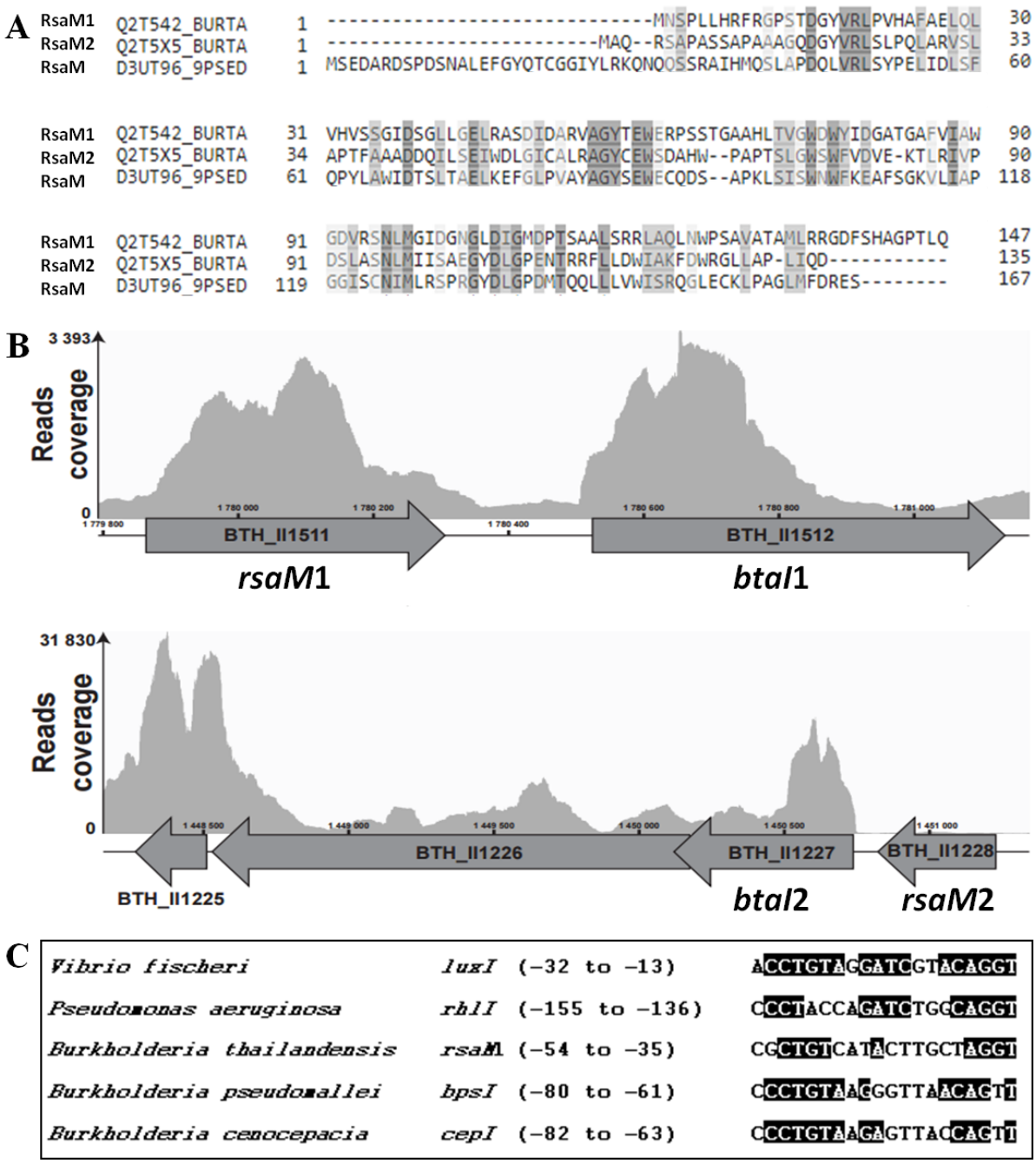

Figure S1. *B. thailandensis* possesses two conserved RsaM-like proteins designated RsaM1 and RsaM2. (A) Sequence alignment of the RsaM1 and RsaM2 proteins of *B. thailandensis* E264 with the *P. fuscovaginae* UPB0736 RsaM homologue. The alignment was generated using Clustal W. Strictly conserved residues are shown on a dark grey background and moderately conserved ones are shown on a light grey background. (B) Genetic arrangement of the RsaM1- and RsaM2-encoding genes with *btaI1* and *btaI2*, respectively. (C) The *rsaM1* gene that codes for RsaM1 possesses in its promoter region a putative *lux* box sequence, which is homologous to characterized *lux* box sequences in Proteobacteria. The start and stop positions of each sequence are indicated in parentheses relative to the translational start site.

