Expanded View Figures

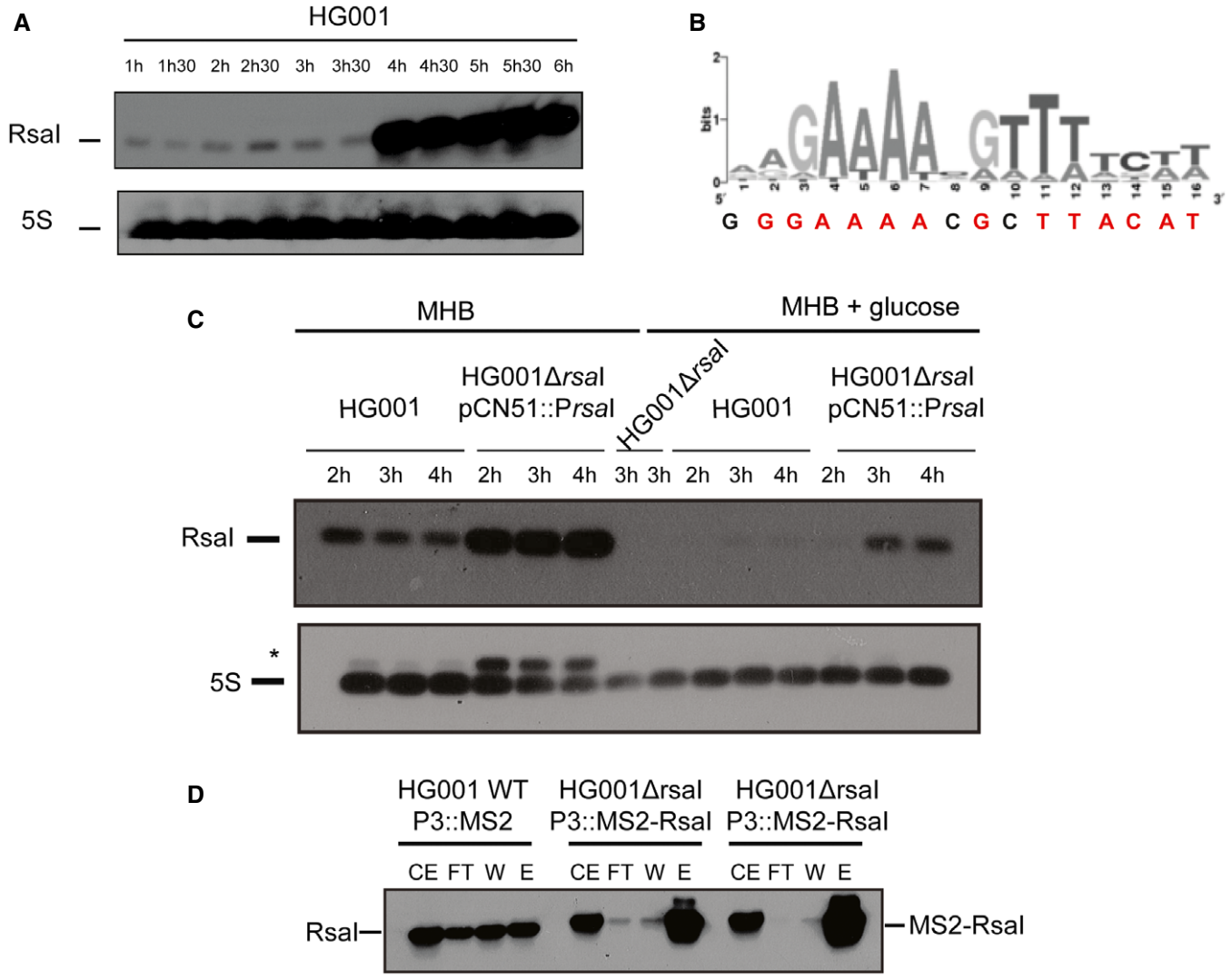


Figure EV1. Analysis of the RsaI expression.

