

Figure S1. Raw data points for the evolution process of six acid-adapted (AA) strains.

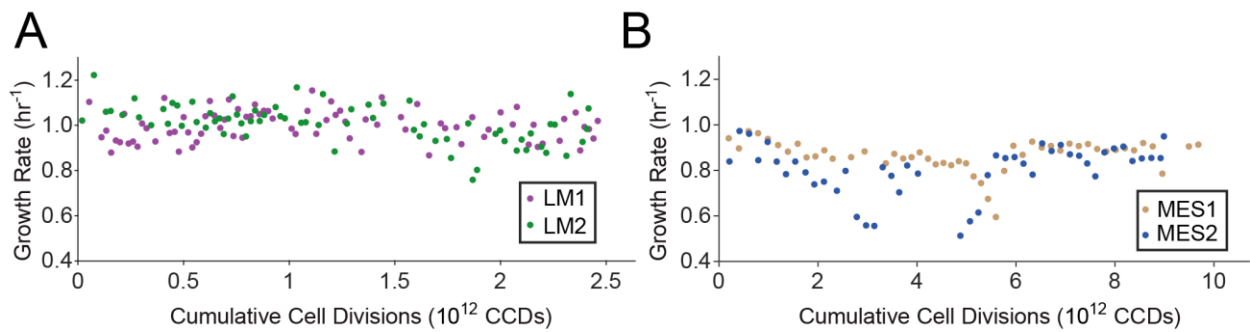


Figure S2. Growth rate versus cumulative cell divisions through the evolution process for control strains. (A) Control strains evolved in lowered magnesium concentration. (B) Control strains evolved in MES buffer.

Table S1. Periodic pH measurements of culture media over the course of evolution.

Day	3	12	25	27	35
Condition					
pH 5.5 MES start	5.55	5.45		5.51	5.52
pH 5.5 MES end	5.38	4.86 (0.26), 4.79 (0.39)		5.14 (0.28)	5.16 (0.37)
pH 7 MES start	7.00	7.04			
pH 7 MES end	6.84	6.54 (0.39)	6.55 (0.26)		
pH 7 low Mg start	7.07	7.10		6.98	7.07
pH 7 low Mg end	6.02	5.76 (0.35)	4.98 (0.28)	5.86 (0.37)	5.40 (0.49)

Aliquots were taken from both clean media (start) and those already used for cell culture (end). Days for sampling were chosen at random, and the used media were obtained from tubes containing cells ready for next passage. The OD600 measurements of the culture media are shown in parenthesis.

Table S2. All mutations identified in the clones of acid-evolved strains under pH 5.5.

Gene	Mutation	Protein change	Flask number	AA1	AA2	AA3	AA4	AA5	AA6
<i>yejH</i>	T→G	D105E (GAT→GAG)	56	X					
<i>rho</i>	C→A	R102S (CGC→AGC)	87	X					
<i>tam</i>	IS4	coding (5-16/759 nt)	114	X					
<i>ilvL</i>	Δ2 bp	coding (33-34/99 nt)	114	X					
<i>rpoC</i>	C→A	A397E (GCG→GAG)	111		X				
<i>rpoC</i>	G→C	G444A (GGT→GCT)	88, 118			X			
<i>yraJ</i>	IS2	coding (1893-1896/2517 nt)	88			X			
<i>yiaA</i>	IS2	coding (16-20/438 nt)	118			X			
<i>nagA</i>	G→C	S90* (TCA→TGA)	84				X		
<i>ycjX</i>	G→T	G207G (GGG→GGT)	84				X		
<i>rpoC</i>	Δ7 bp	coding (4106-4112/4224 nt)	113				X		
<i>ilvL/ilvX</i>	T→G	intergenic (+49/-38)	69					X	
<i>yjcB</i>	C→T	R12H (CGC→CAC)	69					X	
<i>rpoC</i>	Δ1 bp	coding (4111/4224 nt)	111					X	
<i>metF/katG</i>	C→T	intergenic (+275/-54)	111					X	
<i>csgD/csgB</i>	T→C	intergenic (-194/-561)	111					X	

<i>ydhY/ydhZ</i>	IS5	intergenic (-215/+237)	83, 111						X
<i>nagA</i>	C→T	R149H (CGT→CAT)	83						X
<i>phoQ</i>	G→C	P208R (CCG→CGG)	83						X
<i>rho</i>	C→T	R102C (CGC→TGC)	111						X
<i>rpoC</i>	C→T	S539F (TCT→TTT)	111						X
<i>ycgJ</i>	IS2	coding (1-5/369 nt)	111						X

Flask number stands for the number of cell passages during the evolution. The numbers in bold represent the final flask number (thus the endpoint) in the evolution process of the specific acid-evolved strain. The non-bold numbers are intermediate flasks during the evolution where samples were selected for whole genome sequencing.