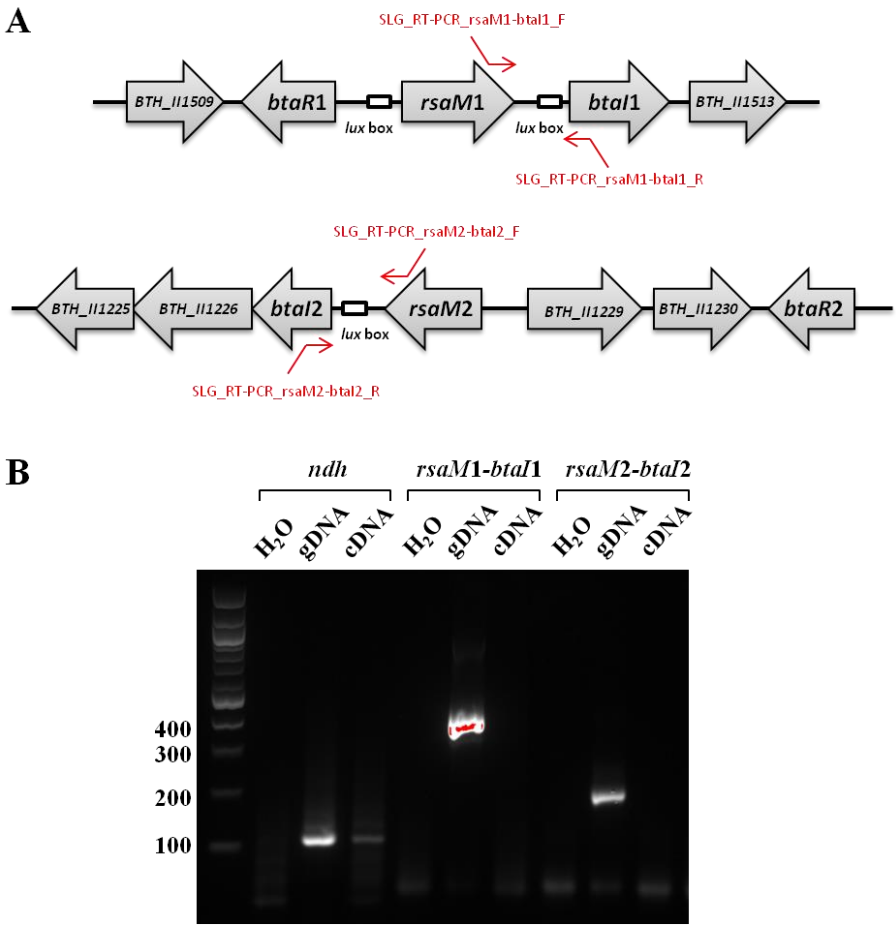

Figure S2. Confirmation of the genetic organization of the *rsaM1* and *rsaM2* genes. (A) The *rsaM1* and *rsaM2* genes are directly adjacent to *btaI1* and *btaI2* on the genome of *B. thailandensis* E264, respectively. The SLG_RT-PCR_rsaM1-btaI1_F and SLG_RT-PCR_rsaM1-btaI1_R primer pair was used to amplify the intergenic region of the *rsaM1* and *btaI1* genes, whereas the SLG_RT-PCR_rsaM2-btaI2_F and SLG_RT-PCR_rsaM2-btaI2_R primer pair was used to amplify the intergenic region of the *rsaM2* and *btaI2* genes. These regions each contain a putative *lux* box sequence as reported previously (1). (B) RT-PCR experiments were performed to examine cotranscription of the *rsaM1* and *rsaM2* genes with *btaI1* and *btaI2*, respectively, and analyzed by agarose gel electrophoresis. The notation *ndh* indicates that the PCR reactions were conducted using the SLG_qRT-PCR_ndh_F and SLG_qRT-PCR_ndh_R primer pair with H_2O as a negative control, genomic DNA (gDNA) as a positive control, or complementary DNA (cDNA) of *B. thailandensis* E264. These reactions testify to the efficiency of the RT-PCR experiments as revealed by the