bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1

2

3 # ScmR, a global regulator of gene expression, quorum sensing, pH
4 # homeostasis, and virulence in *Burkholderia thailandensis*

5

6 Servane Le Guillouzer, Marie-Christine Groleau, Florian Mauffrey[†], and Eric Déziel#

7

8 Centre Armand-Frappier Santé Biotechnologie, Institut National de la Recherche Scientifique
9 (INRS), Laval, Québec, Canada

10

11 [†]Present address : Université de Genève, Geneva, Switzerland.

12

13 #Corresponding author. Mailing address: INRS- Armand-Frappier Santé Biotechnologie, 531
14 Boulevard des Prairies, Laval, Québec H7V 1B7, Canada.
15 e-mail: eric.deziel@iaf.inrs.ca
16 Phone: (450) 687-5010,

17

18 Running title: The ScmR regulator in *Burkholderia thailandensis*

19

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

20 ## Abstract

21 The nonpathogenic soil saprophyte *Burkholderia thailandensis* is a member of the
22 *Burkholderia pseudomallei-thailandensis-mallei* (*Bptm*) group, which also comprises the
23 closely related human pathogens *Burkholderia pseudomallei* and *Burkholderia mallei*
24 responsible for the diseases melioidosis and glanders, respectively. ScmR, a recently
25 identified LysR-type transcriptional regulator (LTTR) in *B. thailandensis* acts as a global
26 transcriptional regulator throughout the stationary phase, and modulates the production of
27 a wide range of secondary metabolites, including *N*-acyl-L-homoserine lactones (AHLs) and
28 4-hydroxy-3-methyl-2-alkylquinoline (HMAQ), virulence in the model host *Caenorhabditis*
29 *elegans*, as well as several quorum sensing (QS)-dependent phenotypes. We have
30 investigated the role of ScmR in *B. thailandensis* strain E264 during the exponential phase.
31 We used RNA-Sequencing (RNA-Seq) transcriptomic analyses to identify the ScmR regulon,
32 which was compared to the QS-controlled regulon, showing a considerable overlap between
33 the ScmR-regulated genes and those controlled by QS. We characterized several genes
34 modulated by ScmR, using quantitative reverse transcription-PCR (qRT-PCR) or mini-CTX-*lux*
35 transcriptional reporters, including the oxalate biosynthetic gene *obc1* required for pH
36 homeostasis, the orphan LuxR-type transcriptional regulator BtaR5-encoding gene, the *bsa*
37 (*Burkholderia* secretion apparatus) type III secretion system (T3SS) genes essential for both
38 *B. pseudomallei* and *B. mallei* pathogenicity, as well as the *scmR* gene itself. We confirmed
39 that the transcription of *scmR* is under QS control, presumably ensuring fine-tuned
40 modulation of gene expression. Finally, we demonstrate that ScmR influences virulence
41 using the fruit fly model host *Drosophila melanogaster*. We conclude that ScmR represents a
42 central component of the *B. thailandensis* QS regulatory network.

43

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

44 **Importance**

45 Coordination of the expression of genes associated with bacterial virulence and
46 environmental adaptation is often dependent on quorum sensing (QS). The QS circuitry of
47 the nonpathogenic bacterium *Burkholderia thailandensis*, which is widely used as a model
48 system for the study of the human pathogen *Burkholderia pseudomallei*, is complex. We
49 found that the recently identified LysR-type transcriptional regulator (LTTR), ScmR, which is
50 highly conserved and involved in the control of virulence/survival factors in the *Burkholderia*
51 genus, is a global regulator mediating gene expression through the multiple QS systems
52 coexisting in *B. thailandensis*, as well as independently of QS. We conclude that ScmR
53 represents a key QS modulatory network element, ensuring tight regulation of the
54 transcription of QS-controlled genes, particularly those required for acclimatization to the
55 environment.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

56 **Introduction**

57 Quorum sensing (QS) is a global regulatory mechanism of gene expression depending on
58 bacterial density (1). Gram-negative bacteria often possess homologues of the LuxI/LuxR
59 system initially characterized in the bioluminescent marine bacterium *Vibrio fischeri* (2). The
60 signaling molecules *N*-acyl-L-homoserine lactones (AHLs), which are typically produced by
61 LuxI-type synthases, accumulate in the environment as bacterial growth progresses until a
62 threshold concentration is reached allowing bacteria to synchronize their activities and to
63 function as multicellular communities. These AHLs activate LuxR-type transcriptional
64 regulators that modulate the transcription of QS target genes, which contain a *lux* box
65 sequence in their promoter region (3).

66 *Burkholderia thailandensis* is a nonpathogenic soil saprophyte belonging to the *Burkholderia-*
67 *pseudomallei-thailandensis-mallei* (*Bptm*) group, which also comprises the closely related
68 pathogens *Burkholderia pseudomallei* and *Burkholderia mallei* responsible for melioidosis
69 and glanders, respectively (4). *B. thailandensis* is considered the avirulent version of *B.*
70 *pseudomallei* (5), and is thus commonly used as a surrogate model for the study of *B.*
71 *pseudomallei*, which is considered a potential bioterrorism agent and whose manipulation is
72 consequently restricted to biosafety level 3 (BSL3) laboratories (6). The members of the
73 *Bptm* group carry multiple LuxI/LuxR QS systems that are associated with the biosynthesis of
74 numerous AHL signaling molecules (4, 7-9). These QS systems are referred to as the
75 BtaI1/BtaR1 (QS-1), BtaI2/BtaR2 (QS-2), and BtaI3/BtaR3 (QS-3) QS systems in *B.*
76 *thailandensis* (10, 11). The QS-1 system is composed of the BtaR1 transcriptional regulator
77 and the BtaI1 synthase, which synthesizes *N*-octanoyl-L-homoserine lactone (C_8-HSL) (12,
78 13). The BtaR2 transcriptional regulator and the BtaI2 synthase that catalyze the

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

79 biosynthesis of both *N*-3-hydroxy-decanoyl-L-homoserine lactone ($3OHC_{10}$-HSL) and *N*-3-
80 hydroxy-octanoyl-L-homoserine lactone ($3OHC_8$-HSL) constitute the QS-2 system (12, 14).
81 The QS-3 system is composed of the BtaR3 transcriptional regulator and the BtaI3 synthase
82 is also responsible for $3OHC_8$-HSL production (12, 13). Furthermore, *B. thailandensis*, *B.*
83 *pseudomallei*, and *B. mallei* carry orphan *luxR* homologues, namely, *btaR4* (*malR*) and *btaR5*
84 in *B. thailandensis* (15, 16).

85 QS is involved in the regulation of several virulence factors in *B. pseudomallei* and *B. mallei*,
86 and is essential to their full capacity to cause infections (7, 8, 17, 18). Other QS-controlled
87 phenotypic traits among the *Bptm* group members have been reported, such as colony
88 morphology, the development of biofilm, self-aggregation, motility, pH homeostasis, as well
89 as production of secondary metabolites (9, 10, 13, 14, 16, 18-25).

90 A LysR-type transcriptional regulator (LTTR) involved in secondary metabolism regulation,
91 hence designated ScmR, was recently identified in the *Bptm* group members (26). LTTRs are
92 part of a large family and display a well conserved structure with a N-terminal DNA-binding
93 helix-turn-helix motif and a C-terminal cofactor-binding domain (27). LTTRs are typically
94 negatively autoregulated and frequently positively modulate expression of adjacent genes
95 (27). Nevertheless, LTTRs were also described as global regulators acting positively or
96 negatively (27). Mao *et al.* (26) demonstrated that ScmR constitutes a global regulator of
97 gene expression in *B. thailandensis* and influences the production of a wide range of
98 secondary metabolites, including AHLs and the putative 4-hydroxy-3-methyl-2-alkylquinoline
99 (HMAQ) signaling molecules, virulence in the nematode worm model *Caenorhabditis*
100 *elegans*, as well as several QS-dependent phenotypes. Additionally, expression of the *scmR*
101 gene is under QS control (10, 26).

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

102 The central goal of the present study was to further characterize the molecular mechanism
103 of action of the *B. thailandensis* E264 ScmR transcriptional regulator. We found that ScmR is
104 a global regulator mediating gene expression through the QS-1, QS-2, and/or QS-3 systems,
105 as well as independently of QS. Furthermore, we identified novel genes modulated by ScmR,
106 including the oxalate biosynthetic gene *obc1* that is essential for pH homeostasis in the
107 *Burkholderia* genus, the orphan LuxR-type transcriptional regulator BtaR5-encoding gene,
108 and the *bsa* (*Burkholderia* secretion apparatus) type III secretion system (TTSS) genes
109 required for both *B. pseudomallei* and *B. mallei* pathogenicity. Moreover, we showed that
110 *scmR* is negatively autoregulated, and we confirmed that its transcription is QS-controlled,
111 ensuring tight regulation of gene expression by ScmR in *B. thailandensis*. Finally, we
112 demonstrate that ScmR represses virulence using the fruit fly model *Drosophila*
113 *melanogaster*. All in all, this study contributes to a better appreciation of the ScmR
114 regulatory mechanism of the expression of genes in *B. thailandensis*, and in particular those
115 related to virulence of *B. pseudomallei*.

116

117 ## Results

118 ### The ScmR regulon comprises many QS-controlled genes

119 ScmR was recently described as a global transcriptional regulator impacting gene expression
120 during the stationary phase of bacterial growth in *B. thailandensis* (26). We used RNA-Seq
121 transcriptomic analyses to further characterize the regulon of the ScmR transcriptional
122 regulator. We identified the ScmR-regulated genes by comparing the transcripts in the wild-
123 type and in the *scmR*- mutant strains of *B. thailandensis* E264 throughout the logarithmic

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

124 growth phase. We found that ScmR both positively and negatively influence the expression
125 of genes located on the two *B. thailandensis* E264 chromosomes (**Fig. 1A**). Using a 3-fold
126 difference in transcription as a cut-off, we identified 907 genes that were positively
127 modulated by ScmR, and 397 genes that were negatively modulated by ScmR (**Fig. 1A**).
128 These findings confirm that ScmR constitutes a global regulator of gene expression in *B.*
129 *thailandensis* E264 (26). Our RNA-Seq analyses identified genes known to be controlled by
130 ScmR or genes encoding functions known to be controlled by ScmR. Indeed, Mao *et al.* (26)
131 recently demonstrated that ScmR stimulates the production of HMAQ,which includes
132 putative signals. RNA-Seq confirmed that expression of the *hmqABCDEFG* operon, which is
133 required for HMAQs production (28), is activated by ScmR (**Table S1**). Furthermore, ScmR
134 represses the production of burkholdac, a hybrid polyketide/nonribosomal peptide and a
135 potent inhibitor of some histone deacetylases (HDACs) (26). Consistently, expression of the
136 *bhc* gene cluster, responsible for burkholdac biosynthesis (29), was increased in the *scmR*-
137 mutant compared to the wild-type strain (**Table S1**). Moreover, we observed that ATP
138 synthesis and stress response genes were downregulated in the absence of ScmR (**Table S1**),
139 as recently reported (26). Finally, RNA-Seq showed that transcription of the putative
140 exopolysaccharide (EPS) genes *bceABCDEFGHIJ* and *bceNOPRSTU* is affected by ScmR (**Table**
141 **S1**). This is in agreement with the finding that ScmR influences colony morphology, as well as
142 pellicle and biofilm formation of *B. thailandensis* E264 (26).