A Northern blot experiments showing the expression of RsaI in HG001 strain during growth phase. Total RNA was prepared from samples taken after various time points of growth in BHI medium at 37°C. Hybridization against 5S rRNA was used as loading control on the same samples which have been migrated on another agarose gel because RsaI and 5S rRNA have very similar sizes.

B The *cre*-site consensus was defined in *Bacillus cereus* (van der Voort *et al*, 2008). The sequence found in the 5′ region of RsaI is represented below the graph, the conserved residues are in red.

C Northern blot experiments showing the expression of RsaI in HG001 strain, the isogenic HG001Δ*rsa*I mutant strain, and the same mutant strain complemented with a plasmid expressing RsaI under the control of its own promoter (HG001Δ*rsa*I pCN51::PrsaI). Total RNA was prepared from samples taken after various time points of growth in MHB medium in the absence or presence of 1% glucose. For the mutant HG001Δ*rsa*I strain (used as a negative control), total RNA was prepared after 4 h of growth in MHB medium in the absence or in the presence of glucose. Hybridization against 5S rRNA was used as loading control on the same membrane. *Traces of RsaI signal after re-hybridization of the membrane with the 5S probe were still observed.

D MS2-RsaI is specifically retained by affinity chromatography containing the MS2-MBP protein. The Northern blot was performed using a DIG-labeled RsaI probe (Table EV3) to visualize RsaI and MS2-RsaI following the MS2 chromatography affinity. CE is for crude extract, FT for flow-through, W for washing, and E for elution. For CE/FT/W samples, 5 µg of total RNA was loaded on a 1.5% agarose gel while for E sample, only 0.5 µg of total RNA was used. The two replicates are shown for the RNA purified from the strain HG001Δ*rsa*I::MS2-RsaI.

Source data are available online for this figure.