presence of a PCR amplification product in the cDNA lane. The notation *rsaM1-btaI1* indicates that the PCR reactions were carried out with the SLG_RT-PCR_rsaM1-btaI1_F and SLG_RT-PCR_rsaM1-btaI1_R primer pair, whereas the notation *rsaM2-btaI2* indicates that the PCR reactions were carried out with the SLG_RT-PCR_rsaM2-btaI2_F and SLG_RT-PCR_rsaM2-btaI2_R primer pair. The absence of PCR amplification products in the cDNA lanes reveals that neither *rsaM1* nor *rsaM2* are cotranscribed with the *btaI1* and *btaI2* genes, respectively. The molecular marker is the DNA Ladder 100 bp (New England Biolabs, Inc., Whitby, ON, Canada).

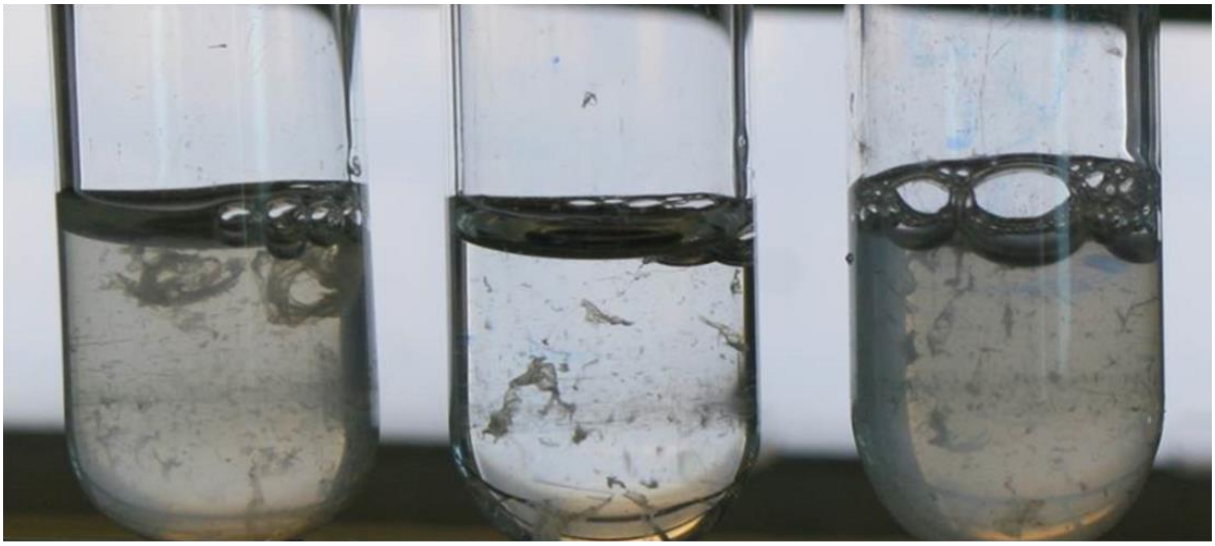

Figure S3. Cell aggregation in the wild-type and the *rsaM1*- and *rsaM2*- mutant strains of *B. thailandensis* E264. To assess auto-aggregation of the *B. thailandensis* E264 wild-type strain and *rsaM1*- and *rsaM2*- mutant strains, overnight bacterial cultures were diluted in M9 minimal medium supplemented with 0.4% glucose (wt/vol) and morpholinepropanesulfonic acid (MOPS) buffer (50 mM; pH 7.0) to an initial OD_{600} of 0.01 and grown with shaking for 24 hrs at 37°C as described previously (2).