143 The ScmR transcriptional regulator was shown to influence the biosynthesis of C_8-HSL,
144 $3OHC_{10}$-HSL, and $3OHC_8$-HSL (26), the main AHLs produced by *B. thailandensis* E264 (10, 11,
145 13, 14). Therefore, we assumed that ScmR could intervene in the regulation of gene
146 expression, *inter alia*, by impacting the QS-1, QS-2, and/or QS-3 systems of *B. thailandensis*
147 E264. Indeed, Mao *et al.* (26) demonstrated that QS-dependent phenotypes, including

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

148 colony morphology, as well as the development of biofilm, are influenced by ScmR and we
149 accordingly found several previously reported QS-controlled genes in the ScmR regulon
150 (**Table S1**). Consequently, we also compared the transcripts in the wild-type strain of *B.*
151 *thailandensis* E264 and in the AHL-defective Δ*btaI1*Δ*btaI2*Δ*btaI3* mutant under the same
152 growth conditions to identify the genes specifically modulated by ScmR independently of its
153 effect on QS. Our RNA-Seq analyses indicate that QS positively regulated expression of 1088
154 genes and negatively modulated expression of 547 genes on both chromosomes of *B.*
155 *thailandensis* E264 (**Fig. 1B**). Importantly, we confirmed the involvement of QS in the
156 regulation of genes affected by AHLs or genes encoding functions affected by AHLs. In *B.*
157 *thailandensis*, QS stimulates contact-dependent growth inhibition (CDI) (10, 30), and we
158 indeed observed that the transcription of the CDI genes was decreased in the absence of
159 AHLs (**Table S1**). Furthermore, RNA-Seq indicated that the transcription of the bactobolin
160 biosynthetic genes (14), as well as the *obc1* gene expression, encoding the oxalate
161 biosynthetic enzyme Obc1 that is essential to pH homeostasis (19), are activated by QS
162 (**Table S1**), as previously reported (10). Moreover, RNA-Seq confirmed that expression of
163 both flagellar genes and methyl-accepting chemotaxis protein genes was upregulated in the
164 Δ*btaI1*Δ*btaI2*Δ*btaI3* mutant in comparison with the wild-type strain (10) (**Table S1**), which is
165 consistent with the observation that *B. thailandensis* E264 QS mutants are hypermotile (13).

166 Interestingly, we found a considerable overlap between the genes regulated by ScmR and
167 those QS-controlled (**Table S2**). We identified 681 genes activated by both ScmR and QS (**Fig.**
168 **2A**), whereas 310 genes were repressed by both ScmR and QS (**Fig. 2B**). Other patterns of
169 coregulation were observed including positive regulation by ScmR and negative regulation
170 by QS (**Fig. 2C**), as well as negative regulation by ScmR and positive regulation by QS (**Fig.**
171 **2D**). While we identified 1019 genes that were coregulated by both ScmR and QS, 901 genes

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

172 appeared to be independently regulated by either ScmR or QS under the conditions of our
173 experiments (**Fig. 2**). Altogether, these results support the idea that ScmR regulates the
174 transcription of many genes through modulation of the QS-1, QS-2 and/or QS-3 systems in *B.*
175 *thailandensis* E264. Additionally, we found that ScmR affected the expression of genes
176 encoding transcriptional factors, including the QS-controlled orphan transcriptional regulator
177 BtaR5-encoding gene (**Table S2**). Thus, many genes could be modulated by ScmR indirectly
178 through auxiliary regulators, as recently proposed (26).

179

180 **ScmR modulates AHLs biosynthesis but not the transcription of the AHL synthase-coding**
181 **genes**

182 The influence of ScmR on C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL production was
183 demonstrated throughout the stationary phase of growth (26), but its effect on QS during
184 the logarithmic phase had not been investigated yet. To determine whether the biosynthesis
185 of the main AHLs produced by *B. thailandensis* E264 was impacted by ScmR in the
186 exponential phase, we compared the concentrations of these AHLs in the wild-type strain of
187 *B. thailandensis* E264 and in the *scmR*- mutant. We confirmed that the levels of C_8-HSL were
188 decreased in the absence of ScmR (**Fig. 3A**), as previously reported (26), suggesting that
189 ScmR is an activator on the QS-1 system. In contrast to stationary phase observations (26),
190 however, we detected increasing concentrations of 3OHC_{10}-HSL and 3OHC_8-HSL in the *scmR*-
191 mutant versus the wild-type strain under our conditions (**Figs. 3B** and **C**), indicating that the
192 QS-2 and/or QS-3 system might be repressed by ScmR.

193 ScmR stimulates the production of C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL during the
194 stationary phase, but the expression of the BtaI1-, BtaI2-, and BtaI3-encoding genes

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

195 responsible for the production of these AHLs, nor the transcription of *btaR1*, *btaR2*, and
196 *btaR3* were downregulated in an Δ*scmR* mutant in comparison with the wild-type (26). To
197 gain insights into the ScmR-dependent modulation of C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL
198 biosynthesis, we determined the expression profiles of the AHL synthase-coding genes *btaI1*,
199 *btaI2*, and *btaI3* throughout the bacterial growth phases in cultures of the *scmR*- mutant
200 versus the *B. thailandensis* E264 wild-type strain harboring a chromosomal *btaI1-lux*, *btaI2-*
201 *lux*, or *btaI3-lux* transcriptional fusion. No discernible difference in expression from the
202 *btaI1*, *btaI2*, and *btaI3* promoters was found in the *scmR*- mutant compared to the wild-type
203 strain (data not shown). Accordingly, our RNA-Seq analyses indicated that ScmR had no
204 impact on *btaI1*, *btaI2*, and *btaI3* transcription (**Table S1**). The *btaR1*, *btaR2*, and *btaR3*
205 genes, encoding the BtaR1, BtaR2, and BtaR3 transcriptional regulators, respectively, were
206 not affected by ScmR neither (**Table S1**). Taken together, these data confirm that the effect
207 of ScmR on C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL biosynthesis does not result from
208 modulation of expression of the QS-1, QS-2, and/or QS-3 system genes.

209

210 **ScmR contributes to pH homeostasis**

211 Interestingly, we noticed growth differences between the *B. thailandensis* E264 wild-type
212 strain and the *scmR*-mutant under the conditions of our experiments. Indeed, inactivation of
213 *scmR* results in reduced OD_{600} during the stationary phase, but not during the exponential
214 phase (**Fig. 4**). Since pH was reported to significantly affect the growth of *B. thailandensis*
215 E264, *B. pseudomallei* Bp82, and *Burkholderia glumae* BGR1 (19, 31), we hypothesized that it
216 could be involved in the *scmR*- mutant phenotype. We analyzed the implication of ScmR in
217 pH homeostasis by measuring the pH in cultures of the *B. thailandensis* E264 wild-type strain

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

218 and the *scmR*- mutant throughout the different stages of the bacterial growth. pH in cultures
219 of both the wild-type strain and the *scmR*- mutant was approximately 7.3 during the
220 exponential phase (**Fig. 5A**). On the other hand, pH in wild-type strain cultures decreased to
221 between 7.0 and 6.5 throughout the stationary phase, whereas pH in *scmR*- mutant cultures
222 increased to between 7.5 and 8.0, apparently correlating with the OD_{600} stabilization (**Figs.**
223 **5B** and **C**). To verify whether growth inhibition could be caused by alkaline toxicity, we
224 buffered cultures of the *scmR*- mutant with 100 mM HEPES (pH 7.0) and observed that the
225 effect on the OD_{600} was alleviated (**Figs. 4** and **5**), supporting the hypothesis that culture
226 medium alkalization is the cause of the *scmR*- mutant growth differences.

227 To further characterize the underlying regulatory mechanisms directing pH homeostasis
228 through ScmR, we investigated the effect of ScmR on expression of the *obc1* gene, encoding
229 the oxalate biosynthetic enzyme Obc1, which influences the pH in several *Burkholderia* spp.
230 (19, 31). Oxalic acid was indeed reported to be essential to neutralize alkalization in
231 stationary-phase cultures of the wild-type strain of *B. thailandensis* E264, *B. pseudomallei*
232 Bp82, and *B. glumae* BGR1 (19, 31). Expression of *obc1*, as well as oxalic acid production,
233 were both shown to be QS-controlled (10, 19). Our RNA-Seq analyses indicate that *obc1*
234 transcription was downregulated in the AHL-defective Δ*btaI1*Δ*btaI2*Δ*btaI3* mutant in
235 comparison with the wild-type strain (approximately 35-fold) (**Table S2**), confirming that QS
236 activates the *obc1* gene expression. Furthermore, we noticed a drastic reduction in
237 transcription of *obc1* in the *scmR*- mutant compared to the wild-type strain (approximately
238 72-fold) (**Table S2**), revealing that the transcription of *obc1* is also strongly enhanced by
239 ScmR. To ascertain the involvement of ScmR in the regulation of the *obc1* gene, expression
240 of *obc1* was assessed by qRT-PCR in cultures of the *B. thailandensis* E264 wild-type strain and
241 the *scmR*- mutant buffered or not with HEPES during the logarithmic growth phase. We

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

242 observed that the transcription of *obc1* was completely abolished in the absence of ScmR
243 (**Fig. 6A**), attesting that expression of *obc1* is tightly controlled by ScmR. Moreover, the
244 finding that *obc1* expression is stimulated by ScmR under neutral conditions confirms
245 previous observations that alkaline stress does not induce *obc1* transcription (10, 19). All in
246 all, these findings suggest that ScmR intervenes in pH homeostasis by regulating oxalic acid
247 biosynthesis.

248 Considering the impact of the absence of ScmR on pH, we hypothesized that some of the
249 regulatory effects observed in our RNA-Seq analyses could result from pH imbalance.
250 Consequently, we measured the transcription of several genes that were affected in the
251 *scmR*- mutant strain in comparison with the wild-type strain of *B. thailandensis* E264,
252 namely, BTH_I3204, *bsaN*, BTH_II0639, *btaR5*, and BTH_II1209 encoding a lipoprotein, the
253 T3SS transcriptional regulator BsaN, a lipase, the orphan LuxR-type transcriptional regulator
254 BtaR5, and a hypothetical protein, respectively. Their expression was monitored by qRT-PCR
255 during the exponential phase in cultures of the *B. thailandensis* E264 wild-type and the
256 *scmR*- mutant strains supplemented or not with HEPES. According to our transcriptomic
257 data, expression of the BTH_I3204 and *bsaN* genes is repressed by ScmR (approximately 26-
258 fold and 17-fold, respectively), whereas expression of the BTH_II0639, *btaR5*, and
259 BTH_II1209 genes is stimulated by ScmR (approximately 16-fold, 27-fold, and 274-fold,
260 respectively) (**Table S1**). We observed that buffering *scmR*- mutant cultures did not restore
261 normal expression of any of these genes to wild-type levels, showing that the effects
262 observed on these genes in the *scmR*- mutant does not result from culture medium
263 alkalization.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

264 pH affects the integrity of AHL signaling molecules. AHLs are stable at neutral and acidic pH,
265 while alkaline conditions cause AHLs hydrolysis (32, 33). Therefore, we asked whether ScmR
266 could influence C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL stability by impacting pH homeostasis.
267 Concentrations of C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL were monitored in the *B.*
268 *thailandensis* E264 wild-type strain and the *scmR*- mutant throughout the different stages of
269 bacterial growth. We confirmed that the levels of C_8-HSL were reduced in the *scmR*- mutant
270 in comparison with the wild-type strain in the early stages of the bacterial growth (**Fig. 7A**),
271 whereas 3OHC_{10}-HSL and 3OHC_8-HSL concentrations were increased (**Figs. 7B** and **C**). As
272 expected, the concentrations of all three AHLs were decreased in the *scmR*- mutant cultures
273 in the late stages of bacterial growth (**Fig. 7**). We then examined the effect of pH buffering
274 on AHLs levels in *scmR*- mutant cultures. The production of all three AHLs was increased in
275 buffered cultures of the *scmR*- mutant (**Fig. 7**). Taken together, these observations indicate
276 that the impact of ScmR on the QS-1, QS-2, and/or QS-3 systems might result, *inter alia*,
277 from its influence on pH homeostasis.