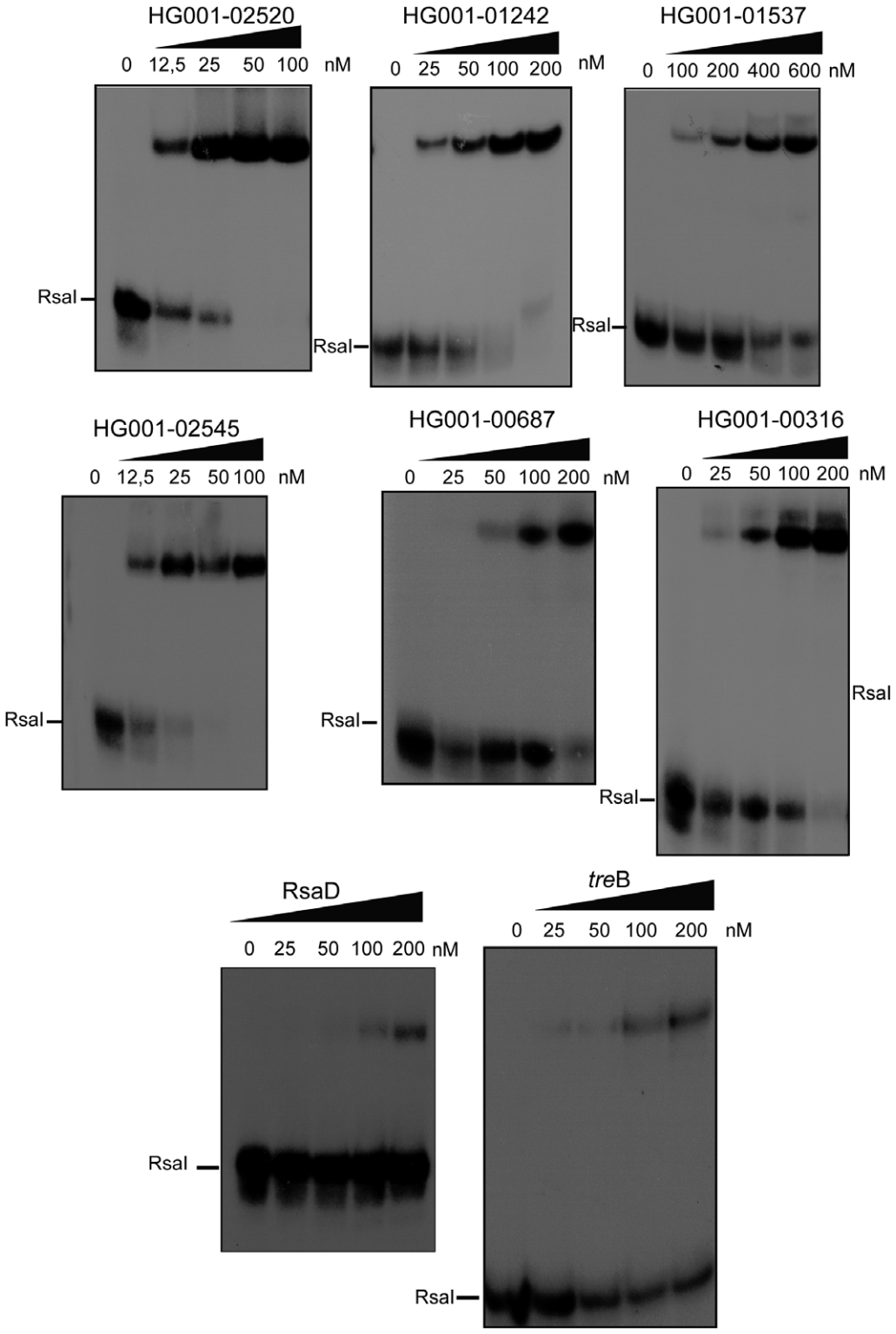


Figure EV2. RsaI interacts with several RNA species.

Gel retardation assays showing the formation of complexes between RsaI and several mRNAs, and the sRNA RsaD. The 5′ end-labeled RsaI was incubated with increasing concentrations of target mRNAs or RsaD as shown on the top of the autoradiographies. Quantification of the autoradiographies showed that RsaI forms stable complexes with many of the mRNAs (Kd < 50 nM) except for TreB and RsaD (Kd > 200 nM).

Source data are available online for this figure.

