Legends of supplementary figures

Fig. S1: Schematic representation of the native A1 locus, of the AapA1-Spa and the AapA1-GFP fusions expressed from plasmid pILL2157, the AapA1-GFP fusions expressed from the native locus and the P*aapA1-lacZ* and P*IsoA1-lacZ* fusions expressed from their respective native loci.

Table S3 presents the corresponding plasmids with, in Table S4, the primers used for the construction and, in Table S2, the resulting strains. SD indicates the Shine-Dalgarno sequence; -10, the position of the -10 box of the promoter; PureI, the promoter of the *ureI* gene from plasmid pILL2157; arrows, the direction of transcription; a red cross on an arrow, an inactivated promoter; SPA, the SPA tag; GFP, green fluorescent protein; kan, the gene conferring kanamycin resistance; a star (*) a STOP codon that has been replaced by a codon coding for Ala. An hatched *IsoA1* arrow indicates that the corresponding RNA is not expressed.

Fig. S2: The AapA1-GFP fusion localizes at the *H. pylori* inner membrane.

A. Western blot analysis of total extract (T), soluble extract (SE), inner membrane (IM) and outer membrane (OM) fractions prepared from *H. pylori* B128 strain carrying a deletion of the A1 TA locus (ΔA1) and expressing the AapA1-GFP fusion either under the control of its native chromosomal promoter (B128 ΔA1::A1-GFP) or from a plasmid under the control of the IPTG-inducible promoter (B128 ΔA1+ pILL2157-A1-GFP). The coomassie stained gel is presented in the upper part of the figure. Below, the western blot was revealed with anti-GFP antibodies for the A1-GFP fusion and with antibodies against the cytoplasmic NikR protein that served as a fractionation control. The AapA1 fusion localizes at the inner membrane of *H. pylori* in both constructs.

B. Fluorescence of a strain expressing GFP from plasmid pILL2157 is presented as a control for the data of **Fig. 2B**.

Fig. S3: Analysis of the effect of the AapA1 toxin on the *H. pylori* membrane potential.

A second membrane potential reactive dye, DIOC-5, was used to analyze live *H. pylori* B128 strains carrying the pA1 or pA1* plasmid. Cells were grown in the presence or absence of 1mM IPTG and analyzed on agarose pads at 0, 4, 8 and 24 h after IPTG addition. While uniformly stained by the DIOC-5 in the absence of IPTG, cells expressing the toxin (pA1) present discrete foci of stronger fluorescence suggesting the local disturbance of the membrane potential of MitoRed staining. For comparison, a 72h-old culture of B128 WT strain forming "aging coccoids" was also analyzed. Bar scale represents 2μm.

Fig. S4: Measurement of *H. pylori* cell length over time.

Mean curve obtained from the analysis of the growth of 46 individual *H. pylori* bacteria (strain HPLEM213 without IPTG, **Table S2**). Bacteria were analyzed by live microscopy, and their size and time of division were measured from their separation following a division to the next division. The curves were analyzed and normalized. Mean division time is 165 min, initial length mean is 2.1 μm, mean length when division occurs is 3.7μm.

Fig. S5: Movies of *H. pylori* morphological transformation.

Representative movies of the transformation of *H. pylori* upon expression of AapA1 toxin. Snapshots of the cells were taken at intervals of 10 or 15min during 10h.

Fig. S6: Response of the *aapA1* and *IsoA1* promoters to different stresses.

β-galactosidase activities expressed by strains expressing the P*aapA1-lacZ* and P*IsoA1-lacZ* fusions from the native locus were measured after 6h treatment with different stresses, $NiCl_2$

(20 and 200 mM), pH 4, Tetracycline (0.1 and 1 mg/ml) or Rifampicin (0.05 and 0.5 mg/ml). β-galactosidase activities are presented as ratio (expressed in %) of activities measured with stress versus activities of untreated samples. Results from 3 independent experiments performed in duplicates are shown. Error bars represent the standard deviation, NS corresponds to non-significant, (P >0.05).

Fig. S7: Half life of the *aapA1* and *IsoA1* RNAs.

RNA decay was determined by plotting normalized intensities (RNA signal relative to time 0) of bands corresponding to full length *aapA1* and *IsoA1* transcripts as a function of time after rifampicin addition. Approximate half-lives (min) measurements from three independent experiments are indicated for each transcript.

Fig. S8: Sequence alignment of the six functional class A TA modules present on the *H. pylori* B128 genome

Sequences alignment of the six functional class A TA module colored according to the percentage identity with their consensus sequence. The -10 box, Shine-Dalgarno (SD) and AapA toxin coding sequence are framed in red and the start and stop codons are indicated above the sequences. *IsoA* antisense RNA sequence is represented by a green bar under the sequence alignment. Note that in B128, two consecutive TA modules are found at the locus A4, here referred as locus A4-1 and locus A4-2.

Fig. S9: Growth and viability of B128 WT strain and multiple isogenic class A TA systems deletion mutants under normal conditions or upon exposure to hydrogen peroxide.

A) Growth of the B128 WT strain, of six isogenic mutants carrying *AapA1-IsoA1* deletions (Δ*A1,* Δ*A2,* Δ*A3,* Δ*A4-2+A4-2,* Δ*A5 or* Δ*A6*) and of a multiple mutant strain carrying deletions of every functional class A TA system (Δ*5*) was followed under normal conditions during 44h. The growth curve of the mutants was similar to that of the parental WT strain.

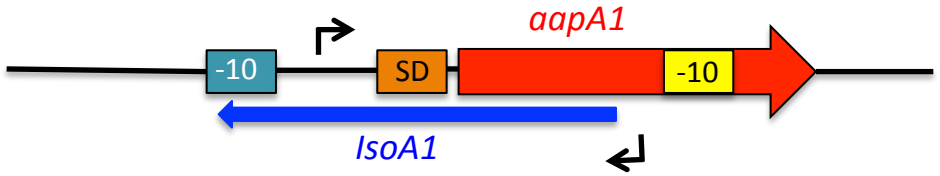
B) Viability of the B128 WT strain and of the Δ5 multiple TA mutant was measured during 72h by determining colony forming units (CFU) by plating on blood agar medium. No significant difference was observed in the kinetics of loss of viability between these strains.

C) Exponentially growing B128 WT strain, Δ*A1* and Δ*5* isogenic mutants were exposed to 1% hydrogen peroxide. Their viability was measured during 12h by counting the colony forming units (CFU/mL) on blood agar plates.