278

279 **QS regulation of *scmR* gene**

280 Transcription of the *scmR* gene is activated by QS (10, 26). It was established that all three
281 AHLs stimulate *scmR* expression (10). Accordingly, our RNA-Seq analyses indicate that *scmR*
282 transcription is diminished in the AHL-null Δ*btaI1*Δ*btaI2*Δ*btaI3* mutant compared to the wild-
283 type strain (approximately 4-fold) (**Table S1**), confirming that expression of *scmR* is positively
284 modulated by QS. However, respective influence of the BtaR1, BtaR2, and BtaR3 regulators
285 on *scmR* expression was not investigated (10). To gain insights into the QS-dependent
286 modulation of the *scmR* gene, we measured its transcription in the Δ*btaR1*, Δ*btaR2*, and

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

287 Δ*btaR3* mutants versus the *B. thailandensis* E264 wild-type strain during the logarithmic
288 growth phase. While no obvious change in *scmR* transcription was visible in the absence of
289 the BtaR2 transcriptional regulator, expression of *scmR* was decreased in both the Δ*btaR1*
290 and Δ*btaR3* mutants (**Fig. 8**). Collectively, these observations indicate that the transcription
291 of *scmR* is stimulated by the QS-1 and QS-3 systems, whereas the QS-2 system is not
292 apparently involved in the modulation of *scmR* expression.

293 While a putative *lux* box sequence was found in the promoter region of the *B. thailandensis*
294 E264 *scmR* gene (26), we do not know whether the BtaR1 and/or BtaR3 transcriptional
295 regulators directly control its transcription. We found a putative *lux* box sequence in the
296 promoter region of *scmR* homologs in both *B. pseudomallei* K96243 and *B. mallei* ATCC
297 23344 (**Fig. S1**). Accordingly, Klaus *et al.* (34) and Majerczyk *et al.* (20) demonstrated that the
298 expression of *scmR* in *B. pseudomallei* Bp82 and *B. mallei* GB8 is stimulated by QS,
299 respectively. *Burkholderia cenocepacia* J2315 also possesses an *scmR* homologue, which was
300 shown to be QS-controlled in *B. cenocepacia* K56-2 (35), but no putative *lux* box sequence
301 was found in its promoter region (36). Altogether, these observations suggest that the QS-
302 dependent regulation of the *scmR* gene is conserved among *Burkholderia* spp.

303 Since *scmR* is directly adjacent to its downstream gene, namely, *ldhA*, encoding a putative
304 lactate dehydrogenase, on the genome of *B. thailandensis* E264, *B. pseudomallei* K96243, *B.*
305 *mallei* ATCC 2344, and *B. cenocepacia* J2315, and transcribed in the same direction (**Fig.**
306 **S2A**), we asked whether they could be cotranscribed. The *scmR* gene is indeed predicted to
307 be arranged in operon with *ldhA* (http://www.burkholderia.com/), and we observed that
308 *ldhA* transcription is also activated by QS (**Table S2**). However, both our transcriptomic data

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

309 (**Fig. S2B**) and RT-PCR experiments (**Fig. S2C**) indicate that *scmR* is not cotranscribed with
310 *ldhA*.

311 Interestingly, expression of *ldhA* was decreased in the *scmR*- mutant compared to the wild-
312 type strain (**Table S2**), highlighting that the *ldhA* gene is positively modulated by ScmR as
313 well. Of note, the reduction in expression of *ldhA* was substantially greater in the *scmR*-
314 mutant (approximately 17-fold) than in the Δ*btaI1*Δ*btaI2*Δ*btaI3* mutant (approximately 3-
315 fold) (**Table S2**), suggesting that QS might activate *ldhA* transcription indirectly via positive
316 regulation of the *scmR* gene.

317 Since LdhA was hypothesized to influence pH homeostasis in *B. thailandensis* E264 (26), we
318 tested its involvement in the ScmR-dependent control of pH homeostasis by measuring the
319 pH in cultures of the *B. thailandensis* E264 wild-type strain and the *scmR*- and *ldhA*- mutants
320 during the stationary phase of growth. While the pH in both the wild-type strain and the
321 *ldhA*- mutant was between 6.5 and 7.0, pH in cultures of the *scmR*- mutant was
322 approximately 9.0 (**Fig. S3A**), showing that LdhA does not affect pH in *B. thailandensis* E264
323 under the conditions of our experiments. Of note, inactivation of the *ldhA* gene was not
324 associated with a change in OD_{600} (**Figs. S3B** and **S3C**). Altogether, these observations
325 indicate that LdhA is not likely involved in the ScmR-dependent control of pH homeostasis in
326 *B. thailandensis* E264.

327

328 ***scmR* is negatively autoregulated**

329 As LTTRs are typically negatively autoregulated (27), we investigated the impact of ScmR on
330 its own transcription. Considering that the use of an *scmR*- mutant to perform our RNA-Seq

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

331 analyses precludes clear assessment, we measured expression of *scmR* in the *B.*
332 *thailandensis* E264 wild-type strain and its *scmR*- mutant strain harboring a chromosomal
333 *scmR-lux* transcriptional fusion. We observed an increase in *scmR* expression in the *scmR*-
334 mutant in comparison with the wild-type strain (**Fig. 9**), revealing that *scmR* is negatively
335 autoregulated.

336 A heterologous host *E. coli* expression reporter system was developed to examine the
337 possibility of direct interaction of ScmR with the promoter region of the *scmR* gene. *E. coli*
338 DH5α recombinant strains were generated containing the chromosomal *scmR-lux*
339 transcriptional reporter and either pMLS7 or pMLS7-*scmR* for constitutive expression of the
340 ScmR transcriptional regulator. In this systems, ScmR did not repress *scmR* transcription
341 (data not shown), suggesting that ScmR does not directly repress its own expression or that
342 additional unknown factor(s), which might be absent in the *E. coli* background, are required
343 for *scmR* negative autoregulation. Indeed, LTTRs generally function in association with
344 ligands to modulate the expression of genes (27).

345

346 **ScmR represses virulence in the fruit fly model *D. melanogaster***

347 The cytotoxin malleilactone was reported to contribute to virulence of both *B. thailandensis*
348 E264 and *B. pseudomallei* Bp82 (15, 34). Interestingly, a Δ*scmR* mutant of *B. thailandensis*
349 E264, which overproduces malleilactone, is more virulent toward the *C. elegans* nematode
350 model host in comparison with the wild-type strain (26). Accordingly, we found that our
351 *scmR*- mutant was significantly more virulent than the wild-type strain using the *D.*
352 *melanogaster* host model ($P < 0.001$) (**Fig. 10**). However, according to our transcriptomic
353 data and in contrast to Mao *et al.* (26) observations, ScmR had no impact on the

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

354 transcription of the *mal* gene cluster (malleilactone biosynthesis) (15, 16), or on the
355 expression of the orphan LuxR-type transcriptional regulator BtaR4 (MalR) which activates
356 *mal* genes, indicating that ScmR might not regulate malleilactone production under our
357 conditions (data not shown). Hence, the ScmR-dependent regulation of pathogenicity in *B.*
358 *thailandensis* E264 is not exclusively mediated through control of the biosynthesis of
359 malleilactone.

360

361 **Discussion**

362 The function of the ScmR transcriptional regulator was recently addressed in *B.*
363 *thailandensis*, revealing its importance in secondary metabolism regulation, as well as its
364 involvement in the modulation of several QS-controlled phenotypes (26). While Mao *et al.*
365 (26) defined the ScmR regulon during the stationary phase, we established the impact of
366 ScmR on the expression of genes during the logarithmic phase of growth. It must be
367 emphasized that the growth stage is an important variable when investigating QS, and this is
368 especially relevant for *B. thailandensis*, a bacterium for which we reported significant
369 differences in QS regulation depending on the growth stage (11). We confirmed that ScmR is
370 a global regulator of gene expression in *B. thailandensis* E264 (**Fig. 1A**). Mao *et al.* (26)
371 highlighted that ScmR modulates the production of the main AHL signaling molecules found
372 in this bacterium, namely, C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL and we confirmed that AHLs
373 biosynthesis is affected by ScmR as well (**Fig. 3**), which hints that ScmR might control the
374 transcription of many genes through its effect on the QS-1, QS-2, and/or QS-3 systems. This
375 is also further supported by the finding that ScmR modulates QS-controlled phenotypic
376 traits, such as colony morphology, as well as pellicle and biofilm formation (26). Consistently,

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

377 we noticed a considerable overlap between the ScmR-regulated genes and those controlled
378 by QS (**Fig. 2**). Furthermore, we attested that the *scmR* gene is regulated by QS (**Fig. 8**),
379 showing that ScmR is deeply integrated into the QS modulatory network of *B. thailandensis*
380 E264. We assume that the QS-dependent regulation of *scmR* transcription allows tightly
381 controlled coordination of the expression of genes.

382 Interestingly, we found that expression of many genes that encode transcriptional
383 regulators, including the orphan QS transcriptional regulator BtaR5-encoding gene (**Fig. 6E**),
384 is modulated either positively or negatively by ScmR (**Table S1**). Consequently, we propose
385 that ScmR controls many genes through different and not mutually exclusive mechanisms:
386 i.e. (i) regulation of AHL signaling molecules biosynthesis, (ii) direct binding of target genes,
387 and (iii) indirect modulation of some genes via intermediate regulators. It will therefore be
388 important to further investigate the molecular mechanism of action of ScmR to decipher
389 between the directly and the indirectly ScmR-regulated genes. Moreover, the
390 characterization of an ScmR-binding motif would contribute to the identification of
391 promoters that are directly modulated versus those that are indirectly modulated.