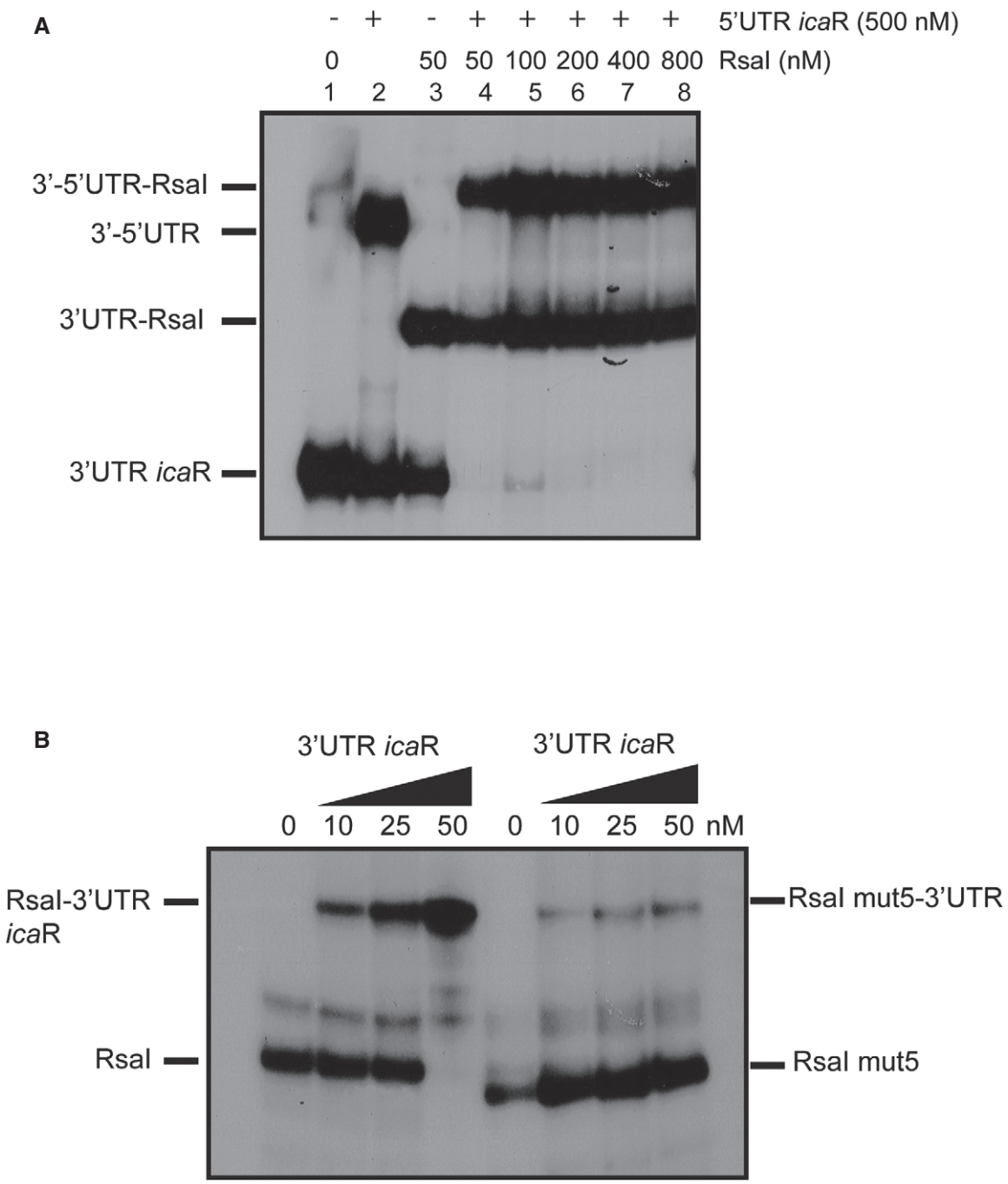


Figure EV3. RsaI interacts with the 3′UTR of *icaR* mRNA.

A Analysis of the effect of RsaI on the interaction between the 5′UTR and the 3′UTR of *icaR*. The 5′ end-labeled of the 3′UTR of *icaR* was incubated with 500 nM of the 5′UTR of *icaR* alone or in the presence of increasing concentrations of RsaI, as shown on the top of the autoradiography.

B Gel retardation assays showing the formation of complexes between RsaI or RsaI mut5 and the 3′UTR of *icaR*. RsaI or RsaI mut5 labeled at their 5′ ends was incubated with increasing concentrations of the 3′UTR of *icaR* (10, 25, and 50 nM). In both gels, the various RNA species present in the different bands are given on one side of the autoradiographies.

Source data are available online for this figure.

Figure EV4. Analysis of the expression of RsaG and RsaD in response to stress.

A Genomic organization of the *uhp*T locus in HG001 strain. This locus is conserved in all *S. aureus* strains (Geissmann *et al*, 2009). The transcription of *uhp*T containing RsaG is induced by the two-component system HptRS, which senses the extracellular concentration of glucose-6 phosphate (G-6P).