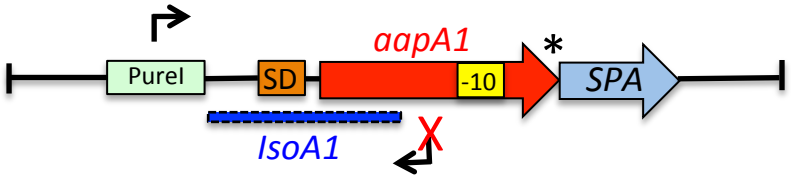
D) Exponentially growing B128 WT strain, Δ*A1* and Δ*5* isogenic mutants were exposed to 1% hydrogen peroxide during 8 h. The percentage of survival was calculated by dividing the number of CFU/mL in the culture after 8h with hydrogen peroxide by the number of CFU/mL after 8 h of incubation without stress.

Figure S1

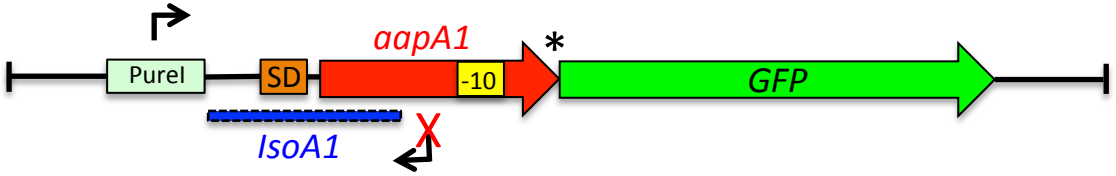
Organization of the A1 locus



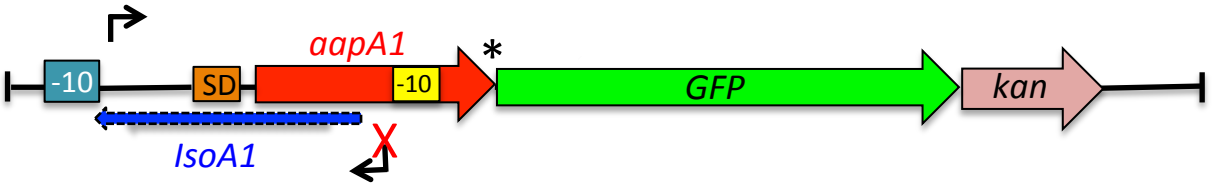
AapA1-SPA translational fusion expressed on plasmid pILL2157 (strain HPLEM066)



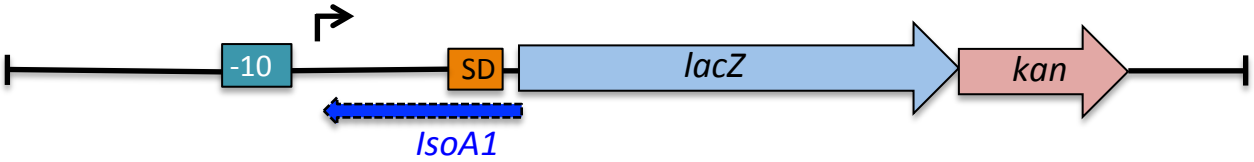
AapA1-GFP translational fusion expressed on plasmid pILL2157 (strain HPLEM115)



AapA1-GFP translational fusion at the native locus (strain HPLEM160)



P*aapA1-lacZ* transcriptional fusion at the native locus (strain HPLEM084)



P*IsoA1-lacZ* transcriptional fusion at the native locus (strain HPLEM142)

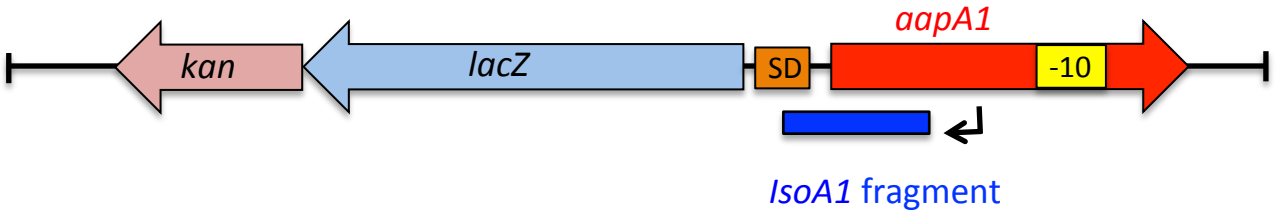
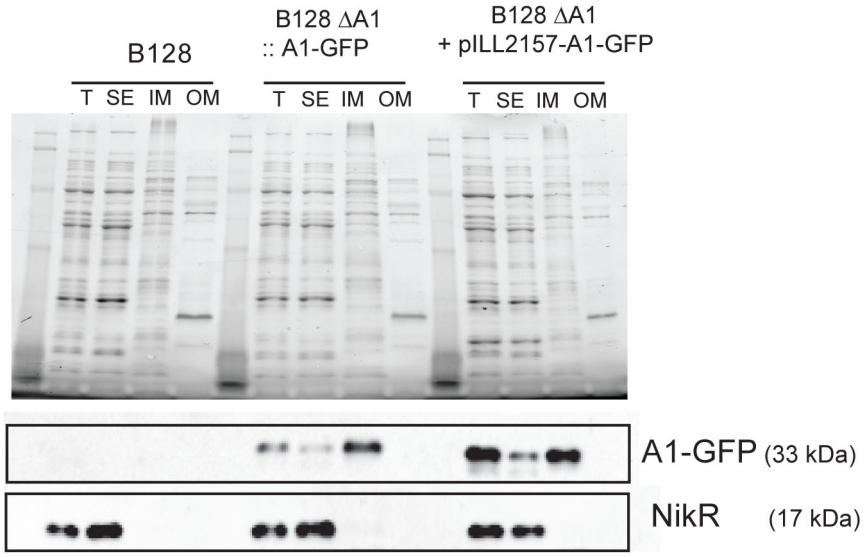


Figure S2

A


B128
B128 ΔA1
:: A1-GFP
B128 ΔA1
+ pILL2157-A1-GFP
T SE IM OM
T SE IM OM
T SE IM OM
A1-GFP (33 kDa)
NikR (17 kDa)