392 The production of oxalic acid, which is required for pH homeostasis, is under QS control in
393 several *Burkholderia* spp. (19, 31). Our RNA-Seq analyses confirmed the implication of AHLs
394 in the regulation of expression of the oxalate biosynthetic gene *obc1*, and we showed that
395 the transcription of *obc1* is stringently modulated by ScmR as well (**Fig. 6A**). Furthermore, we
396 noticed that the impact of ScmR on *obc1* expression was more pronounced than the effect
397 of AHLs (**Table S2**), suggesting that QS activates *obc1* transcription indirectly via positive
398 regulation of the *scmR* gene. Whether the ScmR-dependent control of the *obc1* gene is
399 direct or not remains to be determined. It is also possible that the ScmR-mediated control of

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

400 the homeostasis of pH is not exclusively dependent on regulation of oxalate production. We
401 indeed observed that several genes involved in ATP synthesis are modulated by ScmR (**Table**
402 **S1**), as formerly noticed (26). Additionally, our RNA-Seq analyses revealed that ScmR
403 stimulates expression of the putative lactate dehydrogenase LdhA-encoding gene, which is
404 directly adjacent to *scmR*, and transcribed in the same direction in several *Burkholderia* spp.
405 (26, 37) (**Fig. S2A**). Because lactate dehydrogenase, by reducing pyruvate to lactate, was
406 suggested to affect pH (26), ScmR could also intervene in pH homeostasis through the
407 activation of *ldhA* transcription. Indeed, Silva *et al.* (37) demonstrated that the *Burkholderia*
408 *multivorans* ATCC 17616 homologue, called LdhR, influences pH homeostasis by activating
409 both expression of the *ldhA* gene and lactate production. Still, we noticed no difference in
410 pH between cultures of the *B. thailandensis* E264 wild-type strain and the *ldhA*- mutant (**Fig.**
411 **3A**), suggesting that LdhA is not involved in the ScmR-dependent control of pH homeostasis
412 in *B. thailandensis* E264. Other discrepancies between the *B. thailandensis* E264 ScmR and
413 the *B. multivorans* ATCC 17616 LdhR homologues were reported, such as their negative and
414 positive effects on the development of biofilm, respectively (37). These findings indicate that
415 these two proteins could be functionally different. More experiments will therefore be
416 necessary to further understand the precise underlying molecular mechanism of action of
417 ScmR in the control of pH homeostasis in *B. thailandensis*.

418 Since AHLs are hydrolyzed rapidly under alkaline pH conditions (32, 33), we propose that the
419 impact of ScmR on the production of C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL might result, *inter*
420 *alia*, from its influence on pH homeostasis (**Fig. 7**), This would then explain why no visible
421 change in expression from the *btaI1*, *btaI2*, and *btaI3* promoters was observed in the *scmR*-
422 mutant compared to the wild-type strain (data not shown). However, the ScmR-dependent

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

423 regulation of the QS-1, QS-2, and/or QS-3 systems might be more complex and will need
424 further investigation.

425 In agreement with the fact that LTTRs are typically negatively autoregulated (27), we found
426 that ScmR represses its own expression (**Fig. 9**). Still, we saw no direct effect of the ScmR
427 transcriptional regulator on the promoter region of the *scmR* gene when co-expressed
428 together in a heterologous host. An explanation could be that *scmR* negative autoregulation
429 requires additional modulatory elements, including molecular ligands. Indeed, ligands are
430 recognized as being crucial for the function of LTTRs (27). They frequently participate to a
431 self-inducing loop in which a product or an intermediate product of a given
432 metabolic/synthesis pathway that is commonly stimulated by an LTTR acts as the ligand
433 required for transcriptional regulation (27). Therefore, it will be important to determine the
434 putative ligand(s) of ScmR in order to uncover the precise regulatory mechanism underlying
435 *scmR* negative autoregulation.

436 While the pathogenicity of *B. multivorans* ATCC 17616 in the *Galleria mellonella* larvae
437 model of infection is apparently not regulated by the ScmR homologue LdhR (37), the loss of
438 ScmR in both *B. thailandensis* E264 and *B. pseudomallei* Bp82 resulted in hypervirulence
439 toward the model host *C. elegans* (26, 34). In addition, Melanson *et al.* (38) demonstrated
440 that the rice pathogen *B. glumae* 336gr-1 ScmR homologue, called NtpR, is a negative
441 regulator of toxoflavin production, which is considered the primary virulence factor of *B.*
442 *glumae*, suggesting that similarly to the *B. thailandensis* E264 and *B. pseudomallei* Bp82
443 ScmR homologues, NtpR influences virulence. In *B. thailandensis* and *B. pseudomallei*, ScmR
444 was proposed to modulate the infection process by repressing the biosynthesis of
445 malleilactone (26, 34). We demonstrated that ScmR of *B. thailandensis* E264 contributes to

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

446 pathogenicity using the *D. melanogaster* host model (**Fig. 10**). However, we observed no
447 difference in the transcription of the *mal* gene cluster, which encodes the principal enzymes
448 responsible for malleilactone biosynthesis (15, 34), between the *B. thailandensis* E264 wild-
449 type and the *scmR*- mutant strains, suggesting that ScmR is not necessarily involved in the
450 production of the cytotoxin malleilactone. Any negative effect of ScmR on malleilactone
451 biosynthesis *in vivo* is currently unknown. Still, we do not exclude the possibility that ScmR
452 reduces virulence by modulating the expression of additional virulence/survival factors. For
453 instance, we highlighted that expression of the *bsa* T3SS genes, which are crucial for the
454 pathogenicity of both *B. pseudomallei* and *B. mallei* (39, 40), is repressed by ScmR (**Table S1**).
455 The involvement of other potential virulence factors in the ScmR-mediated control of
456 pathogenicity in *B. thailandensis* is currently under investigation.

457

458 Materials and methods

459 Bacterial strains and culture conditions

460 The bacterial strains used in this study are listed in **Table S3**. Unless otherwise stated, all
461 bacteria were cultured at 37°C in tryptic soy broth (TSB; BD Difco, Mississauga, Ontario,
462 Canada), with shaking (240 rpm) in a TC-7 roller drum (New Brunswick, Canada), or on Petri
463 dishes containing TSB solidified with 1.5% agar. When required, antibiotics were used at the
464 following concentrations: 200 µg/mL tetracycline (Tc), 100 µg/mL kanamycin (Km), and 100
465 µg/mL trimethoprim (Tp) for *B. thailandensis* E264, while Tc was used at 15 µg/mL for *E. coli*
466 DH5α. All measurements of optical density at 600 nm (OD_{600}) were acquired with a Thermo
467 Fisher Scientific NanoDrop ND-1000 spectrophotometer.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

468

469 **Construction of plasmids**

470 Plasmids used in this study are described in **Table S4**. Amplification of the promoter region
471 of *scmR* was performed from genomic DNA from *B. thailandensis* E264 using the appropriate
472 primers (**Table S5**). The amplified product was digested with the FastDigest restriction
473 enzymes *Xho*I and *Bam*HI (Thermo Fisher Scientific) and inserted by T4 DNA ligase (Bio Basic,
474 Inc., Markham, ON, Canada) within the corresponding restriction sites in the mini-CTX-*lux*
475 plasmid (41), generating the transcriptional reporter pSLG01. All primers were from Alpha
476 DNA (Montreal, Quebec, Canada).

477

478 **Construction of reporter strains**

479 Chromosomal integration of the mini-CTX-*scmR-lux* transcriptional reporter at the *attB* locus
480 in *B. thailandensis* E264 strains was performed through conjugation with the auxotrophic *E.*
481 *coli* χ7213, as described previously (12). Successful chromosomal insertion of *scmR-lux* was
482 confirmed by PCR using appropriate primers.

483

484 **RNA isolation**

485 Total RNA of *B. thailandensis* E264 cultures at an OD_{600} of 4.0 was extracted with the
486 PureZOL RNA isolation reagent (Bio-Rad Laboratories, Mississauga, ON, Canada) and treated
487 twice with the TURBO DNA-Free kit (Ambion Life Technologies, Inc., Burlington, ON, Canada)
488 according to the manufacturer’s instructions. Extractions were done on two different

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

489 bacterial cultures for RNA-Sequencing (RNA-Seq) analysis and on three different bacterial
490 cultures for quantitative reverse transcription-PCR (qRT-PCR) and reverse transcription-PCR
491 (RT-PCR) experiments. Quality and purity controls were confirmed by agarose gel
492 electrophoresis and UV spectrophotometric analysis, respectively. Quantification of total
493 RNA was accomplished on a Corbett Life Science Rotor-Gene 6000 thermal cycler using the
494 QuantiFluor RNA system (Promega, Madison, WI, USA), according to the manufacturer's
495 protocol.

496

497 **RNA-Seq libraries construction and sequencing**

498 The RNA-Seq libraries construction and sequencing using an Illumina HiSeq 2000 PE100 were
499 performed by the McGill University and Génome Québec Innovation Centre (Montreal, QC,
500 Canada). The RNA-Seq libraries were prepared using the TruSeq stranded mRNA sample
501 preparation kit (Illumina, Inc., San Diego, CA, USA) and the Ribo-Zero rRNA removal kit
502 (Epicentre, Madison, WI, USA).

503

504 **RNA-Seq mapping and analyses**

505 All computations were made on the supercomputer Briarée from the Université de
506 Montréal, managed by Calcul Québec and Compute Canada. Raw reads were filtered to
507 remove low quality reads using the FASTX toolkit by discarding any reads with more than
508 10% nucleotides with a PHRED score < 20. Reads were then aligned with the reference
509 genome (the GenBank accession no. for chromosome 1 of strain E264 is CP000086.1 and for
510 chromosome 2, it is CP000085.1) using Bowtie (v 2.2.3) with default parameters.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

511 Chromosome 1 and chromosome 2 sequence alignments were separately processed to allow
512 expression analysis between the two chromosomes. SAMtools (v 0.1.18) and BEDtools (v
513 2.20.1) were used for the generation of sam and bam files, respectively. The GC content of *B.*
514 *thailandensis* E264 genes was calculated using BEDtools (v 2.20.1), prior to normalization.
515 Normalization of the read count was done using the RPKM normalization function of the
516 NOIseq package in R (42). To exclude features with low read counts, a low count filter was
517 applied using a CPM method with a CPM value of 1 and a cutoff of 100 for the coefficient of
518 variation. Cutoff values of 3-fold were used to consider differential expression biologically
519 significant.

520

521 **LC-MS/MS quantification of AHLs**

522 The concentrations of AHLs were determined from samples of *B. thailandensis* E264 cultures
523 obtained at different time points during bacterial growth, by liquid chromatography coupled
524 to tandem mass spectrometry (LC-MS/MS). The samples were prepared and analyzed as
525 described previously (43). 5,6,7,8-Tetradeutero-4-hydroxy-2-heptylquinoline (HHQ-d4) was
526 used as an internal standard. All experiments were performed in triplicate and conducted at
527 least twice independently. For experiments with additions of 4-(2-hydroxyethyl)-1-
528 piperazineethanesulfonic acid (HEPES), cultures were buffered or not with 100 mM HEPES
529 (Sigma-Aldrich Co., Oakville, ON, Canada) from a stock prepared in ultrapure water. Water
530 only was added to the controls.

531

532 **Quantitative reverse transcription-PCR and reverse transcription-PCR experiments**

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

533 cDNA synthesis was performed using the iScript reverse transcription supermix (Bio-Rad
534 Laboratories), and amplification was accomplished on a Corbett Life Science Rotor-Gene
535 6000 thermal cycler using the SsoAdvanced universal SYBR green supermix (Bio-Rad
536 Laboratories), according to the manufacturer's protocol. The reference gene was *ndh* (44).
537 The *ndh* gene displayed stable expression under the different genetic contexts tested. All
538 primers used for cDNA amplification are presented in **Table S6**. Differences in gene
539 expression between *B. thailandensis* E264 strains were calculated using the $2^{-\Delta\Delta CT}$ formula
540 (45). A threshold of 0.5 was chosen as significant. All experiments were performed in
541 triplicate and conducted at least twice independently.

542

543 **Measurement of the activity of *scmR-lux* reporter**

544 Expression from the promoter region of *scmR* was quantified by measuring the
545 luminescence of *B. thailandensis* E264 cultures carrying the corresponding chromosomal
546 mini-CTX-*lux* transcriptional reporter, as described previously (11). Overnight bacterial
547 cultures were diluted in TSB to an initial OD_{600} of 0.1 and incubated as indicated above. The
548 luminescence was regularly determined from culture samples using a multi-mode microplate
549 reader (Cytation 3; BioTek Instruments, Inc., Winooski, VT, USA) and expressed in relative
550 light units per optical density of the culture (RLU/OD_{600}). All experiments were performed
551 with three biological replicates and repeated at least twice.