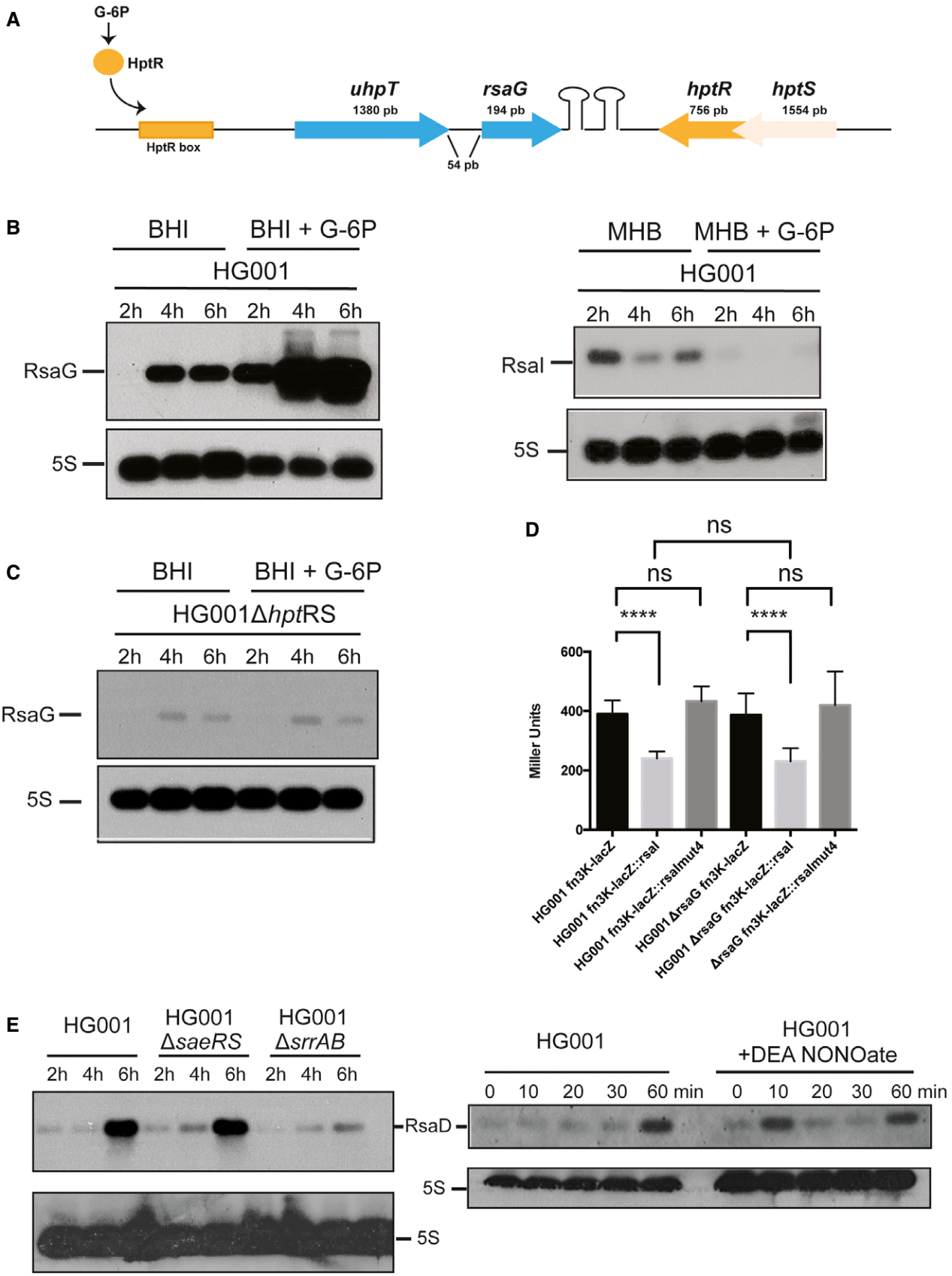
B Northern blot experiment showing RsaG (left panel) or RsaI (right panel) expression in the HG001 strain. Total RNA was extracted at 2, 4, and 6 h of growth in BHI or MHB with or without addition of glucose-6 phosphate (+G-6P). 5S rRNA was used as loading control using the same samples, which have been migrated on another agarose gel.

C Northern blot experiment showing RsaG expression in the HG001Δ*hpt*RS deleted mutant. Total RNA was extracted at 2, 4, and 6 h of growth in BHI with or without addition of glucose-6 phosphate (+G-6P). 5S rRNA was used as loading control using the same samples, which have been migrated on another agarose gel.

D The β-galactosidase activity (Miller Units) have been measured from P*rpo*B::*fn3K*::*lacZ*, P*rpo*B::*fn3K*::*lacZ*::*rsa*I, and P*rpo*B::*HG001_fn3K*::*lacZ*::*rsa*I mut4 expressed in HG001 and HG001Δ*rsa*G strains. The β-galactosidase activity was normalized for bacterial density and the results represented the mean of four independent experiments. ****$P < 0.0001$, ns: not significant.