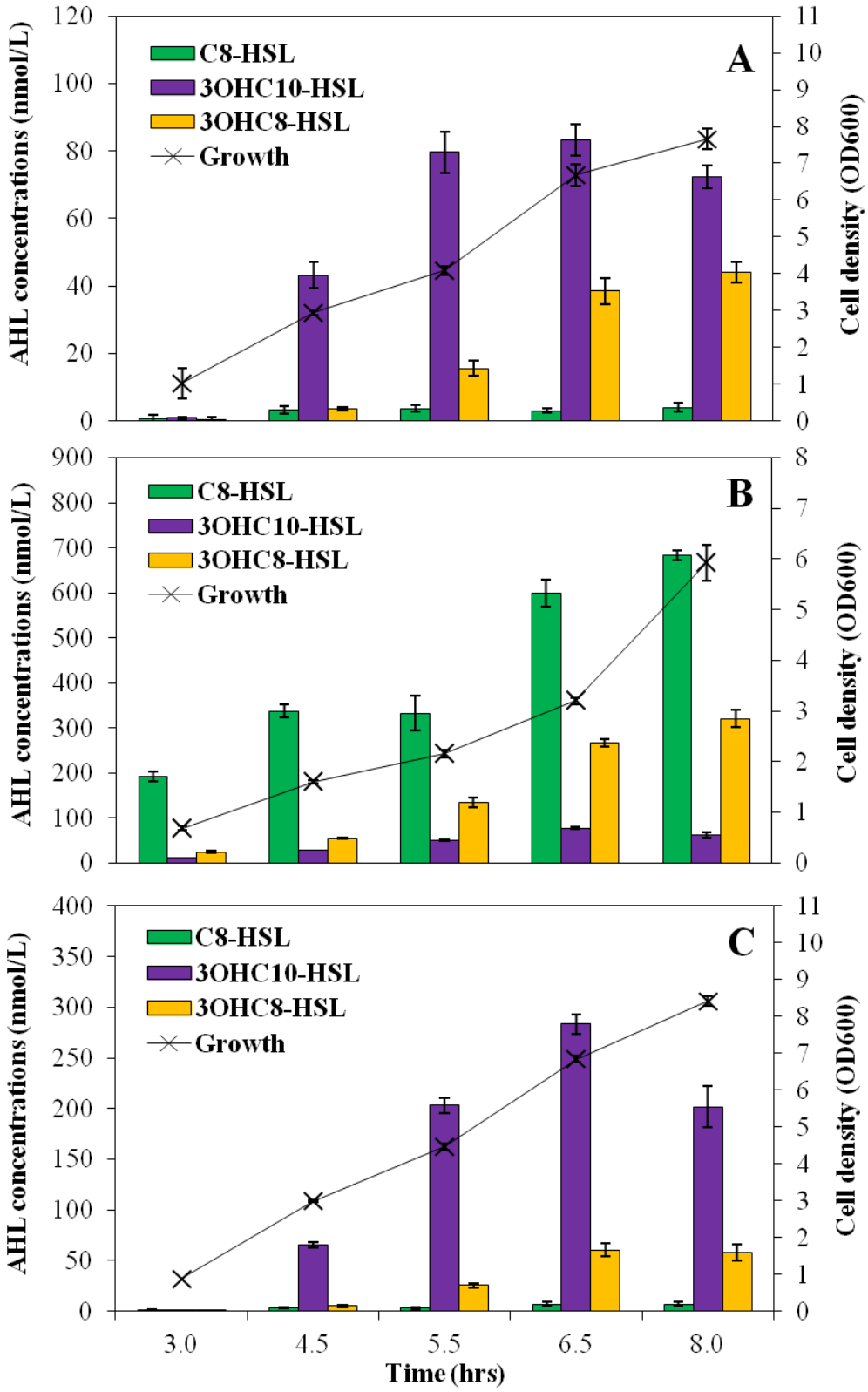

Figure S4. AHL production profiles in the wild-type and the *rsaM1*- and *rsaM2*- mutant strains of *B. thailandensis* E264. The biosynthesis of AHLs (bars) was monitored by LC-MS/MS at various times during growth (lines) in cultures of (A) the *B. thailandensis* E264 wild-type strain and (B) *rsaM1*- and (C) *rsaM2*- mutant strains. The values represent the means for three replicates.