552

553 **Infection of *D. melanogaster***

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

554 The fruit flies were infected by feeding according to the previously described protocol (46).
555 Briefly, 1 g of fruit fly dry medium was put into infection vials. Bacteria were harvested from
556 LB-grown cultures adjusted to an OD_{600} of 4.0 by centrifugation at 10,000 x *g* for 5 min. The
557 pellets were suspended in 0.02X PBS containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$, as well as 500
558 µg/mL ampicillin (Ap) to avoid infection with nonspecific bacteria. Two mL of bacterial
559 suspension were added to the dry food. Six-seven days-old male flies were anesthetized with
560 CO_2 and added to the vials by group of 10. The control vials contained the PBS solution only.
561 Fly survival was scored daily and survival curves were processed with GraphPad Prism 5
562 (GraphPad Software, Inc., San Diego, CA, USA) to perform a statistical log-rank (Mantel-Cox)
563 test.

564

565 **Data analysis**

566 Unless stated otherwise, data are reported as means ± standard deviations (SD). Statistical
567 analyses were performed with the R software version 3.3.3 (http://www.R-project.org/)
568 using one-way analysis of variance (ANOVA). Probability values of less than 0.05 were
569 considered significant.

570

571 Funding information

572 This study was supported by Canadian Institutes of Health Research (CIHR) operating grants
573 MOP-97888 and MOP-142466 to ED. ED holds the Canada Research Chair in
574 Sociomicrobiology. The funders had no role in study design, data collection and
575 interpretation, or the decision to submit the work for publication.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

576

577 ## Acknowledgments

578 We thank Everett Peter Greenberg (Department of Microbiology, University of Washington
579 School of Medecine, Seattle, WA, USA) for providing the *B. thailandensis* E264 strains. We
580 especially thank Sylvain Milot, François D’Heygere, and Koyomi Ozaki for their technical help.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

581 # References

582 1. Fuqua WC, Winans SC, Greenberg EP. 1994. Quorum sensing in bacteria: the LuxR-LuxI family
583 of cell density-responsive transcriptional regulators. J Bacteriol 176:269-75.
584 2. Nealson KH, Platt T, Hastings JW. 1970. Cellular control of the synthesis and activity of the
585 bacterial luminescent system. J Bacteriol 104:313-22.
586 3. Fuqua WC, Greenberg EP. 2002. Listening in on bacteria: acyl-homoserine lactone signalling.
587 Nat Rev Mol Cell Biol 3:685-95.
588 4. Majerczyk CD, Greenberg EP, Chandler JR. 2013. Quorum Sensing in *Burkholderia*. In Vasil, M,
589 and Darwin, A (ed), Regulation of Bacterial Virulence ASM Press, Washington, DC
590 doi:10.1128/9781555818524.ch3:p 40-57.
591 5. Brett PJ, DeShazer D, Woods DE. 1998. *Burkholderia thailandensis* sp. nov., a *Burkholderia*
592 *pseudomallei*-like species. Int J Syst Bacteriol 48 Pt 1:317-20.
593 6. Haraga A, West TE, Brittnacher MJ, Skerrett SJ, Miller SI. 2008. *Burkholderia thailandensis* as
594 a model system for the study of the virulence-associated type III secretion system of
595 *Burkholderia pseudomallei*. Infect Immun 76:5402-11.
596 7. Ulrich RL, Deshazer D, Brueggemann EE, Hines HB, Oyston PC, Jeddeloh JA. 2004. Role of
597 quorum sensing in the pathogenicity of *Burkholderia pseudomallei*. J Med Microbiol 53:1053-
598 64.
599 8. Ulrich RL, Deshazer D, Hines HB, Jeddeloh JA. 2004. Quorum sensing: a transcriptional
600 regulatory system involved in the pathogenicity of *Burkholderia mallei*. Infect Immun
601 72:6589-96.
602 9. Ulrich RL, Hines HB, Parthasarathy N, Jeddeloh JA. 2004. Mutational analysis and biochemical
603 characterization of the *Burkholderia thailandensis* DW503 quorum-sensing network. J
604 Bacteriol 186:4350-60.
605 10. Majerczyk CD, Brittnacher M, Jacobs M, Armour CD, Radey M, Schneider E, Phattarasokul S,
606 Bunt R, Greenberg EP. 2014. Global analysis of the *Burkholderia thailandensis* quorum
607 sensing-controlled regulon. J Bacteriol 196:1412-24.
608 11. Le Guillouzer S, Groleau MC, Déziel E. 2017. The Complex Quorum Sensing Circuitry of
609 *Burkholderia thailandensis* Is Both Hierarchically and Homeostatically Organized. mBio
610 8:e01861-17.
611 12. Le Guillouzer S, Groleau MC, Déziel E. 2018. Two *rsaM* homologues encode central
612 regulatory elements modulating quorum sensing in *Burkholderia thailandensis*. J Bacteriol
613 200:e00727-17.
614 13. Chandler JR, Duerkop BA, Hinz A, West TE, Herman JP, Churchill ME, Skerrett SJ, Greenberg
615 EP. 2009. Mutational analysis of *Burkholderia thailandensis* quorum sensing and self-
616 aggregation. J Bacteriol 191:5901-9.
617 14. Duerkop BA, Varga J, Chandler JR, Peterson SB, Herman JP, Churchill ME, Parsek MR,
618 Nierman WC, Greenberg EP. 2009. Quorum-sensing control of antibiotic synthesis in
619 *Burkholderia thailandensis*. J Bacteriol 191:3909-18.
620 15. Biggins JB, Ternei MA, Brady SF. 2012. Malleilactone, a polyketide synthase-derived virulence
621 factor encoded by the cryptic secondary metabolome of *Burkholderia pseudomallei* group
622 pathogens. J Am Chem Soc 134:13192-5.
623 16. Truong TT, Seyedsayamdost M, Greenberg EP, Chandler JR. 2015. A *Burkholderia*
624 *thailandensis* Acyl-Homoserine Lactone-Independent Orphan LuxR Homolog That Activates
625 Production of the Cytotoxin Malleilactone. J Bacteriol 197:3456-62.
626 17. Valade E, Thibault FM, Gauthier YP, Palencia M, Popoff MY, Vidal DR. 2004. The PmlI-PmlR
627 quorum-sensing system in *Burkholderia pseudomallei* plays a key role in virulence and
628 modulates production of the MprA protease. J Bacteriol 186:2288-94.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

629 18. Song Y, Xie C, Ong YM, Gan YH, Chua KL. 2005. The BpsIR quorum-sensing system of
630 *Burkholderia pseudomallei*. J Bacteriol 187:785-90.
631 19. Goo E, Majerczyk C, An JH, Chandler JR, Seo YS, Ham H, Lim JY, Kim H, Lee B, Jang MS,
632 Greenberg EP, Hwang I. 2012. Bacterial quorum sensing, cooperativity, and anticipation of
633 stationary-phase stress. Proc Natl Acad Sci U S A 109:19775-80.
634 20. Majerczyk CD, Brittnacher MJ, Jacobs MA, Armour CD, Radey MC, Bunt R, Hayden HS,
635 Bydalek R, Greenberg EP. 2014. Cross-species comparison of the *Burkholderia pseudomallei*,
636 *Burkholderia thailandensis*, and *Burkholderia mallei* quorum-sensing regulons. J Bacteriol
637 196:3862-71.
638 21. Tseng BS, Majerczyk CD, Passos da Silva D, Chandler JR, Greenberg EP, Parsek MR. 2016.
639 Quorum Sensing Influences *Burkholderia thailandensis* Biofilm Development and Matrix
640 Production. J Bacteriol 198:2643-50.
641 22. Ulrich RL. 2004. Quorum quenching: enzymatic disruption of *N*-acylhomoserine lactone-
642 mediated bacterial communication in *Burkholderia thailandensis*. Appl Environ Microbiol
643 70:6173-80.
644 23. Ooi WF, Ong C, Nandi T, Kreisberg JF, Chua HH, Sun G, Chen Y, Mueller C, Conejero L, Eshaghi
645 M, Ang RM, Liu J, Sobral BW, Korbsrisate S, Gan YH, Titball RW, Bancroft GJ, Valade E, Tan P.
646 2013. The condition-dependent transcriptional landscape of *Burkholderia pseudomallei*. PLoS
647 Genet 9:e1003795.
648 24. Ramli NS, Eng Guan C, Nathan S, Vadivelu J. 2012. The effect of environmental conditions on
649 biofilm formation of *Burkholderia pseudomallei* clinical isolates. PLoS One 7:e44104.
650 25. Mongkolrob R, Taweechaisupapong S, Tungpradabkul S. 2015. Correlation between biofilm
651 production, antibiotic susceptibility and exopolysaccharide composition in *Burkholderia*
652 *pseudomallei bpsI*, *ppk*, and *rpoS* mutant strains. Microbiol Immunol 59:653-63.
653 26. Mao D, Bushin LB, Moon K, Wu Y, Seyedsayamdost MR. 2017. Discovery of *scmR* as a global
654 regulator of secondary metabolism and virulence in *Burkholderia thailandensis* E264. Proc
655 Natl Acad Sci U S A 114:E2920-E2928.
656 27. Maddocks SE, Oyston PC. 2008. Structure and function of the LysR-type transcriptional
657 regulator (LTTR) family proteins. Microbiology 154:3609-23.
658 28. Vial L, Lépine F, Milot S, Groleau MC, Dekimpe V, Woods DE, Déziel E. 2008. *Burkholderia*
659 *pseudomallei*, *B. thailandensis*, and *B. ambifaria* produce 4-hydroxy-2-alkylquinoline
660 analogues with a methyl group at the 3 position that is required for quorum-sensing
661 regulation. J Bacteriol 190:5339-52.
662 29. Biggins JB, Gleber CD, Brady SF. 2011. Acyldepsipeptide HDAC inhibitor production induced in
663 *Burkholderia thailandensis*. Org Lett 13:1536-9.
664 30. Majerczyk CD, Schneider E, Greenberg EP. 2016. Quorum sensing control of Type VI secretion
665 factors restricts the proliferation of quorum-sensing mutants. Elife 5.
666 31. Goo E, An JH, Kang Y, Hwang I. 2015. Control of bacterial metabolism by quorum sensing.
667 Trends Microbiol 23:567-76.
668 32. Byers JT, Lucas C, Salmond GP, Welch M. 2002. Nonenzymatic turnover of an *Erwinia*
669 *carotovora* quorum-sensing signaling molecule. J Bacteriol 184:1163-71.
670 33. Yates EA, Philipp B, Buckley C, Atkinson S, Chhabra SR, Sockett RE, Goldner M, Dessaux Y,
671 Camara M, Smith H, Williams P. 2002. *N*-acylhomoserine lactones undergo lactonolysis in a
672 pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia*
673 *pseudotuberculosis* and *Pseudomonas aeruginosa*. Infect Immun 70:5635-46.
674 34. Klaus JR, Deay J, Neuenswander B, Hursh W, Gao Z, Bouddhara T, Williams TD, Douglas J,
675 Monize K, Martins P, Majerczyk C, Seyedsayamdost MR, Peterson BR, Rivera M, Chandler JR.
676 2018. Malleilactone Is a *Burkholderia pseudomallei* Virulence Factor Regulated by Antibiotics
677 and Quorum Sensing. J Bacteriol 200.
678 35. O'Grady EP, Viteri DF, Malott RJ, Sokol PA. 2009. Reciprocal regulation by the CepIR and CciIR
679 quorum sensing systems in *Burkholderia cenocepacia*. BMC Genomics 10:441.