B

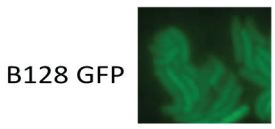

B128 GFP

Figure S3

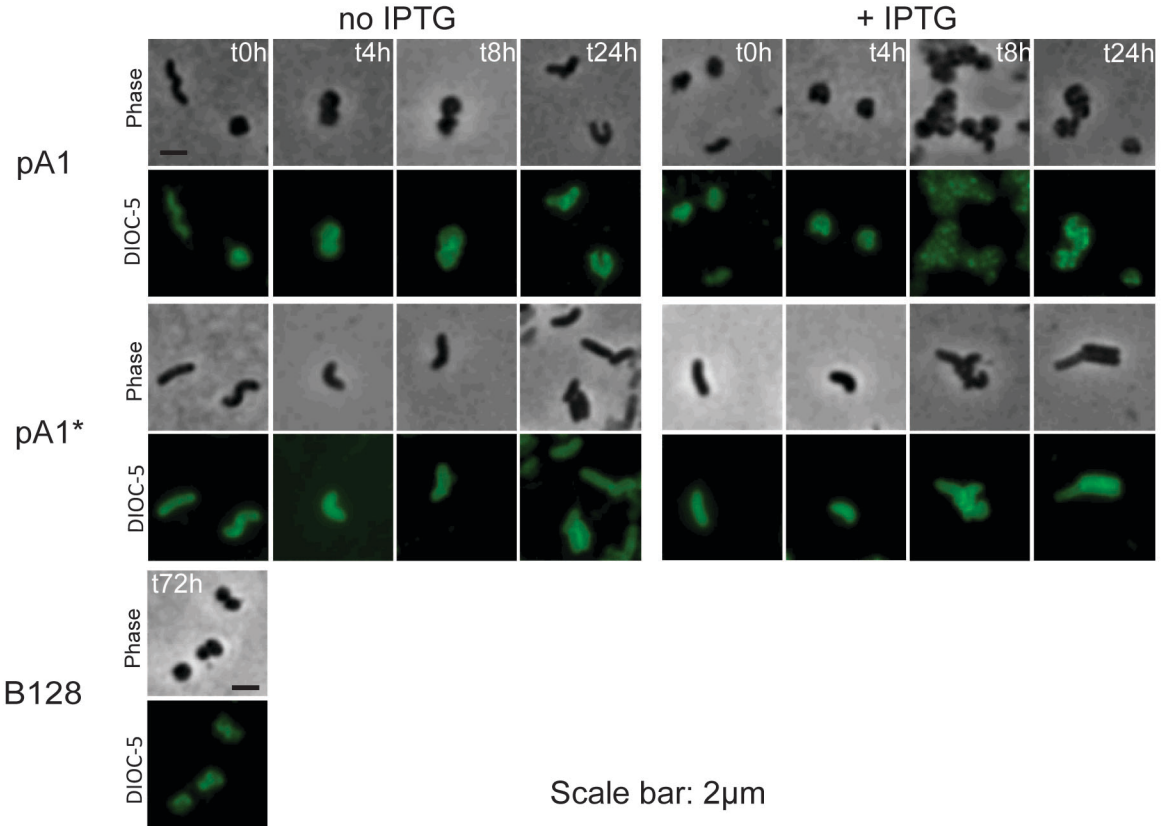

no IPTG
+ IPTG
t0h
t4h
t8h
t24h
t72h
Phase
DIOC-5
pA1
pA1*
B128
Scale bar: 2µm

Figure S4

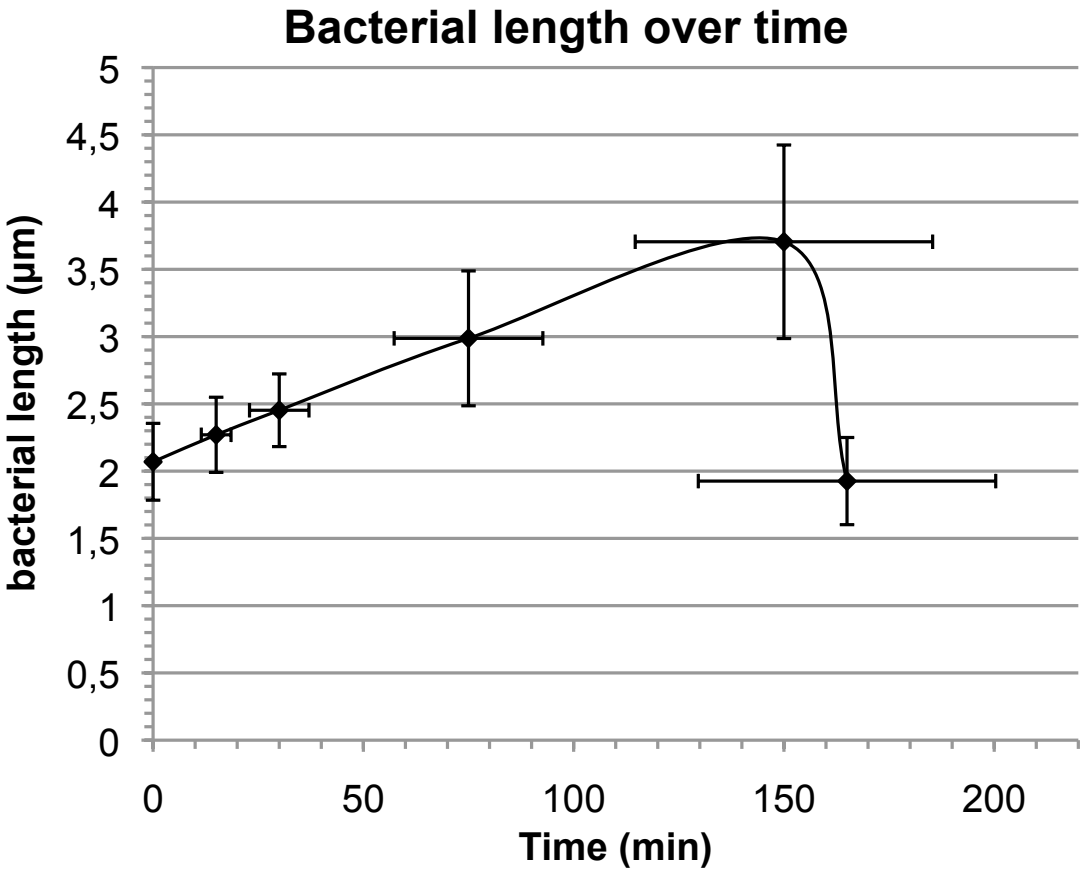
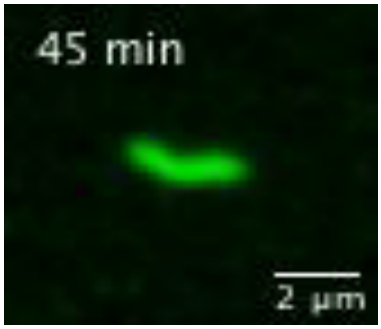

Bacterial length over time
bacterial length (µm)
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
0
50
100
150
200
Time (min)

