGAGGG--GGAAGGAAAUCAAAGGAUUAAAAACU-----GCUUGAAGGAAUCAA-CGUC--GU-UGAUUUUGGAAGUUUCGUCGUUACCAA-AGACCAAGAACUGCGCA---AAAAUUUGGUGUU  -114
874)
```

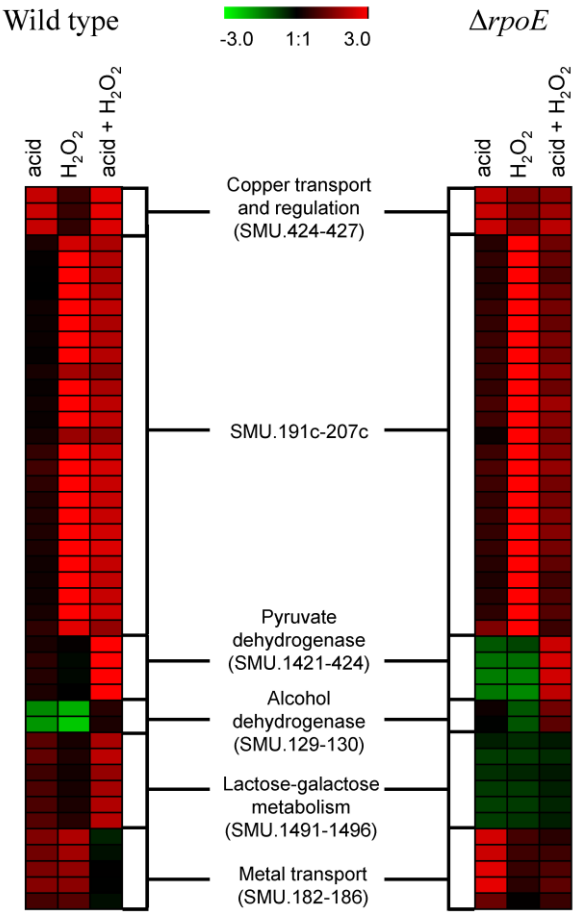
r2.9 **SMU.564** hypothetical protein

Score: -78 Pvalue: 0.00935811

```
sRNA              21   AAUUGAAUGUGA--GAUAG-C-GCCGACUGACUAACAAUCUGGAUAAG-----GAAAUCACAACCUUUCUAAAUUUCAA-UUAUU   95
                       ||||||||||:|  |||   | |||   |||  || | ||||| || |     ||||| ||| | |:| | | ||||||  || ||
mRNA (SMU.564)   -113   UUAACUUACAUUUGCUAAAAGACGGAGAACUUUUUAUCAGACCGAUACGAAAACUUUA-UGUCGUAGACAGU-AAAGUUCAAGAA  -195
```

S7. Predicted smu-r2 promoter and terminator (A), secondary structure (B), and interaction with target mRNAs (C). A: The putative -35 and -10 regions were marked by green colour. The sequence of smu-r2 was underlined. The predicted transcription start was indicated by bold, while the putative terminator was marked by red colour.

Figure S8



S8. Hierarchical clustering of differentially expressed genes under stresses in *S. mutans* wild type and Δ*rpoE* mutant strains.

Gene expression ratios are calculated as log2 fold changes under stresses comparing to the control (log-phase cells, before treatment). Red: up-regulated genes; green: down-regulated genes.

Table T1. Inhibition of growth of *S. mutans* wild type, Δ*rpoE*, and Δ*rpoE*comp strains by tetracycline and kanamycin.

A: Tetracycline (μg/ml) [a]

	1	0.8	0.6	0.4	0.2
WT	+	+	+	+	+
Δ*rpoE*	-	-	-	+[b]	+
Δ*rpoE*comp	+[c]	+	+	+	+

B: Kanamycin (μg/ml)

	100	80	60	40	20
WT	+	+	+	+	+
Δ*rpoE*	-	-	+	+	+
Δ*rpoE*comp	-	+[c]	+	+	+

[a] +: Visible growth; -: no visible growth.

[b] Δ*rpoE* mutant forms flocks at the presence of 0.4 μg/ml tetracycline.

[c] Δ*rpoE*comp strain showed weaker growth than the wild type strain.

Table T2. Differentially expressed non-coding regions in the Δ*rpoE* mutant compared to the wild type strain under 5 experimental conditions, including normal growth at log-phase or early stationary-phase, growth under acid stress, H_2O_2 stress, and acid+H_2O_2-stressed growth.