E Northern blot experiment showing the steady-state level of RsaD. On the left, RsaD expression was analyzed at 2, 4, and 6 h of growth, in HG001 wild-type strain and in Δ*sae*RS and Δ*srr*AB mutant strains. On the right, the expression of RsaD was monitored in response to nitric oxide (NO) stress. The culture performed in BHI medium until OD_{600} = 0.2 at 37°C was then treated with the addition of 100 μM Na-diethylamine (+DEA NONOate). 5S rRNA was used as loading control using the same samples, which have been migrated on another agarose gel.

Source data are available online for this figure.


A
G-6P
HptR
HptR box
uhpT
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rsaG
194 pb
54 pb
hptR
756 pb
hptS
1554 pb
B
BHI
BHI + G-6P
HG001
2h 4h 6h 2h 4h 6h
RsaG
5S
MHB
MHB + G-6P
HG001
2h 4h 6h 2h 4h 6h
RsaI
5S
C
BHI
BHI + G-6P
HG001ΔhptRS
2h 4h 6h 2h 4h 6h
RsaG
5S
D
ns
ns
ns
****
****
600
400
200
0
Miller Units
HG001 fn3K-lacZ
HG001 fn3K-lacZ::rsaI
HG001 fn3K-lacZ::rsaImut4
HG001 ΔrsaG fn3K-lacZ
HG001 ΔrsaG fn3K-lacZ::rsaI
ΔrsaG fn3K-lacZ::rsaImut4
E
HG001
HG001 ΔsaeRS
HG001 ΔsrrAB
2h 4h 6h 2h 4h 6h 2h 4h 6h
RsaD
5S
5S
HG001
HG001 +DEA NONOate
0 10 20 30 60 min 0 10 20 30 60 min

Figure EV4.

Figure EV5. RsaI represents a signature of a metabolic change as the result of glucose consumption.

A Growth curves of the wild-type 132 (132) and mutant Δ*rsa*I (132D) strains containing the vector pES (empty) or pES::*rsa*I (expressing RsaI from a constitutive promoter) in BHI.

B Northern blot experiment showing RsaI expression in the wild-type 132 (132) or mutant Δ*rsa*I (132Δ*rsa*I) strains containing the vector pES or pES::*rsa*I. Total RNA was extracted at 2, 4, and 6 h of growth in BHI. 5S rRNA was used as the loading control using the same samples, which have been migrated on another agarose gel. Quantification of RsaI normalized to 5S rRNA was done with ImageQuant TL software (GE Healthcare Life Sciences).

C Northern blot experiments showing the expression of RsaI in the wild-type HG001 strain, the mutant HG001Δ*rsa*I strain, and the same mutant strain complemented with a plasmid expressing RsaI from its own promoter (HG001Δ*rsa*I pCN51::P*rsa*I). All the other strains have been transformed with the pCN51 plasmid. Total RNA was prepared from samples taken after 6 h of growth in BHI medium. Hybridization against 5S rRNA was used as loading control using the same membrane. Three replicates were carried out (lanes 1–3), and the same samples were used for the differential transcriptomic analysis.

Source data are available online for this figure.