Table S3. Mutations identified in the clones of control strains evolved under lowered magnesium concentration or in MES buffer

Gene	Mutation	Protein change	Flask number	LM1	LM2	MES1	MES2
<i>oxyR</i>	T→G	C199G (TGT→GGT)	122	X			
<i>dsbG/ahpC</i>	IS5	intergenic (- 311/-58)	122	X			
<i>oxyR</i>	C→A	A204E (GCA→GAA)	109		X		
<i>alaC/ypdA</i>	C→A	intergenic (- 281/-95)	109		X		
<i>ilvL/ilvX</i>	T→G	intergenic (+49/- 38)	53, 47			X	X
<i>cbl</i>	G→T	Q225K (CAG→AAG)	47				X
<i>potG</i>	C→T	T107T (ACC→ACT)	47				X
<i>casA</i>	C→T	P60P (CCG→CCA)	47				X

Table S4. Differentially expressed genes under acid stress for strain AA2

Gene	Locus	log2FoldChange	Corrected p-value
exbD	b3005	7.19	7.36E-05
feoA	b3408	6.57	4.55E-04
ygaM	b2672	4.85	1.29E-14
ftnA	b1905	4.29	4.28E-12
zupT	b3040	4.17	3.43E-08
dpiA	b0620	3.88	1.30E-12
feoB	b3409	3.64	1.21E-13
clcB	b1592	3.57	1.21E-05
narG	b1224	3.56	6.19E-18
narK	b1223	3.55	1.26E-07
ynjF	b1758	3.45	4.55E-06
gatZ	b2095	3.42	2.46E-08
gatA	b2094	3.29	8.46E-04
ycbC	b0920	3.25	4.92E-14
focA	b0904	3.22	2.83E-11
wrbA	b1004	3.19	5.69E-03
hisD	b2020	2.90	1.14E-06
frdB	b4153	2.89	2.65E-04
pfkA	b3916	2.75	9.28E-07
frdA	b4154	2.74	4.34E-10
cueO	b0123	2.72	3.23E-08
gpmM	b3612	2.68	5.88E-09
sucC	b0728	2.68	8.55E-06
yhbT	b3157	2.68	4.68E-03
sucB	b0727	2.66	7.88E-07
ycfP	b1108	2.61	5.60E-03
sugE	b4148	2.59	3.48E-03
hisC	b2021	2.58	5.03E-04
gmk	b3648	2.57	1.23E-04
glnH	b0811	2.54	1.11E-05
yhbQ	b3155	2.47	1.71E-09
thrB	b0003	2.38	2.80E-05
hfq	b4172	2.35	7.44E-03
exbB	b3006	2.34	2.66E-05
copA	b0484	2.27	4.48E-11
raiA	b2597	2.21	1.31E-04
atpG	b3733	2.21	1.08E-04