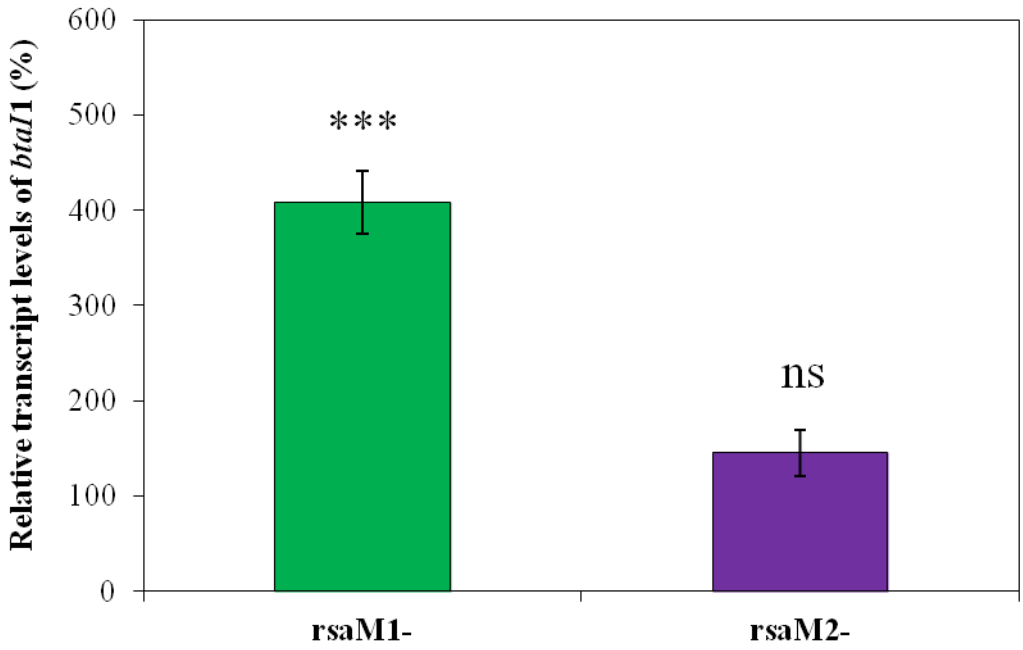

Figure S5. The impact of RsaM1 and RsaM2 on *btaI1* transcription. The relative transcript levels of *btaI1* were assessed by qRT-PCR in cultures of the wild-type and the *rsaM1*- and *rsaM2*- mutant strains of *B. thailandensis* E264. The results are presented as relative quantification of transcription of the gene compared to the wild-type strain, which was set at 100%. The values represent the means for three replicates. ***, $P < 0.001$; ns, nonsignificant.