Figure S5



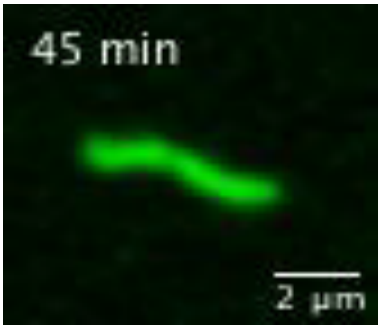


Figure S6

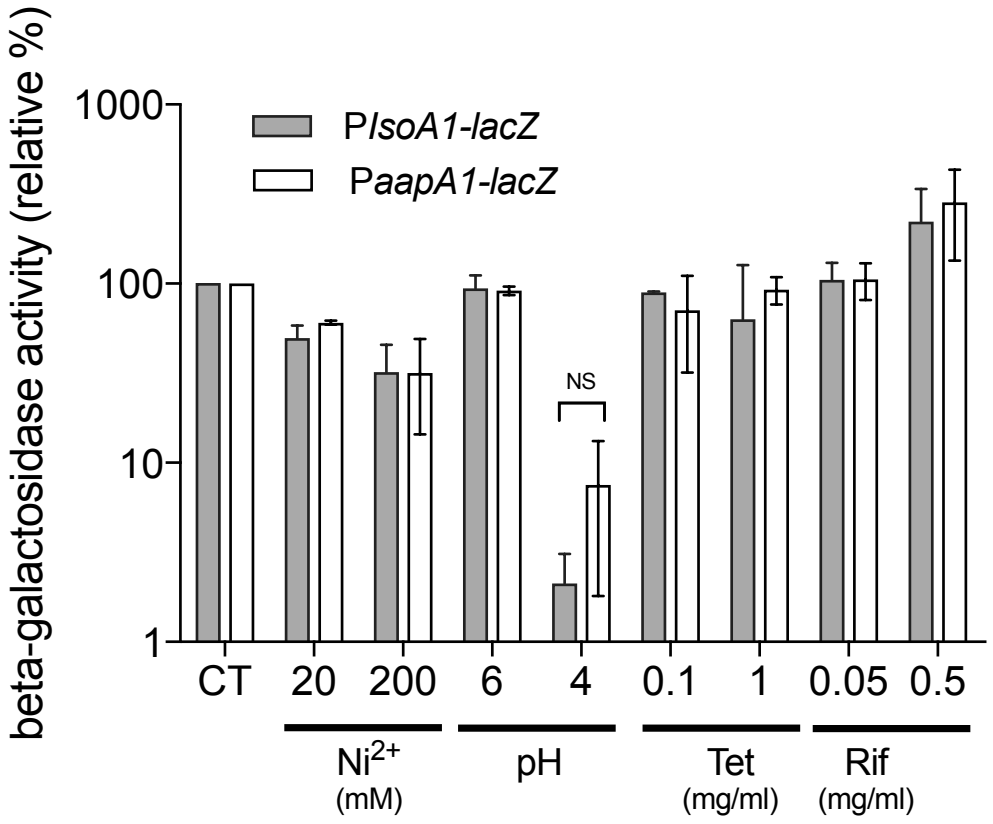

beta-galactosidase activity (relative %)
1000
100
10
1
PIsoA1-lacZ
PaapA1-lacZ
NS
CT
20
200
6
4
0.1
1
0.05
0.5
Ni2+
(mM)
pH
Tet
(mg/ml)
Rif
(mg/ml)

Figure S7

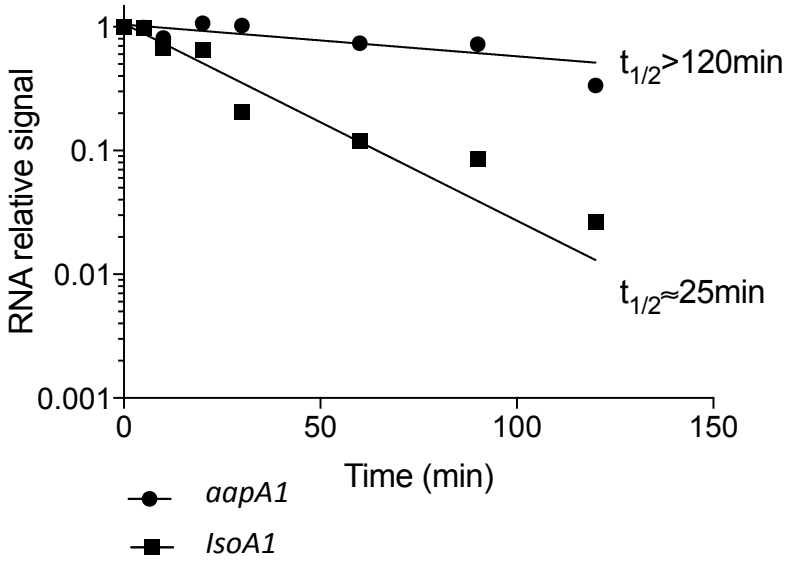

RNA relative signal
1
0.1
0.01
0.001
0
50
100
150
Time (min)
$t_{1/2}$>120min
$t_{1/2}$≈25min
aapA1
IsoA1

Figure S8

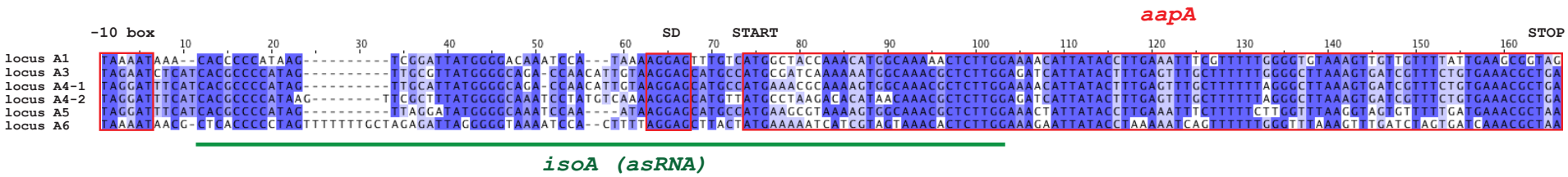

aapA
-10 box
SD
START
STOP
locus A1
locus A3
locus A4-1
locus A4-2
locus A5
locus A6
isoA (asRNA)