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

680 36. Chambers CE, Lutter EI, Visser MB, Law PP, Sokol PA. 2006. Identification of potential CepR
681 regulated genes using a cep box motif-based search of the *Burkholderia cenocepacia*
682 genome. BMC Microbiol 6:104.
683 37. Silva IN, Ramires MJ, Azevedo LA, Guerreiro AR, Tavares AC, Becker JD, Moreira LM. 2017.
684 Regulator LdhR and d-Lactate Dehydrogenase LdhA of *Burkholderia multivorans* Play Roles in
685 Carbon Overflow and in Planktonic Cellular Aggregate Formation. Appl Environ Microbiol 83.
686 38. Melanson RA, Barphagha I, Osti S, Lelis TP, Karki HS, Chen R, Shrestha BK, Ham JH. 2017.
687 Identification of new regulatory genes involved in the pathogenic functions of the rice-
688 pathogenic bacterium *Burkholderia glumae*. Microbiology 163:266-279.
689 39. Warawa J, Woods DE. 2005. Type III secretion system cluster 3 is required for maximal
690 virulence of *Burkholderia pseudomallei* in a hamster infection model. FEMS Microbiol Lett
691 242:101-8.
692 40. Ulrich RL, DeShazer D. 2004. Type III secretion: a virulence factor delivery system essential
693 for the pathogenicity of *Burkholderia mallei*. Infect Immun 72:1150-4.
694 41. Becher A, Schweizer HP. 2000. Integration-proficient *Pseudomonas aeruginosa* vectors for
695 isolation of single-copy chromosomal *lacZ* and *lux* gene fusions. Biotechniques 29:948-50,
696 952.
697 42. Tarazona S, Garcia-Alcalde F, Dopazo J, Ferrer A, Conesa A. 2011. Differential expression in
698 RNA-seq: a matter of depth. Genome Res 21:2213-23.
699 43. Chapalain A, Groleau MC, Le Guillouzer S, Miomandre A, Vial L, Milot S, Déziel E. 2017.
700 Interplay between 4-Hydroxy-3-Methyl-2-Alkylquinoline and *N*-Acyl-Homoserine Lactone
701 Signaling in a *Burkholderia cepacia* Complex Clinical Strain. Front Microbiol 8:1021.
702 44. Subsin B, Chambers CE, Visser MB, Sokol PA. 2007. Identification of genes regulated by the
703 *cepIR* quorum-sensing system in *Burkholderia cenocepacia* by high-throughput screening of a
704 random promoter library. J Bacteriol 189:968-79.
705 45. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time
706 quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-8.
707 46. Pilatova M, Dionne MS. 2012. *Burkholderia thailandensis* is virulent in *Drosophila*
708 *melanogaster*. PLoS One 7:e49745.

709

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

710 ## Figure legends

711 **Figure 1. Numbers of ScmR-regulated genes and those QS-controlled in *B. thailandensis***
712 **E264 according to transcriptomic analyses obtained by RNA-Seq.** (A) Total numbers of
713 genes showing positive or negative modulation in the *scmR*- mutant compared to the wild-
714 type strain of *B. thailandensis* E264. (B) Total numbers of genes showing positive or negative
715 modulation in the Δ*btaI1*Δ*btaI2*Δ*btaI3* mutant in comparison with the wild-type strain of *B.*
716 *thailandensis* E264.

717

718 **Figure 2. The ScmR regulon comprises many QS-controlled genes.** (A) Number of genes
719 positively regulated by ScmR and/or activated by QS. (B) Number of genes negatively
720 modulated by ScmR and/or repressed by QS. (C) Number of genes stimulated by ScmR
721 and/or negatively controlled by QS. (D) Number of genes inhibited by ScmR and/or positively
722 controlled by QS.

723

724 **Figure 3. ScmR influences the production of the main AHLs found in *B. thailandensis* E264**
725 **during the logarithmic phase of growth.** C_8-HSL, 3OHC_{10}-HSL, and 3OHC_8-HSL biosynthesis
726 was measured using LC-MS/MS in cultures of the wild-type and of the *scmR*- mutant strains
727 of *B. thailandensis* E264. The values are means ± standard deviations (error bars) for three
728 replicates. Values that are significantly different are indicated by asteriks as follows: ***, $P <$
729 0.001; **, $P < 0.01$; *, $P < 0.05$.

730

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

731 **Figure 4. Impact of *scmR* inactivation on bacterial growth.** The *B. thailandensis* E264 wild-
732 type strain and the *scmR*- mutant strain growth curves. Cultures were buffered with 100 mM
733 HEPES. Water only was added to the controls. The error bars represent the standard
734 deviations of the averages for three replicates.

735

736 **Figure 5. ScmR influences pH homeostasis.** (A) pH value was measured with a pH electrode
737 and meter (Mettler-Toledo, Mississauga, ON, Canada), at various times during growth in
738 cultures of the *B. thailandensis* E264 wild-type strain and the *scmR*- mutant strain. Cultures
739 were buffered with 100 mM HEPES. Water only was added to the controls. (B) The cell
740 density was monitored by measuring turbidity, expressed in 600 nm absorption units
741 (OD_{600}). (C) Colony-forming units (CFUs) were determined by plate-counting methods. The
742 error bars represent the standard deviations of the averages for three replicates.

743

744 **Figure 6. Expression of several ScmR-regulated genes in cultures buffered or not with**
745 **HEPES of the *B. thailandensis* E264 wild-type and the *scmR*- mutant strains.** The relative
746 transcript levels of (A) *obc1*, (B) BTH_I3204, (C) *bsaN*, (D) BTH_II0639, (E) *btaR5*, and (F)
747 BTH_II1209 were assessed by qRT-PCR in cultures of the wild-type and of the *scmR*- mutant
748 strains of *B. thailandensis* E264. Cultures were buffered with 100 mM HEPES. Water only was
749 added to the controls. The results are presented as relative quantification of transcription of
750 the gene compared to the wild-type strain, which was set at 100%. The error bars represent
751 the standard deviations of the averages for three replicates.

752

bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

753 **Figure 7. Effect of culture medium alkalization on the levels of AHLs in *B. thailandensis***
754 **E264 cultures.** The concentrations of (A) C_8-HSL, (B) 3OHC_{10}-HSL, and (C) 3OHC_8-HSL was
755 assessed using LC-MS/MS throughout the bacterial growth phases in cultures of the wild-
756 type and of the *scmR*- mutant strains of *B. thailandensis* E264. Cultures were buffered with
757 100 mM HEPES. Water only was added to the controls. The error bars represent the
758 standard deviations of the averages for three replicates.

759

760 **Figure 8. The QS-1 and QS-3 systems activate the transcription of *scmR*.** The relative
761 transcript levels of *scmR* were assessed by qRT-PCR in cultures of the wild-type and of the
762 Δ*btaR1*, Δ*btaR2*, and Δ*btaR3* mutant strains of *B. thailandensis* E264. The results are
763 presented as relative quantification of transcription of the gene compared to the wild-type
764 strain, which was set at 100%. The error bars represent the standard deviations of the
765 averages for three replicates.

766

767 **Figure 9. The *scmR* gene is negatively autoregulated.** The luciferase activity of the
768 chromosomal *scmR-lux* transcriptional fusion was monitored at various times during growth
769 in cultures of the *B. thailandensis* E264 wild-type strain and the *scmR*- mutant strain.
770 Cultures were buffered with 100 mM HEPES. Water only was added to the controls. The
771 error bars represent the standard deviations of the averages for three replicates. The
772 luminescence is expressed in relative light units per optical density of the culture
773 (RLU/OD_{600}).

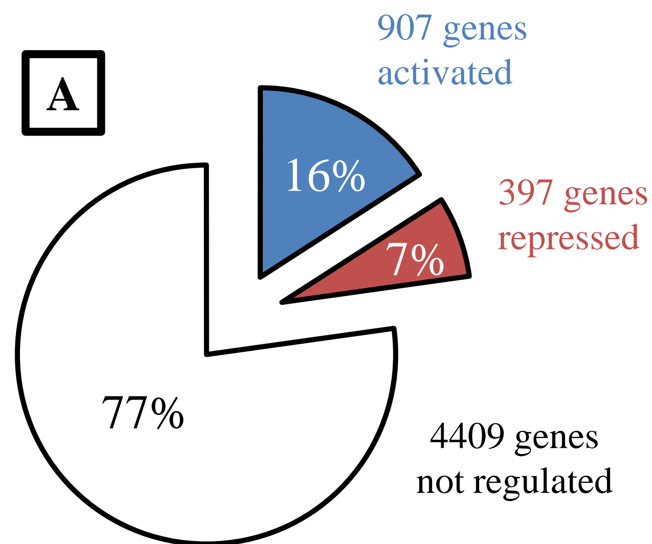
774

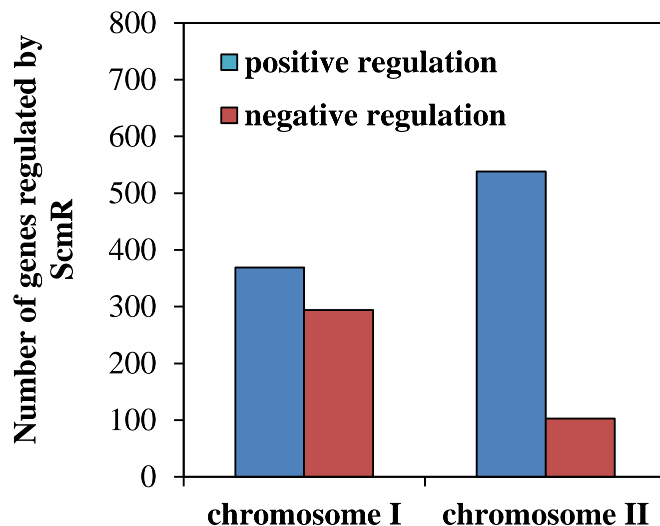
bioRxiv preprint doi: https://doi.org/10.1101/871871; this version posted December 11, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

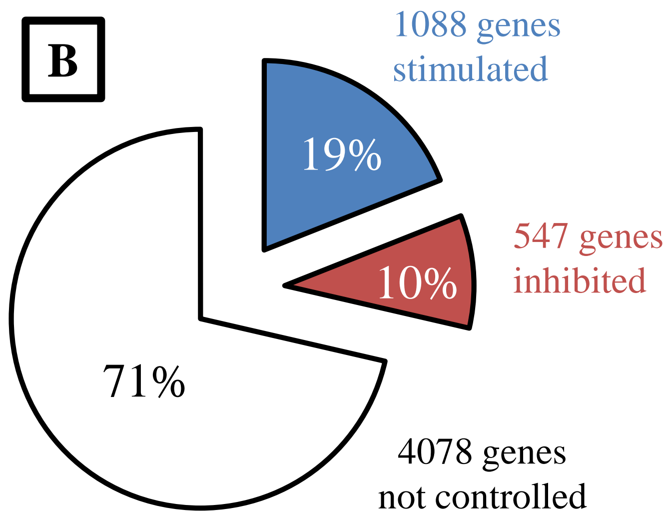
775 **Figure 10. Virulence of the wild-type strain and of the *scmR*- mutant strain of *B.***