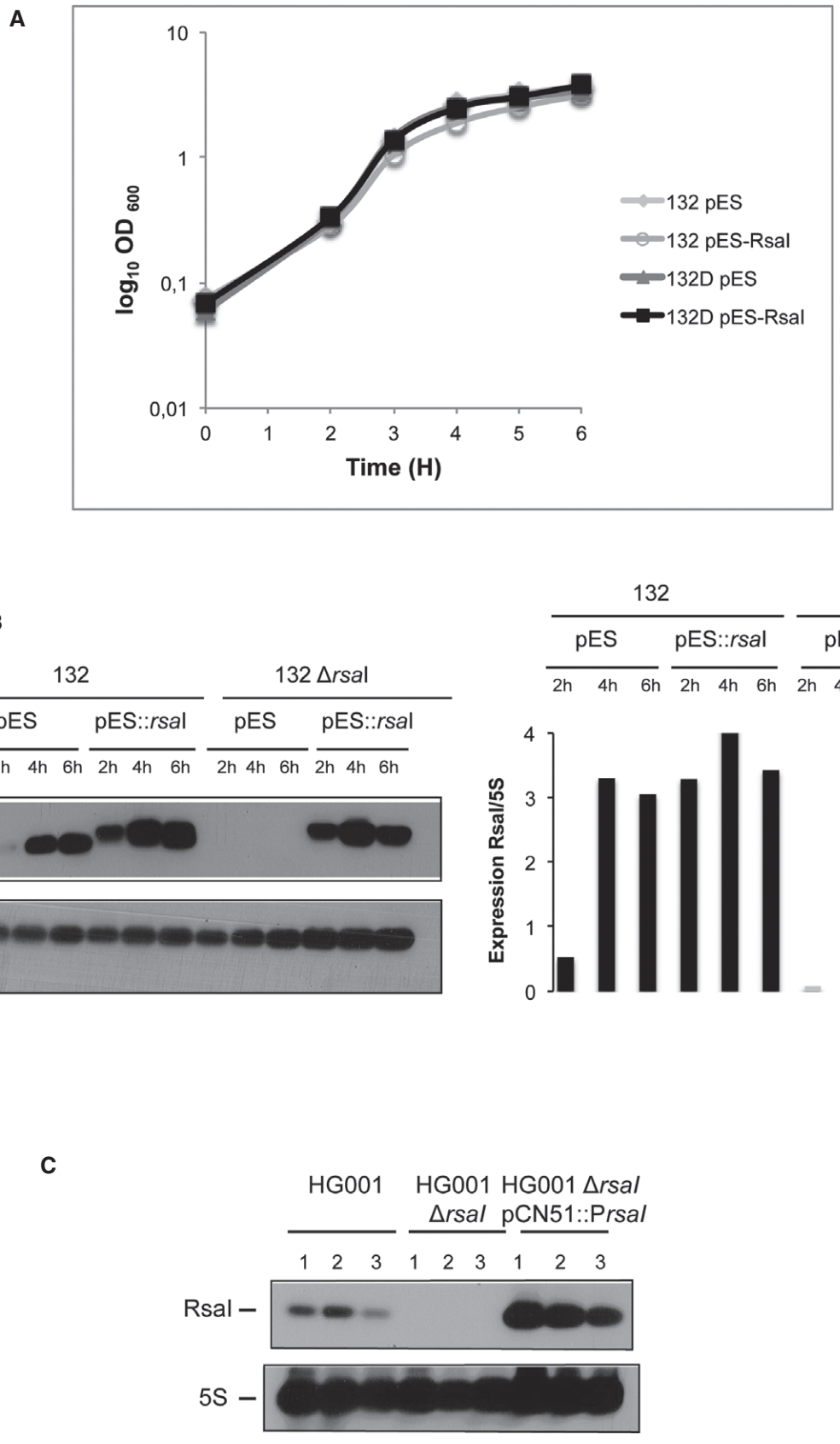
A
10
1
0,1
0,01
log10 OD 600
0 1 2 3 4 5 6
Time (H)
132 pES
132 pES-RsaI
132D pES
132D pES-RsaI
B
132
132 ΔrsaI
pES
pES::rsaI
pES
pES::rsaI
2h 4h 6h 2h 4h 6h 2h 4h 6h 2h 4h 6h
RsaI
5S
132
132 ΔrsaI
pES
pES::rsaI
pES
pES::rsaI
2h 4h 6h 2h 4h 6h 2h 4h 6h 2h 4h 6h
Expression RsaI/5S
4
3
2
1
0
C
HG001
HG001 ΔrsaI
HG001 ΔrsaI pCN51::PrsaI
1 2 3 1 2 3 1 2 3
RsaI
5S

Figure EV5.