		Log2 fold change (Δ*rpoE* / wild type)						
#	non-coding regions_start_stop / upstream gene-function / down-stream gene-function	log-phase	stationary-phase	acid-stress	H_2O_2-stress	Acid+H_2O_2-stress	Gene Dir type	Terminator start_stop
1.	NC_004350__530356_530591	1.52	1.65	1.18	1.13	1.37	<-->	530364_530404
	SMU.566c-hypothetical protein	-0.30	-0.18	0.28	-0.51	-0.43		
	SMU.567-putative glutamine ABC transporter, permease	-0.50	-0.26	-0.48	-0.37	0.21		
2.	NC_004350__739911_740429	1.16	1.55	1.45	1.19	2.19	->->	739940_739955
	SMU.788-putative RNA methyltransferase	0.39	0.69	0.34	0.30	0.84		
	SMU.789-hypothetical protein	0.35	0.31	0.30	0.05	0.98		
3.	NC_004350__862809_862923	1.18	1.52	1.37	1.27	1.67	->->	862854_862874
	SMU.906-putative ABC transporter, ATP-binding protein	0.50	0.35	0.48	0.28	1.06		
	SMU.909-putative permease	0.40	0.54	0.39	0.36	0.36		
4.	NC_004350__1894335_1894524	1.25	1.58	1.82	1.17	1.66	<-->	-
	SMU.2027-putative transcriptional regulator	0.22	0.50	1.28	0.14	-0.51		
	SMU.2028-levansucrase precursor; beta-D-fructosyltransferase	0.25	0.12	-1.67	0.71	-1.12		
5.	NC_004350__1912245_1912417	1.93	1.16	1.69	1.13	1.68	<-->	-
	SMU.2038-putative PTS system, trehalose-specific IIABC component	-0.23	-0.96	-0.65	-1.16	0.91		
	SMU.2040-putative transcriptional regulator; repressor of the trehalose operon	-0.03	-0.24	0.14	0.24	0.13		

6.	NC_004350__1919190_1919336	1.53	1.71	1.42	1.29	2.25	<-<-	-
	SMU.2044-putative stringent response protein, ppGpp synthetase	-0.49	-0.15	-0.14	0.03	-0.86		
	SMU.2046c-hypothetical protein	1.51	0.26	0.87	-0.30	1.23		
7.	NC_004350__368829_368962	1.83	2.31	2.35	1.51	2.31	<-->	-
	SMU.392c-hypothetical protein	0.71	1.18	1.03	0.73	0.71		
	SMU.393-hypothetical protein	-0.30	0.13	1.25	-0.57	0.92		
8.	NC_004350__381368_381675	1.03	2.46	2.48	1.54	3.31	<-->	-
	SMU.406c-hypothetical protein	-0.18	0.02	0.23	-0.29	0.36		
	SMU.407-hypothetical protein	0.22	0.13	0.15	0.54	0.71		
9.	NC_004350__401703_402053	1.34	1.48	1.32	1.29	1.64	<-->	-
	SMU.429c-hypothetical protein	-0.24	-0.04	-0.23	-0.43	-0.39		
	SMU.431-putative ABC transporter, ATP-binding protein	0.60	0.41	0.52	0.53	-0.07		

-: no terminator was found.

Table T3. Differentially expressed genes in the Δ*rpoE* mutant compared to the wild type under log-phase or early stationary-phase growth conditions.