ftsZ	b0095	2.15	2.41E-04
deaD	b3162	2.15	9.84E-09
hisG	b2019	2.12	2.52E-04
cyoE	b0428	2.11	4.70E-03
cydB	b0734	2.09	1.22E-04
sucD	b0729	2.07	3.44E-05
udp	b3831	2.06	3.30E-03
yjhX	b4566	2.05	8.95E-03
ybaQ	b0483	2.03	5.26E-05
proB	b0242	2.01	2.83E-03
ydgJ	b1624	1.99	5.83E-04
yfbR	b2291	1.98	1.90E-04
nirB	b3365	1.98	1.24E-06
hisB	b2022	1.95	4.69E-03
thrC	b0004	1.94	6.60E-04
ycfD	b1128	1.94	8.34E-04
manY	b1818	1.87	3.98E-05
yieP	b3755	1.84	6.45E-03
ddpF	b1483	1.83	1.54E-03
hemG	b3850	1.83	2.31E-03
cdh	b3918	1.80	6.11E-04
thrA	b0002	1.79	1.51E-05
ppc	b3956	1.78	6.63E-05
yciU	b1248	1.77	3.38E-04
ydgA	b1614	1.76	6.00E-03
atpC	b3731	1.69	6.66E-03
dapB	b0031	1.69	9.19E-03
yhfA	b3356	1.68	9.70E-04
pgk	b2926	1.66	3.07E-03
rlmE	b3179	1.66	3.22E-03
thiC	b3994	1.63	5.03E-04
cysA	b2422	1.63	5.37E-03
yhjX	b3547	1.61	6.12E-04
fhuA	b0150	1.61	6.16E-06
asnA	b3744	1.61	7.09E-04
manZ	b1819	1.60	3.92E-03
gltB	b3212	1.57	1.19E-04
cysH	b2762	1.56	7.71E-03
dcuR	b4124	1.56	7.14E-04

leuC	b0072	1.54	2.20E-03
nuoN	b2276	1.53	5.98E-03
bglJ	b4366	1.48	3.07E-07
crp	b3357	1.44	7.93E-03
brnQ	b0401	1.44	9.36E-03
pbpG	b2134	1.44	8.46E-03
glyS	b3559	1.42	3.06E-03
pykA	b1854	1.41	8.87E-04
yjjL	b4356	1.41	1.16E-04
gadA	b3517	1.41	5.57E-03
intZ	b2442	1.40	6.83E-04
nuoL	b2278	1.38	8.98E-03
codA	b0337	1.36	8.15E-03
infB	b3168	1.36	3.54E-03
rhtC	b3823	1.35	1.40E-04
gshA	b2688	1.33	5.38E-03
slyB	b1641	1.33	7.81E-03
hypD	b2729	1.31	6.89E-03
gltD	b3213	1.30	7.44E-03
rhtB	b3824	1.29	9.57E-04
ydiU	b1706	1.28	2.57E-03
lpIT	b2835	1.27	1.70E-03
ybiP	b0815	1.26	2.50E-04
fxsA	b4140	1.23	3.27E-03
aceB	b4014	1.22	4.83E-04
ynfM	b1596	1.21	2.39E-03
insD1	b3045	1.18	9.00E-03
pck	b3403	1.16	7.81E-03
yidR	b3689	1.15	5.90E-05
mltD	b0211	1.15	4.75E-03
ackA	b2296	1.14	3.13E-03
rhIB	b3780	1.12	3.20E-03
pykF	b1676	1.11	1.09E-03
rpoN	b3202	1.10	7.93E-03
tpx	b1324	1.00	6.39E-04
ysaA	b3573	-1.01	5.40E-03
yaD	b3552	-1.10	6.45E-03
glnD	b0167	-1.12	2.41E-04
yfcJ	b2322	-1.14	9.94E-03

gntU	b4476	-1.14	6.90E-03
araH	b4460	-1.16	7.96E-03
yafJ	b0223	-1.20	1.19E-04
eutR	b2437	-1.21	3.73E-03
yqil	b3048	-1.25	9.00E-03
yfeH	b2410	-1.28	3.42E-03
yfjD	b4461	-1.29	3.73E-03
rmf	b0953	-1.30	6.27E-03
ydhB	b1659	-1.30	2.05E-04
galR	b2837	-1.32	1.45E-05
yhjG	b3524	-1.32	6.15E-03
sfsB	b3188	-1.34	1.14E-03
nadB	b2574	-1.35	9.79E-04
rhaR	b3906	-1.37	8.95E-03
trg	b1421	-1.38	7.44E-03
hisI	b2026	-1.39	2.08E-03
trmL	b3606	-1.39	2.32E-03
rtcR	b3422	-1.41	9.72E-04
ebgA	b3076	-1.47	2.31E-03
yafC	b0208	-1.49	1.48E-03
djlB	b0646	-1.53	9.19E-03
yghB	b3009	-1.54	1.31E-03
yifB	b3765	-1.55	2.06E-05
modC	b0765	-1.58	8.62E-03
tatD	b4483	-1.60	6.52E-05
ybhR	b0792	-1.65	8.74E-03
yfcl	b2305	-1.65	2.06E-05
erfK	b1990	-1.67	6.49E-03
rimO	b0835	-1.68	5.22E-03
ybdD	b4512	-1.69	3.21E-04
slmA	b3641	-1.71	1.30E-03
ttdT	b3063	-1.72	6.07E-06
yadS	b0157	-1.73	4.20E-03
tqsA	b1601	-1.73	3.74E-06
ratB	b2618	-1.74	5.04E-03
yajR	b0427	-1.74	2.35E-04
purM	b2499	-1.78	4.25E-03
cbl	b1987	-1.80	1.43E-05
marB	b1532	-1.86	3.61E-03