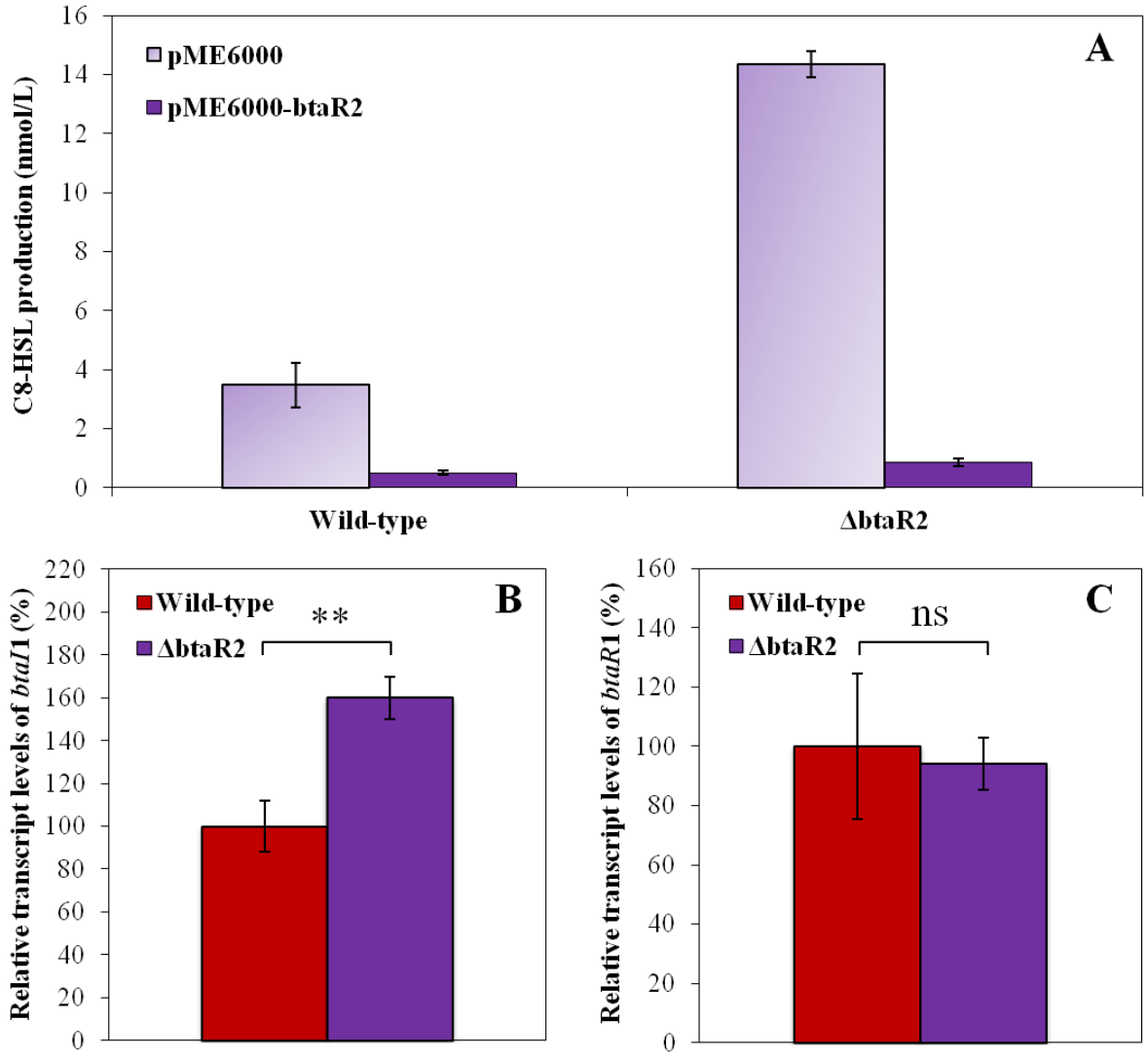

Figure S6. The biosynthesis of C_8-HSL and *btaI1* transcription are negatively regulated by BtaR2. (A) C_8-HSL production was quantified using LC-MS/MS during the logarithmic growth in cultures of the wild-type strain and the Δ*btaR2* mutant strain of *B. thailandensis* E264 carrying either pME6000 (light purple bars) or pME6000-*btaR2* (dark purple bars). The values represent the means for three replicates. The relative transcript levels of (B) *btaI1* and (C) *btaR1* from the wild-type *B. thailandensis* E264 strain and its Δ*btaR2* mutant strain were estimated by qRT-PCR during the logarithmic growth. The results are presented as relative quantification of transcription of the gene compared to the wild-type strain, which was set at 100%. **, $P < 0.01$; ns, nonsignificant.

Table S1. Bacterial strains used in this study.

Strains	Description	Reference
E. coli		
χ7213	*thr*-1, *leuB*6, *fhuA*21, *lacY*1, *glnV*44, *recA*1, Δ*asdA*4, Δ(*zhf*-2::Tn*10*), *thi*-1, RP4-2-Tc :: Mu [λ *pir*]	Lab collection
DH5α	F-, φ80d*lacZ*ΔM15, Δ(*lacZYA*-*argF*)U169, *deoR*, *recA*1, *endA*1, *hsdR*17(rk-, mk+), *phoA*, *supE*44, λ-, *thi*-1, *gyrA*96, *relA*1	Lab collection
B. thailandensis		
E264	Wild-type	(3)
JBT107	E264 Δ*btaR1*	(2)
JBT108	E264 Δ*btaR2*	(2)
JBT109	E264 Δ*btaR3*	(2)
JBT112	E264 Δ*btaI1* Δ*btaI2* Δ*btaI3*	(2)
JBT101	E264 Δ*btaI1*	(2)
JBT102	E264 Δ*btaI2*	(2)
JBT103	E264 Δ*btaI3*	(2)
BT03295	E264 *rsaM1*::IS*lacZ*-PrhaBo-Tp/FRT; Tp^R	(4)
BT04218	E264 *rsaM2*::IS*lacZ*-PrhaBo-Tp/FRT; Tp^R	(4)
ED1020	E264 (pME6000)	This study
ED3477	E264 (pME6000-*btaR2*)	This study
ED3478	E264 Δ*btaR2* (pME6000)	This study
ED1015	E264 Δ*btaR2* (pME6000-*btaR2*)	This study
ED3330	E264::*btaI1-lux*	(1)