Figure S9

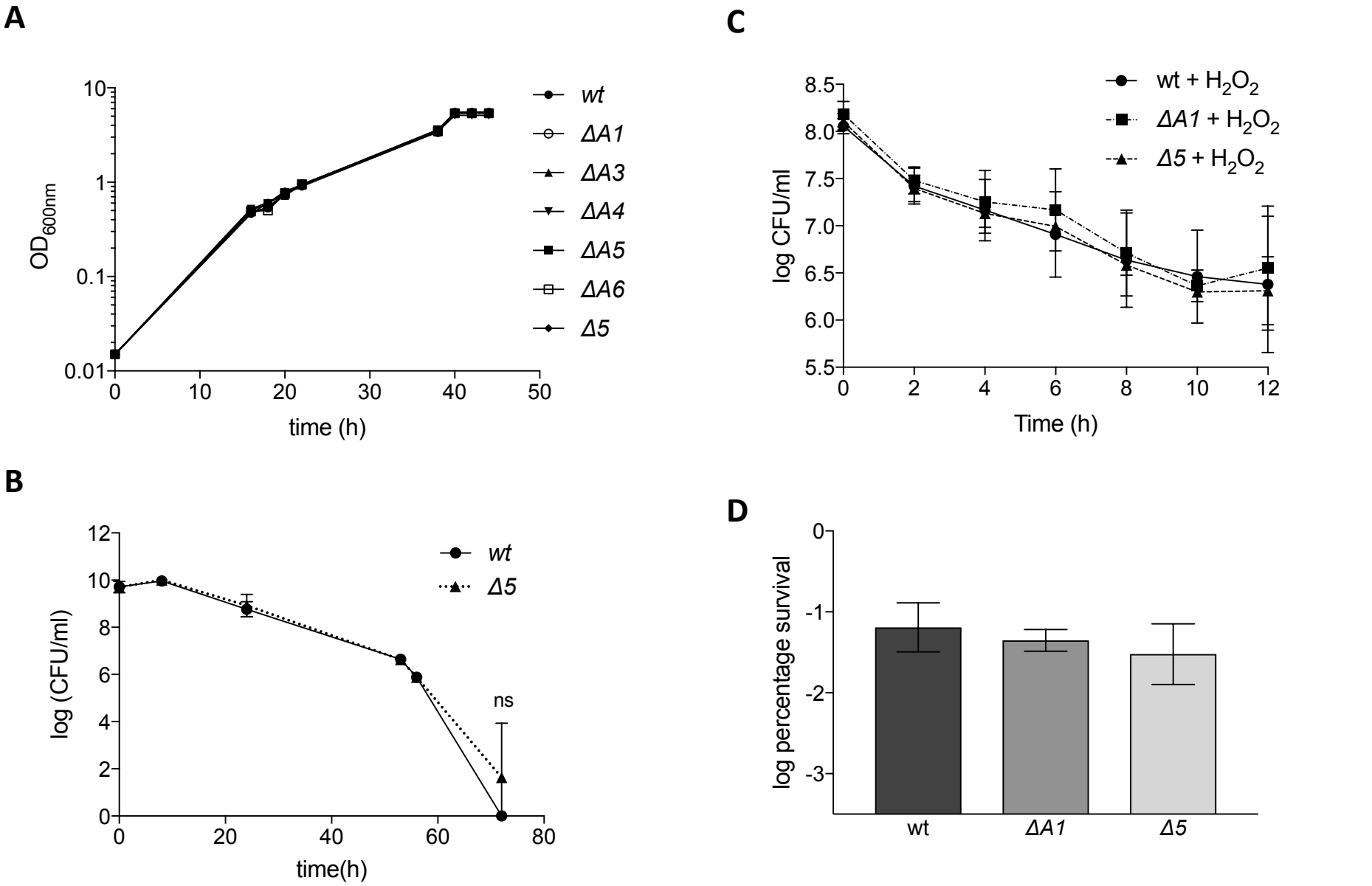

A
10
1
0.1
0.01
OD600nm
0
10
20
30
40
50
time (h)
wt
ΔA1
ΔA3
ΔA4
ΔA5
ΔA6
Δ5
B
12
10
8
6
4
2
0
log (CFU/ml)
0
20
40
60
80
time(h)
wt
Δ5
ns
C
8.5
8.0
7.5
7.0
6.5
6.0
5.5
log CFU/ml
0
2
4
6
8
10
12
Time (h)
wt + H2O2
ΔA1 + H2O2
Δ5 + H2O2
D
0
-1
-2
-3
log percentage survival
wt
ΔA1
Δ5

Table S1: Summary of the muropeptide composition of peptidoglycan extracted from B128 WT strain during exponential phase or after 72h culture and of B128 Δ*aapA1-IsoA1* + pA1 strain after 6 or 16 hours of induction of the toxin.

Panel A: Summary of the muropeptide composition of peptidoglycan extracted from *H. pylori* B128 WT strain during exponential phase (16h, first column); of 72h culture "(aging" coccoids, second column) and of toxin-induced coccoids of B128 Δ*aapA1-IsoA1* + pA1 strain after 6 h (third column) or 16 hours of induction (fourth column). The relative abundance of muropeptides in each sample was calculated according to Glauner *et al.* [23]. Arrows show major changes in the muropeptide composition observed 72°C growth of strain B128 WT.

		Area - % of each muropeptide[a]			
		B128		B128 Δ*aapA1-IsoA1* + pA1	
		Exponential phase (16h)	72h	6 hours induction	16 hours induction
Peak n°	*Monomers*	65.84	67.69	72.33	64.76
3	GM2	7.42	30.83 ↗	6.25	18.24↗
1	GM3	6.16	1.40 ↘	5.26	2.77↘
4	GM4	11.77	4.95	15.60	11.11
5	GM5	37.26	23.73↘	41.18	28.98↘
2	GM4+gly[b]	3.23	6.78	4.05	3.66
	Dimers	14.11	14.71	12.05	15.62
7	GM3 + GM4	1.76	0.94	1.32	1.47
8	GM4 + GM4+gly[b]	0.40	1.38	0.46	0.66
9	GM4 + GM4	5.06	5.65	5.15	6.43
10	GM5 + GM4	6.89	6.73	5.12	7.05
	Anhydromuropeptides	20.04	17.60	15.62	19.62
12	G(anh)M2	0.61	1.45	0.66	1.17
6	G(anh)M3	1.08	0.32	0.65	0.70
11	G(anh)M4	3.54	0.52	2.29	1.57
13	G(anh)M5	3.65	1.62	2.93	2.76
14	G(anh)[c]M3 + GM4	1.52	1.40	1.09	1.51
15	G(anh)[c]M4 + GM4+gly[b]	0.23	0.71	0.22	0.37
16	G(anh)[c]M4 + GM4	4.59	4.81	3.94	5.30
17	G(anh)[c]M5 + GM4	3.38	4.61	2.62	4.34
18	G(anh)M3+ G(anh)M4	0.27	0.34	0.19	0.28
19	G(anh)M4+ G(anh)M4	0.68	0.93	0.62	0.91
20	G(anh)M5+ G(anh)M4	0.49	0.88	0.41	0.71