xylG	b3567	-1.87	1.13E-03
ycgR	b1194	-1.88	1.77E-06
zraP	b4002	-1.88	2.08E-03
kbaY	b3137	-1.91	9.80E-03
apaH	b0049	-1.94	8.61E-06
ykiA	b0392	-1.97	4.82E-03
gntX	b3413	-1.98	1.90E-03
yicL	b3660	-2.01	7.46E-05
ybdM	b0601	-2.04	2.69E-08
glcA	b2975	-2.06	1.57E-03
ycgG	b1168	-2.07	6.63E-04
yhhL	b3466	-2.09	4.78E-05
insQ	b1432	-2.10	6.14E-05
abgR	b1339	-2.15	4.14E-03
yabl	b0065	-2.15	3.83E-03
appY	b0564	-2.33	3.03E-03
yjgR	b4263	-2.38	4.27E-03
allD	b0517	-2.50	1.90E-03
yhil	b3487	-2.51	8.18E-03
osmB	b1283	-2.67	4.43E-03
alsB	b4088	-2.81	4.16E-03
feaR	b1384	-2.84	7.81E-03
ybcF	b0521	-2.88	1.39E-08
phoE	b0241	-2.89	5.16E-09
ygjI	b3078	-3.02	7.93E-03
mglA	b2149	-3.04	1.58E-07
xylF	b3566	-3.46	1.47E-03
panM	b3459	-3.98	8.05E-07
yadN	b0141	-4.32	3.51E-03

Table S5. Differentially expressed genes under acid stress for strain AA6

Gene	Locus	log2FoldChange	Corrected p-value
nuoE	b2285	2.97	6.48E-03
atpC	b3731	2.50	1.54E-04
gatZ	b2095	2.30	2.57E-03
cysN	b2751	2.25	1.12E-03
fliY	b1920	2.16	4.55E-03
dksA	b0145	2.13	9.41E-03
atpG	b3733	2.11	4.30E-04
rho	b3783	2.09	4.38E-06
sucC	b0728	2.08	7.15E-04
hisJ	b2309	2.08	3.84E-03
ydjN	b1729	2.00	1.16E-03
nuoM	b2277	1.99	1.36E-03
sucD	b0729	1.99	7.98E-05
cysP	b2425	1.93	2.24E-03
gltB	b3212	1.90	4.38E-06
dauA	b1206	1.90	1.12E-03
cysD	b2752	1.89	8.30E-03
nuoN	b2276	1.84	7.55E-04
fabF	b1095	1.78	4.66E-04
rplS	b2606	1.74	8.38E-03
cysI	b2763	1.74	2.02E-03
tyrS	b1637	1.72	7.15E-03
sucB	b0727	1.67	4.77E-03
speA	b2938	1.66	1.20E-04
nuoL	b2278	1.62	2.92E-03
thrA	b0002	1.61	3.21E-04
aspC	b0928	1.57	3.80E-03
argG	b3172	1.52	4.30E-04
argH	b3960	1.48	5.10E-03
tsf	b0170	1.46	4.77E-03
infB	b3168	1.44	3.39E-03
rnb	b1286	1.42	6.22E-03
argA	b2818	1.42	6.80E-03
secD	b0408	1.36	5.10E-03
gltD	b3213	1.34	7.87E-03
ppc	b3956	1.32	7.99E-03
rpoD	b3067	1.27	1.11E-03

aceB	b4014	1.06	4.09E-03
ydbH	b1381	-1.70	3.01E-03
malF	b4033	-1.91	3.59E-03