Locus Tag	Gene symbol	Gene description	Log2 fold change (Δ*rpoE* / wild type)	
			Log-phase	Early stationary-phase
Carbohydrate transport and metabolism, energy production and conversion				
SMU.870	*fruR*	putative transcriptional regulator	-2.71	-0.70
SMU.871	*pfkB*	putative fructose-1-phosphate kinase	-2.70	-0.64
SMU.872	*fxpC*	putative fructose-PTS II ABC	-2.75	-0.51
SMU.877	*agaL*	alpha-galactosidase	3.60	0.96
SMU.878	*msmE*	multiple sugar-binding ABC transporter, sugar-binding protein	3.60	0.65
SMU.879	*msmF*	multiple sugar-binding ABC transporter, permease	3.25	0.36
SMU.880	*msmG*	multiple sugar-binding ABC transporter, permease	3.41	0.58
SMU.881	*gtfA*	sucrose phosphorylase	3.04	0.22
SMU.882	*msmK*	multiple sugar-binding ABC transporter, ATP-binding protein	3.00	0.21
SMU.883	*dexB*	dextran glucosidase DexB	3.10	0.23
SMU.885	*galR*	galactose operon repressor GalR	2.00	0.64
SMU.886	*galK*	galactokinase	3.86	0.37
SMU.887	*galT*	galactose-1-phosphate uridylyltransferase	3.76	0.48

SMU.888	*galE*	UDP-galactose 4-epimerase, GalE	1.72	0.88
SMU.113	*pfk*	putative fructose-1-phosphate kinase	0.59	-4.25
SMU.114	*fruC*	putative fructose-PTS IIBC	0.63	-4.17
SMU.115	*fruD*	putative fructose-PTS IIA	0.43	-3.05
SMU.116	*lacD2*	tagatose 1,6-diphosphate aldolase	0.79	-3.47
SMU.1956c	*levX*	hypothetical protein	1.49	2.91
SMU.1957	*levG*	putative frutose/mannose-PTS IID	1.34	2.71
SMU.1958c	*levF*	putative frutose/mannose-PTS IIC	1.18	2.55
SMU.1960c	*levE*	putative frutose/mannose-PTS IIB	1.58	3.13
SMU.1961c	*levD*	putative frutose/mannose-PTS IIA	1.50	3.17
SMU.78	*fruA*	fructan hydrolase	3.14	2.64
SMU.79	*fruB*	fructan hydrolase	2.46	2.56
SMU.1421	*pdhC*	branched-chain alpha-keto acid dehydrogenase subunit E2	1.18	-0.99
SMU.1422	*pdhB*	putative pyruvate dehydrogenase E1 component beta subunit	1.75	-1.29
SMU.1423	*pdhA*	putative pyruvate dehydrogenase	1.78	-2.04
SMU.1424	*pdhD*	putative dihydrolipoamide dehydrogenase	1.70	-2.15
SMU.148	*adhE*	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	0.98	-1.35
		Amino acid transport		
SMU.1062	*opuAb*	putative proline/glycine betaine ABC transporter, permease protein	-1.55	-1.84
SMU.1063	*opuAa*	putative proline/glycine betaine ABC transporter, ATP-binding	-1.54	-1.93

SMU.2116	*opuCa*	putative osmoprotectant amino acid ABC transporter, ATP-binding	-1.14	-0.95
SMU.2117	*opuCb*	putative osmoprotectant ABC transporter; permease	-1.19	-1.02
SMU.2118	*opuCc*	putative osmoprotectant-binding protein	-1.17	-1.04
SMU.2119	*opuCd*	putative osmoprotectant ABC transporter; permease protein	-1.2	-1.09
		Iron transport		
SMU.995	-	ferrichrome ABC transporter, permease	0.89	0.88
SMU.996	-	ferrichrome ABC transporter, permease	0.95	1.12
SMU.997	-	ferrichrome ABC transporter, ATP-binding	1.09	1.35
SMU.998	-	ferrichrome ABC transporter, periplasmic ferrichrome-binding	1.06	1.27

Table T4. Confirmation of differential gene expression by quantitative PCR in comparison to the microarray results.

	Microarray Fold change (Δ*rpoE* / wild type)						Real-time PCR Fold change (Δ*rpoE* / wild type)				
	M0/W0	M1/W1	M2/W2	M3/W3	M4/W4	ajusted p value	M0/W0	M1/W1	M2/W2	M3/W3	M4/W4
levD	2.83	8.99	0.95	0.33	0.65	1.25 E-08	6.88 ±0.06	19.76 ±0.08	0.38 ±0.48	0.25 ±0.04	0.17 ±0.52
msmE	12.15	1.57	0.44	0.99	1.44	1.35 E-12	23.17 ±0.22	1.74 ±0.42	0.31 ±0.13	0.72 ±0.01	0.80 ±0.06
hisC	0.25	0.21	0.31	0.48	0.21	6.07 E-15	0.24 ±0.02	0.21 ±0.07	0.29 ±0.04	0.39 ±0.01	0.20 ±0.02
fruC	1.55	0.06	1.47	1.28	0.99	1.92 E-10	2.48 ±0.01	0.40 ±0.01	1.50 ±0.01	3.54 ±0.01	1.59 ±0.00