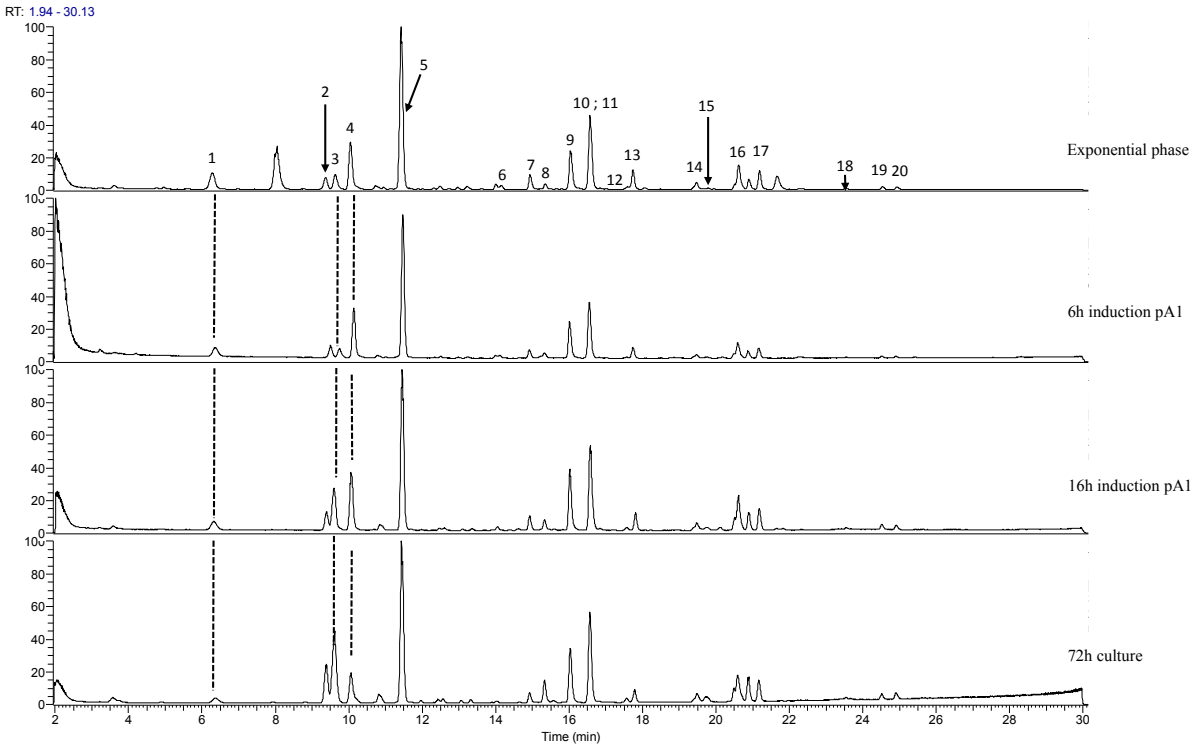
GM2: GlcNAc-Mur*N*Ac-dipeptide; GM3: GlcNAc-Mur*N*Ac-tripeptide; GM4: GlcNAc-Mur*N*Ac-tetrapeptide; GM5: GlcNAc-Mur*N*Ac-pentapeptide; AnhM : *N*-acetyl-anhydromuramic acid.

[a] Percentages were calculated as in Glauner *et al.* [23].

[b] GM4+gly: GlcNAc-Mur*N*Ac-Tetrapeptide with an additional glycine moiety.

[c](anh): dimer with only one anhydroMur*N*Ac moiety with indeterminate position.

Panel B: Total ion current chromatograms of the HPLC profiles of muropeptides of the four samples indicated above. Peak numbers correspond to those of **Panel A**.



Supplementary Table S2: List of strains used in this study.

	Strain designation	Genotype	Plasmid	Antibiotic resistance markers [a]	Reference or source
B128	HPLEM001	Parental wild type strain	-	-	McClain *et al.*[48]
B128 Δ*rpsL::rpsL1*	HPLEM015	Δ*rpsL::rpsL1*	-	Str	Fischer *et al.*[24]
B128 Δ *TA A1::Kan + pA1-isoA1*	837	Δ*AapA1-isoA1::aphA-3*	pA1-isoA1	Kan, Cm	Arnion *et al.*[20]
B128 Δ *TA A1 ::Kan + pA1*	838	Δ*AapA1-isoA1::aphA-3*	pA1	Kan, Cm	Arnion *et al.*[20]
B128 Δ *TA A1::Kan + pA1**	HPLEM086	Δ*AapA1-isoA1::aphA-3*	pA1*	Kan, Cm	Arnion *et al.*[20]
B128 Δ *TA A1::Kan + pA1-SPA*	HPLEM066	Δ*AapA1-isoA1::aphA-3*	p-A1-SPA	Kan, Cm	Arnion *et al.*[20]
B128 Δ*rpsL::rpsL1* Δ*A1 PureA-GFP* Δ*flaA::Apra + pA1*	HPLEM213	Δ*rpsL::rpsL1* Δ*A1* Δ*flaA::Apra* Δ*ureA::GFP-mut2*	pA1	Str, Kan, Apr, Cm	This study
B128 Δ*rpsL::rpsL1* Δ*TA A1 + pA1-GFP*	HPLEM155	Δ*rpsL::rpsL1* Δ*A1*	pA1-GFP	Str, Cm	This study
B128 Δ*TA A1-isoA1:: AapA1-222nt-GFP-253nt*	HPLEM160	Δ*A1-isoA1:: AapA1-222nt-gfp-mut2-253nt*	-	Kan	This study
B128 Δ *TA A1 AapA1-isoA1::PaapA1-lacZ-Kan*	HPLEM084	Δ *AapA1-isoA1::PaapA1-lacZ-Kan*	-	Kan	This study
B128 Δ *TA A1 AapA1-isoA1::PisoA1-lacZ-Kan*	HPLEM142	Δ *AapA1-isoA1::PisoA1-lacZ-Kan*	-	Kan	This study
B128 Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	HPLEM159	Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	-	Str, Kan	This study
B128 Δ*rpsL::L1* Δ*TA A1*	HPLEM214	Δ*rpsL::L1* Δ*TA A1*	-	Str	This study
B128 Δ*rpsL::L1* Δ*TA A3*	HPLEM080	Δ*rpsL::L1* Δ*TA A3*	-	Str	This study
B128 Δ*rpsL::L1* Δ*TA A4::Kan*	HPLEM157	Δ*rps ::L1* Δ*TA A4::Kan*	-	Str, Kan	This study
B128 Δ*rpsL::L1* Δ*TA A5*	HPLEM067	Δ*rpsL::L1* Δ*TA A5*	-	Str	This study
B128 Δ*rps ::L1* Δ*TA A6*	HPLEM216	Δ*rps ::L1* Δ*TA A6*	-	Str	This study
B128 Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	HPLEM159	Δ*rpsL::rpsL1* Δ*TA* Δ*A5* Δ*A3* Δ*A1* Δ*A6* Δ*A4::Kan*	-	Str, Kan	This study

[a] Str : Strepromycin ; Kan : Kanamycin ; Apr : Apramycin ; Cm : Chloramphenicol

Supplementary Table S3: List of plasmids used in this study.