ED3331	E264::*btaI2-lux*	(1)
ED3332	E264::*btaI3-lux*	(1)
ED3469	E264 *rsaM1*-::*btaI1-lux*	This study
ED3470	E264 *rsaM1*-::*btaI2-lux*	This study
ED3471	E264 *rsaM1*-::*btaI3-lux*	This study
ED3472	E264 *rsaM2*-::*btaI1-lux*	This study
ED3473	E264 *rsaM2*-::*btaI2-lux*	This study
ED3474	E264 *rsaM2*-::*btaI3-lux*	This study

Table S2. Plasmids used in this study.

Plasmids	Description	Source
pME6000	Broad-host-range cloning vector; Tc^R	(5)
pMCG21	*btaR2* inserted in *Bam*HI-*Hind*III restriction sites in pME6000; Tc^R	This study
pSLG02	*btaI1* promoter inserted in the restriction sites *Xho*I-*Bam*HI in the mini-CTX-*lux* integration vector with promoterless *luxCDABE*; Tc^R	(1)
pSLG03	*btaI2* promoter inserted in the restriction sites *Xho*I-*Bam*HI in the mini-CTX-*lux* integration vector with promoterless *luxCDABE*; Tc^R	(1)
pSLG04	*btaI3* promoter inserted in the restriction sites *Xho*I-*Bam*HI in the mini-CTX-*lux* integration vector with promoterless *luxCDABE*; Tc^R	(1)

Table S3. Primers used for PCR.

Genes	Oligonucleotides	Sequences (5’ to 3’)
btaR2	btaR2F	GCGGGATCCATGCGAGGATATGGAGATGC
	btaR2R	CGCAAGCTTTCGAGATATCCCGCCTATTG

Table S4. Primers used for RT-PCR and qRT-PCR.

Genes	Oligonucleotides	Sequences (5’ to 3’)
ndh	SLG_qRT-PCR_ndh_F	ACCAGGGCGAATTGATCTC
	SLG_qRT-PCR_ndh_R	GATGACGAGCGTGTCGTATT
btaR1	SLG_qRT-PCR_btaR1_F	AGCTCGAACATGATCGTCTG
	SLG_qRT-PCR_btaR1_R	TGAAGCGTCAGATGGTTGAT
btaR2	SLG_qRT-PCR_btaR2_F	GAGAAATTCCGCAACGAGAG
	SLG_qRT-PCR_btaR2_R	GCCGTCCACTTCAACACAT
btaR3	SLG_qRT-PCR_btaR3_F	CGACTACTTCACCATCGATCC
	SLG_qRT-PCR_btaR3_R	GCTGATGCCGTTGTCGAG
btaI1	btaI1RTF	CTTCGAACGGGATCAATACG
	btaI1RTR	CATGTCGTGTGCGACCAG
rsaM1	SLG_qPCR_BTH_II1511_F	TGAATTCACCACTGCTCCAC
	SLG_qPCR_BTH_II1511_R	ATTCCGGACGATACGTGAAC
rsaM2	SLG_qPCR_BTH_II1228_F	GGACGGATACGTTCGTCTGT
	SLG_qPCR_BTH_II1228_R	AGGTCCCAGATTTCCGAGAG
rsaM1-btaI1	SLG_RT-PCR_rsaM1-btaI1_F	GTCGCTACCGCGATGCTT
	SLG_RT-PCR_rsaM1-btaI1_R	CCGATAAAGGCCCAGATCA
rsaM2-btaI2	SLG_RT-PCR_rsaM2-btaI2_F	GGCGATTTCTATTGGATTGG
	SLG_RT-PCR_rsaM2-btaI2_R	CTTGACGGTGGAATCCAGTT

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