776 ***thailandensis* E264 toward the fruit fly *D. melanogaster*.**

777







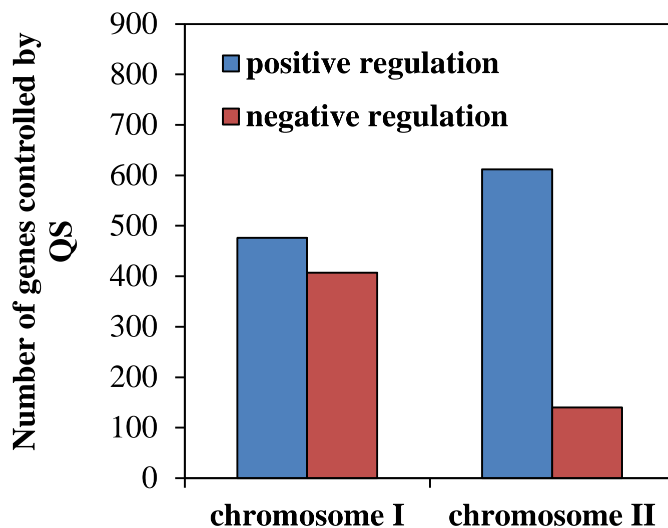


Fig. 1. Numbers of ScmR-regulated genes and those QS-controlled in *B. thailandensis* E264 according to transcriptomic analyses obtained by RNA-Seq. (A) Total numbers of genes showing positive or negative modulation in the *scmR*- mutant compared to the wild-type strain of *B. thailandensis* E264. (B) Total numbers of genes showing positive or negative modulation in the Δ*btaI1*Δ*btaI2*Δ*btaI3* mutant in comparison with the wild-type strain of *B. thailandensis* E264.

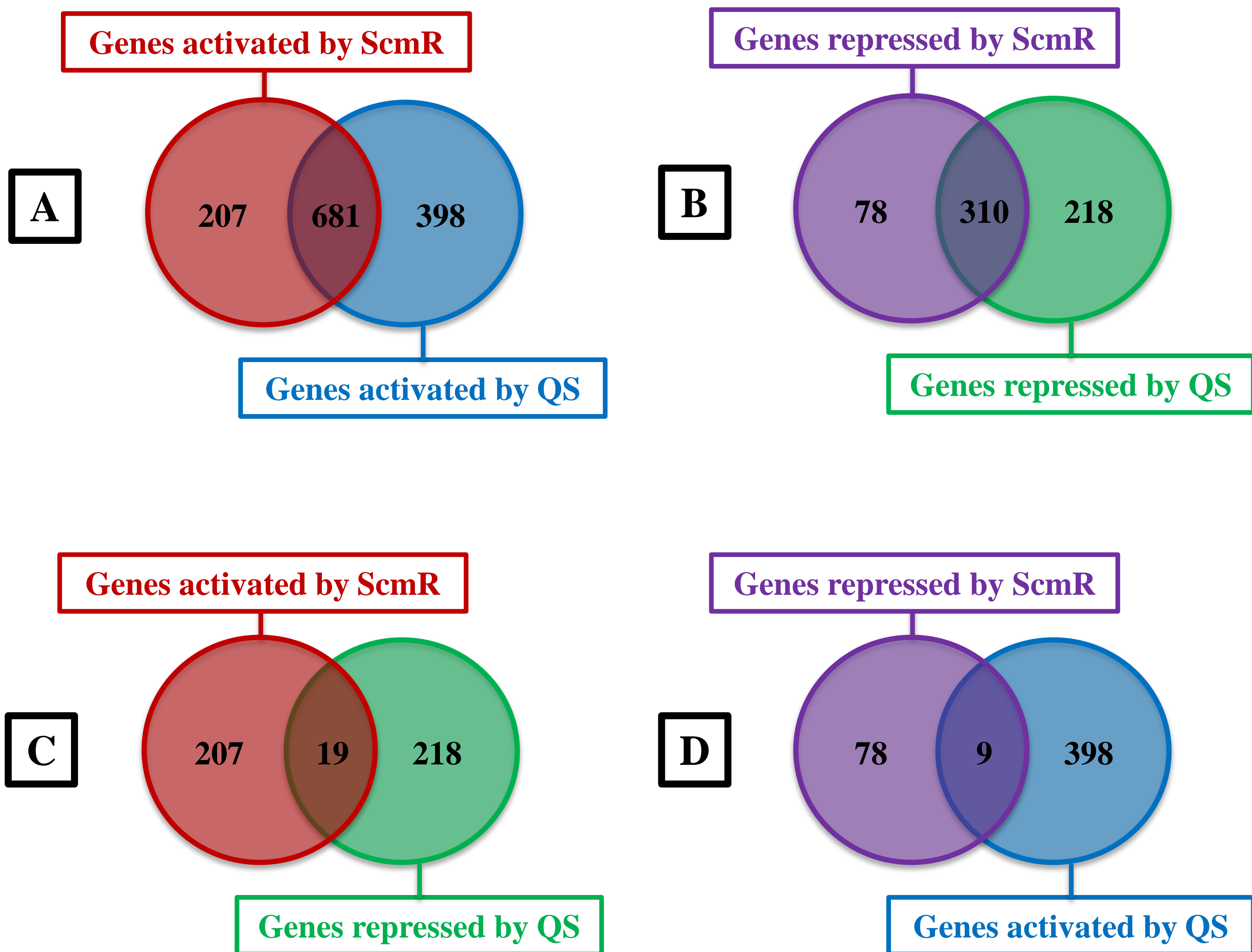


Fig. 2. The ScmR regulon comprises many QS-controlled genes. (A) Number of genes positively regulated by ScmR and/or activated by QS. (B) Number of genes negatively modulated by ScmR and/or repressed by QS. (C) Number of genes stimulated by ScmR and/or negatively controlled by QS. (D) Number of genes inhibited by ScmR and/or positively controlled by QS.

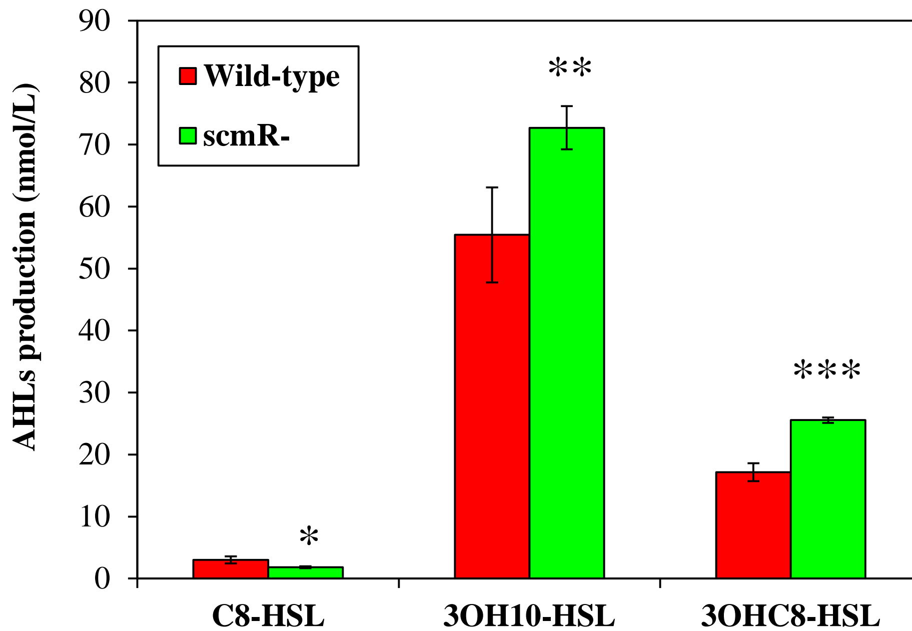


Fig. 3. ScmR influences the production of the main AHLs found in *B. thailandensis* E264 during the logarithmic phase of growth. C_8-HSL, 3OHC$_{10}$-HSL, and 3OHC$_8$-HSL biosynthesis was measured using LC-MS/MS in cultures of the wild-type and of the *scmR*- mutant strains of *B. thailandensis* E264. The values are means ± standard deviations (error bars) for three replicates. Values that are significantly different are indicated by asteriks as follows: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

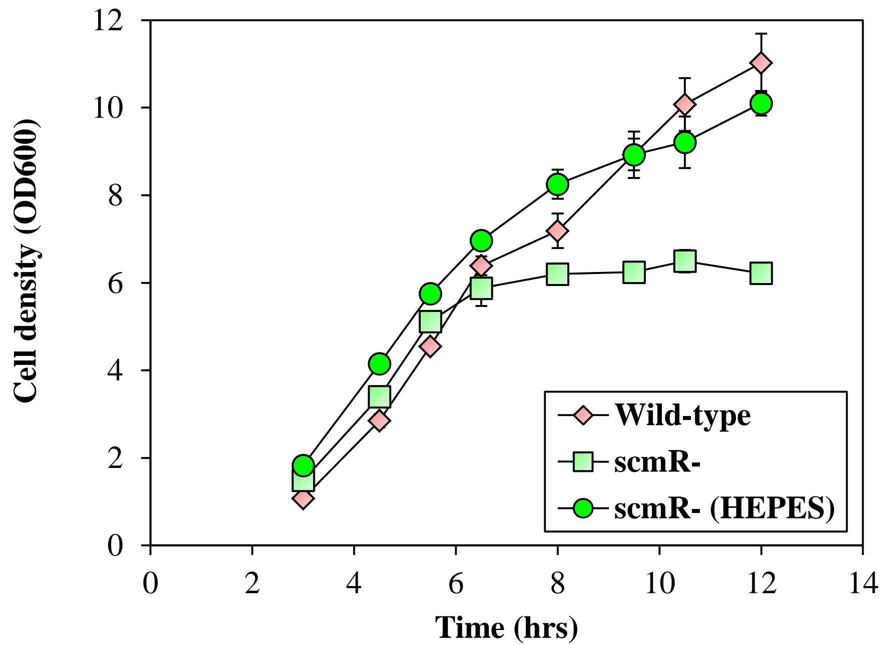


Fig. 4. Impact of *scmR* inactivation on bacterial growth. The *B. thailandensis* E264 wild-type strain and the *scmR*- mutant strain growth curves. Cultures were buffered with 100 mM HEPES. Water only was added to the controls. The error bars represent the standard deviations of the averages for three replicates.