Name	Description	Resistance (*)	Reference or source
pILL2157	Derivative of the pHeL-2 *E. coli-H. pylori* shuttle vector, carries *lacZ* under the control of p*ureI* with 2 LacI-binding sites	Cm	Boneca *et al.*[21]
pILL2157bis	pILL2157 without *lacZ* (pILL2157bis)	Cm	This study
pDifWT-RC	*rpsL-cat* cassette flanked by *difH*		Debowski *et al.*[49]
pA1-isoA1	*AapA1-isoA1* locus cloned into pILL2157bis	Cm	Arnion *et al.*[20]
pA1	*AapA1-isoA1* locus cloned into pILL2157bis with *isoA1* promoter inactivated	Cm	Arnion *et al.*[20]
pA1*	*AapA1-isoA1* locus cloned into pILL2157bis with isoA1 promoter inactivated and AapA1 start codon mutated to ATT	Cm	Arnion *et al.*[20]
pA1-SPA	*AapA1-isoA1* locus cloned into pILL2157bis with *isoA*1 promoter inactivated and SPA tag fused at the C-terminus of the AapA1 peptide	Cm	Arnion *et al.*[20]
pGEM ΔTA A1	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A1* for markerless deletion of A1 locus.	Amp/Cm	This study
pGEM ΔTA A3	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A3* for markerless deletion of A3 locus.	Amp/Cm	This study
pGEM ΔTA A5	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A5* for markerless deletion of A5 locus.	Amp/Cm	This study
pGEM ΔTA A6	Suicide plasmid with *difH-rpsL-cat-difH* flanked by upstream and downstream region *A6* for markerless deletion of A6 locus.	Amp/Cm	This study
pJET-pureA-GFP-mut2-Kan-ureA	Suicide plasmid with *gfp-mut2* under the control of *ureA* promoter for chromosomal integration at the *ureA* locus	Amp/Kan	Corbinais *et al.*[53]
pA1-GFP	*gfp-mut2* cloned in translational fusion between AapA1 sequence 222 nt (from 0 to the 222 nt aapA1) and 253 nt (from 223 to the 253 nt) into vector pILL2157bis. AapA1-222nt-GFP-253nt is under the control of the *pureI* promoter.	Cm	This study

(*) Cm: Chloramphenicol; Amp: Ampicillin; Kan: Kanamycin

Supplementary Table S4: list of primers used in this study.

Name	Sequence 5'→3'	Description
oLEM001	GTAAGCATTGCCGACAAACAC	***rpsl::rpsl1*** Forward primer to amplify upstream region of *rpsL.*
oLEM002	CCAATTGATTTATGGTAGGCACTATTTTTCCTTATTC	***rpsl::rpsl1*** Reverse primer to amplify upstream region of *rpsL.* Primer contains a homolougous region to *rpsL1.*
oLEM003	GTGCCTACCATAAATCAATTGG	***rpsl::rpsl1*** Forward primer to amplify *rpsL1* from pDifWT-RC.
oLEM004	CTAACGGATTTGTCTGTATG	***rhpA* deletion** Reverse primer to amplify *rpsL1* from pDifWT-RC.
oLEM005	CATACAGACAAATCCGTTAGAGGAAAACAAAAACATGAGAAG	***rpsl::rpsl1*** Forward primer to amplify downstream region of rpsL. Primer contains a homologous region to *rpsL1.*
oLEM006	CCATTCTAACTCCAATTACCAG	***rpsl::rpsl1*** Reverse primer to amplify downstream region of *rpsL.*
oLEM192	GGATGTATAGACCGTTATGG	***flaA::apr*** Forward primer to amplify upstream region of *flaA.*
oLEM193	cactccCTAgTTAgTCACcatTGTTGTAACTCCTTG	***flaA ::apr*** Reverse primer to amplify upstream region of *flaA*. Primer contains a homolougous region to *apr* resistance cassette with a stop codon and an RBS.
oLEM120	TGAcTAAcTAGgagtgcaATGtcgtgcaa	***flaA::apr*** Forward primer to amplify *apr* resistance cassette with stop codon upstream of the RBS.
oLEM066	cgatccgctccacgtgttgcc	***flaA::apr*** Reverse primer to amplify *apr* resistance cassette.
oLEM194	ggcaacacgtggagcggatcgCAAGCCAATACCGTTCAAC	***flaA::apr*** Forward primer to amplify downstream region of *flaA*. Primer contains a homologous region to *apr* resistance cassette.
oLEM195	CATAGCATAAAATCGCATCC	***flaA::apr*** Reverse primer to amplify downstream region of *flaA*.
oLEM015	CTCCCACCGCAATTGATTG	**Δ*A1* with marker less system** Forward primer to amplify upstream region of *A1* Toxin antitoxin locus.
oLEM035	CATACTCGAGGCTTGATTGAGTGCATCAAAAC	**Δ*A1* with marker less system** Reverse primer to amplify upstream region of *A1* Toxin antitoxin locus. Primer contains a *Xho*I restriction site.
oLEM036	CATAGGATCCCGAAGTTTCTGTAAAACGATAG	**Δ*A1* with marker less system** Forward primer to amplify downstream region of A1 Toxin antitoxin locus. Primer contains a *Bam*HI restriction site.
oLEM018	CTCAATGCGTTTAGGATTAATC	**Δ*A1* with marker less system** Reverse primer to amplify downstream region of *A1* Toxin antitoxin locus.
oLEM019	CATTCAAAGATGTTGGTAG	**Δ*A3* with marker less system** Forward primer to amplify upstream region of *A3* Toxin antitoxin locus.
oLEM037	CATACTCGAGCTAGATCGCATCCAATACG	**Δ*A3* with marker less system** Reverse primer to amplify upstream region of *A3* Toxin antitoxin locus. Primer contains a *Xho*I restriction site.
oLEM038	CATAGGATCCCAAGAGCGTTCCCTTAAGC	**Δ*A3* with marker less system** Forward primer to amplify downstream region of *A3* Toxin antitoxin locus. Primer contains a *Bam*HI restriction site.

Name	Sequence 5'→3'	Description
oLEM022	CTTGAAAGGCTTCAATCAAG	**Δ*A3* with marker less system** Reverse primer to amplify downstream region of *A3* Toxin antitoxin locus.
oLEM027	CATGCTTGTCAAACCACAG	**Δ*A5* with marker less system** Forward primer to amplify upstream region of *A5* Toxin antitoxin locus.
oLEM041	CATACTCGAGGCTCTTAAATGCAACCAC	**Δ*A5* with marker less system** Reverse primer to amplify upstream region of *A5* Toxin antitoxin locus. Primer contains a *Xho*I restriction site.
oLEM042	CATAGGATCCCTAAGAGCGTTCCCCTAAG	**Δ*A5* with marker less system** Forward primer to amplify downstream region of *A5* Toxin antitoxin locus. Primer contains a *Bam*HI restriction site.
oLEM030	CTCAGTATGTGAATTTAGCG	**Δ*A5* with marker less system** Reverse primer to amplify downstream region of *A5* Toxin antitoxin locus.
oLEM031	GCCAAGCACCATCTTCTTTATG	**Δ*A6* with marker less system** Forward primer to amplify upstream region of *A6* Toxin antitoxin locus
oLEM043	CATACTCGAGGCTGCAAACCACTCATTTAAAG	**Δ*A6* with marker less system** Reverse primer to amplify upstream region of *A6* Toxin antitoxin locus. Primer contains a *Xho*I restriction site.
oLEM044	CATAGGATCCGGGTTATCCTTAAGTGGA	**Δ*A6* with marker less system** Forward primer to amplify downstream region of *A6* Toxin antitoxin locus. Primer contains a *Bam*HI restriction site
oLEM034	CTCATTACGACACTATTGC	**Δ*A6* with marker less system** Reverse primer to amplify downstream region of *A6* Toxin antitoxin locus.
oLEM045	CATACTCGAGatttaaaagtttgaaaagtgcag	**marker less system** Forward primer to amplify *rpsl-cat* cassette from pDifWT-RC. Primer contains a *Xho*I restriction site.
oLEM046	CATAGGATCCtcatttagttatgaaaactgcac	**marker less system** Reverse primer to amplify *rpsl-cat* cassette from pDifWT-RC. Primer contains a *Bam*HI restriction site.
oLEM023	GAGGCTGTAAGGATAAGG	**Δ*A4::Kan*** Forward primer to amplify upstream region of *A4* Toxin antitoxin locus.
oLEM107	gTTAgTCAcccgggtaccCAAACGCTAAAACGAGGCAC	**Δ*A4::Kan*** Reverse primer to amplify upstream region of *A4* Toxin antitoxin locus. Primer contains a homologous region to the Kanamycin resistance cassette.
oLEM009	GGTACCCGGGTGACTAAC	**Δ*A4::Kan*** Forward primer to amplify the Kanamycin resistance cassette.
oLEM010	CATTATTCCCTCCAGGTAC	**Δ*A4::Kan*** Reverse primer to amplify the Kanamycin resistance cassette
oLEM108	gtacctggagggaataATGGTTGGTCATTTTGGTATAAAAC	**Δ*A4::Kan*** Forward primer to amplify downstream region of *A4* Toxin antitoxin locus. Primer contains a homologous region to the Kanamycin resistance cassette.
oLEM026	CCCTAATAGTAGAAAATGGAG	**Δ*A4::Kan*** Reverse primer to amplify downstream region of *A4* Toxin antitoxin locus.
oLEM073	GATCATTAAAGGCTCCTTTTG	***pA1-GFP*** Forward primer to amplify upstream region of pureI in pILL2157bis.
oLEM080	CAAAATGCCCGCTTCAATAAAAC	***pA1-GFP*** Reverse primer to amplify *aapA1* where stop codon of the A1 toxin has been replaced by Ala codon .

Name	Sequence 5'→3'	Description
oLEM081	GAAGCGGGCATTTTGTAAAACGAAGTTTCTGGCAAACGATAG	***pA1-GFP*** Forward primer to amplify 3'-UTR of aapA1 (from 142 to 222 nt). Contains a homologous region to oLEM080 and 2 stop codons each mutated in Ala codon to enable the GFP translational fusion.
oLEM083	GTTCTTCTCCTTTACTCATGAAAACCCTTAAAGCTAAAAG	***pA1-GFP*** Reverse primer to amplify 3'-UTR region of *aapA1*. Primer also contains a homologous region to *gfp-mut2*.
oLEM082	ATGAGTAAAGGAGAAGAAC	***pA1-GFP*** Forward primer to amplify *gfp-mut2*.
oLEM084	GTCATTTGTATAGTTCATCC	***pA1-GFP*** Reverse primer to amplify *gfp-mut2*.
oLEM085	GGATGAACTATACAAATGACTTTAAAGCTTATCCTTAAC	***pA1-GFP*** Forward primer to amplify *aapA1* 3'-UTR (from 222 nt to 253 nt). Primer contains a homologous region to *gfp-mut2*.
oLEM099	CGGTACCCTAGTATTCCAAGCAAAGAATG	***pA1-GFP*** Reverse primer to amplify *aapA1* 3'-UTR from pA1. Primer contains a KpnI restriction site for cloning in pILL2157bis.
oLEM047	atgACTAGTACCATGATTACG	***lacZ transcriptional fusion*** Forward primer to amplify *lacZ* from pILL2157bis.
oLEM069	GAAGGAAAAGcatatgACTAG	***lacZ transcriptional fusion*** Forward primer to amplify *lacZ* with RBS from pILL2157bis
oLEM048	GGGATCCTTATTTTTGACAC	***lacZ transcriptional fusion*** Reverse primer to amplify *lacZ* from pILL2157bis.
oLEM059	GTGTCAAAAATAAGGATCCCGGTACCCGGGTGACTAAC	***lacZ-Kan*** Forward primer to amplify Kan cassette with oLEM010. Primer contains a homologous region to *lacZ*.
oLEM049	CGTAATCATGGTACTAGTcatGACAAACTCCTTTTATGG	***PaapA1-lacZ*** Reverse primer to amplify upstream region of *aapA1* with oLEM015. Primer contains a homologous region to *lacZ*.
oLEM060	gtacctggagggaataATGCGAAGTTTCTGTAAAACGATAG	***PaapA1-lacZ*** Forward primer to amplify downstream region of *aapA1* with oLEM018. Primer contains a homologous region to *Kan*.
oLEM070	CTAGTcatatgCTTTTCCTTCAACATGGCAAAAACTCTTGG	***PisoA1-lacZ*** Reverse primer to amplify the upstream region of isoA1 promoter with oLEM018. Primer contains a homologous region to RBS-*lacZ*.
oLEM071	gtacctggagggaataATGCAACAATCTTTCTAAAACC	***PisoA1-lacZ*** Forward primer to amplify downstream region of isoA1with oLEM015. Primer contains a homologous region to Kanamycin resistance cassette.
oLEM213	AGTTTTTGCCATGTTTGGTA	***Northern Blot*** *AapA1 probe*
oLEM214	GAGTTTGTCATGGCTACCAA	***Northern Blot*** *isoA1 probe*
oLEM215	TCGGAATGGTTAACTGGGTAGTTCCT	***Northern Blot*** *5S probe*