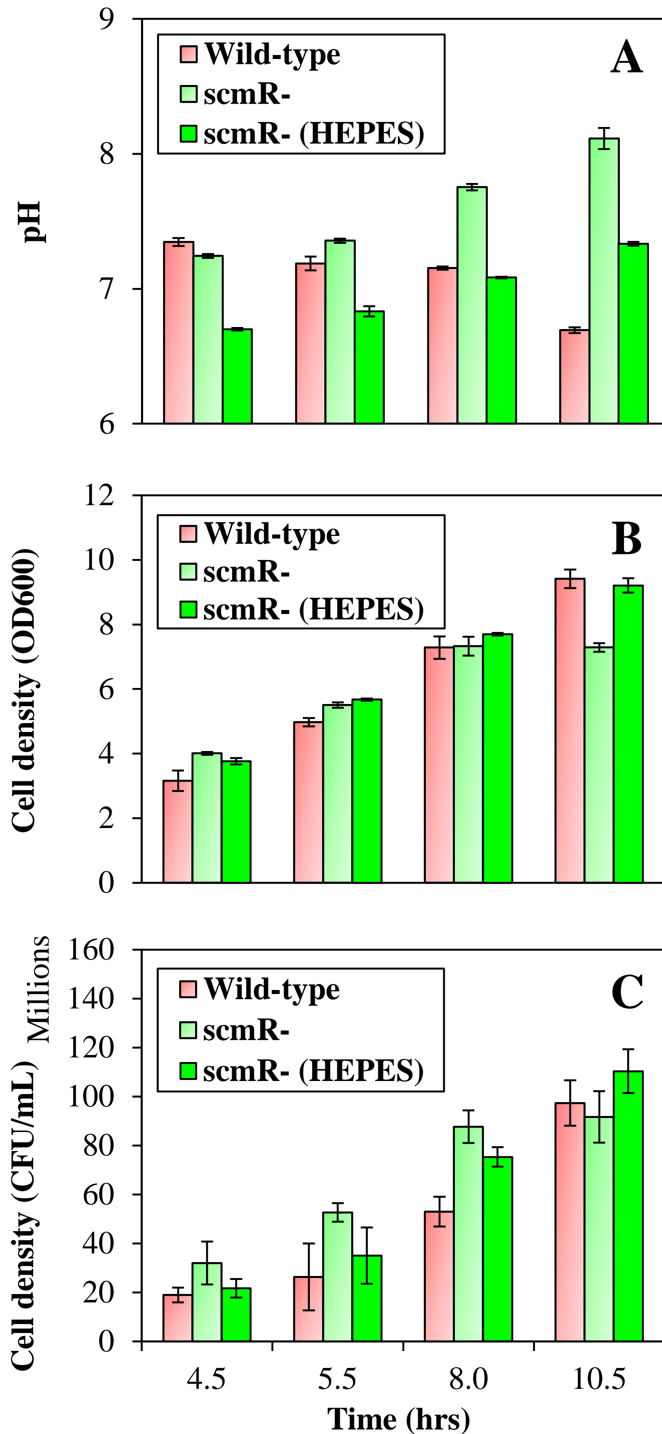


Fig. 5. ScmR influences pH homeostasis. (A) pH value was measured with a pH electrode and meter (Mettler-Toledo, Mississauga, ON, Canada), at various times during growth in cultures of the *B. thailandensis* E264 wild-type strain and the *scmR*- mutant strain. Cultures were buffered with 100 mM HEPES. Water only was added to the controls. (B) The cell density was monitored by measuring turbidity, expressed in 600 nm absorption units (OD_{600}). (C) Colony-forming units (CFUs) were determined by plate-counting methods. The error bars represent the standard deviations of the averages for three replicates.

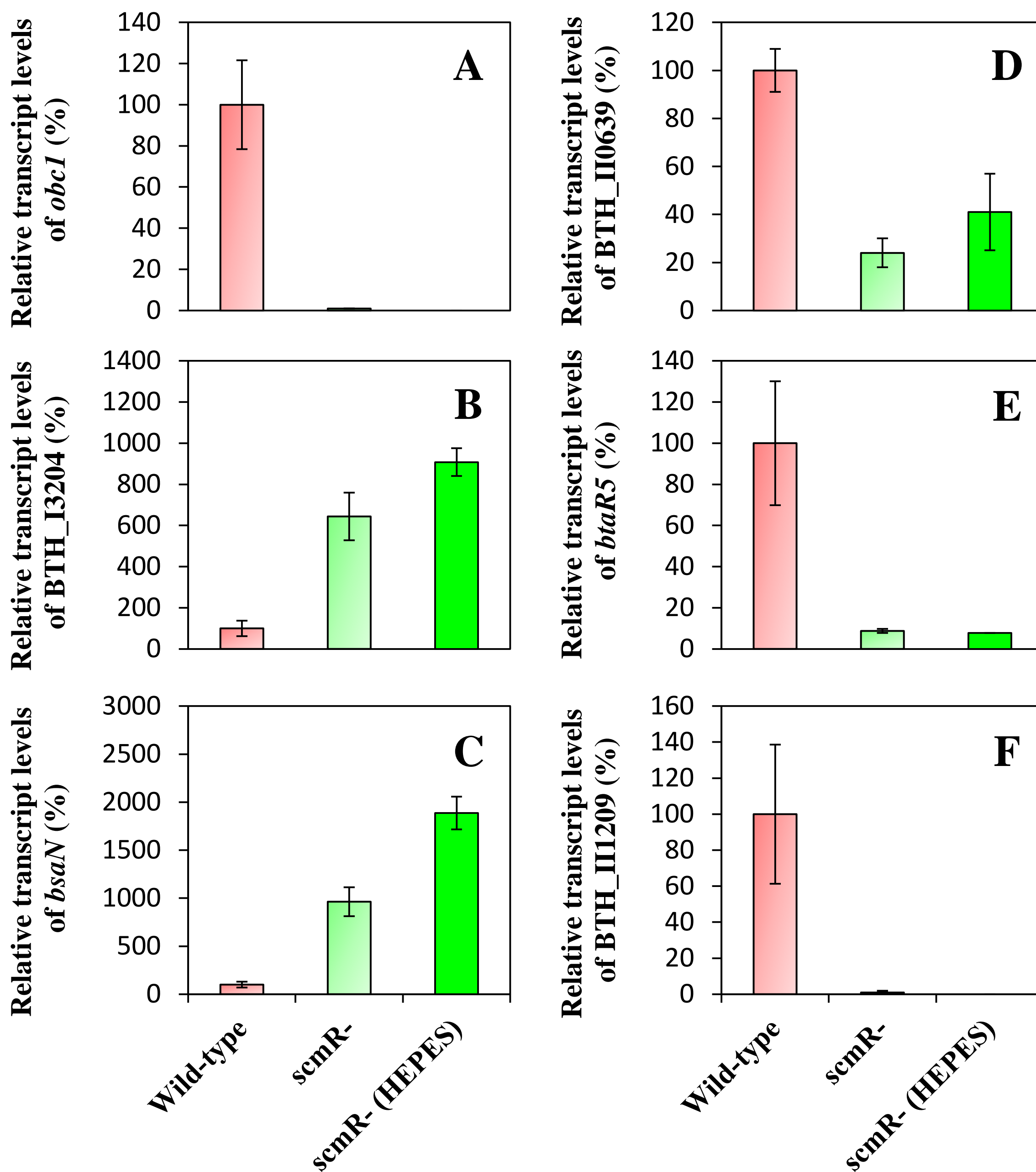


Fig. 6. Expression of several ScmR-regulated genes in cultures buffered or not with HEPES of the *B. thailandensis* E264 wild-type and the *scmR*- mutant strains. The relative transcript levels of (A) *obc1*, (B) BTH_I3204, (C) *bsaN*, (D) BTH_II0639, (E) *btaR5*, and (F) BTH_II1209 were assessed by qRT-PCR in cultures of the wild-type and of the *scmR*- mutant strains of *B. thailandensis* E264. Cultures were buffered with 100 mM HEPES. Water only was added to the controls. The results are presented as relative quantification of transcription of the gene compared to the wild-type strain, which was set at 100%. The error bars represent the standard deviations of the averages for three replicates.

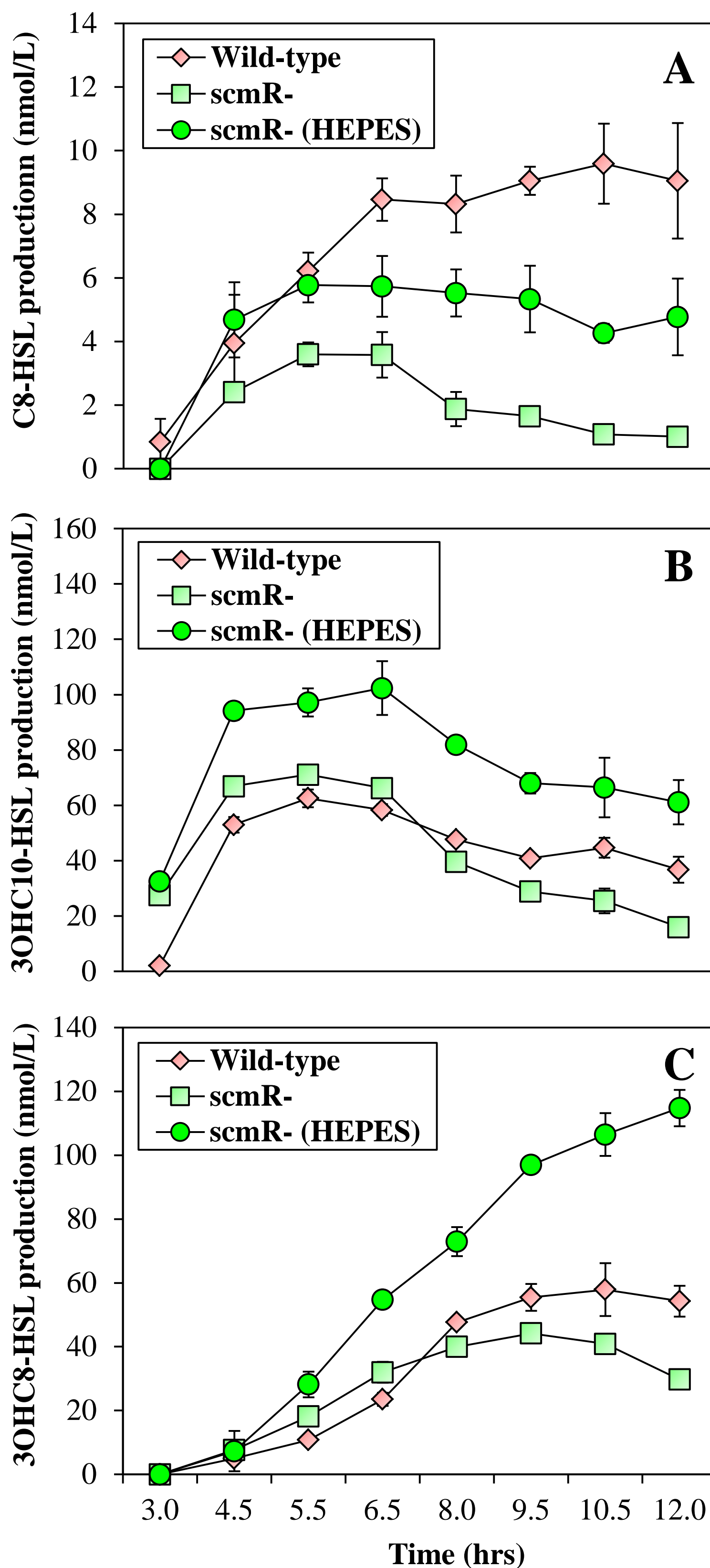


Fig. 7. Effect of culture medium alkalization on the production of AHLs in *B. thailandensis* E264. The biosynthesis of (A) C_8-HSL, (B) $3OHC_{10}$-HSL, and (C) $3OHC_8$-HSL was assessed using LC-MS/MS throughout the bacterial growth phases in cultures of the wild-type and of the *scmR*- mutant strains of *B. thailandensis* E264. Cultures were buffered with 100 mM HEPES. Water only was added to the controls. The error bars represent the standard deviations of the averages for three replicates.

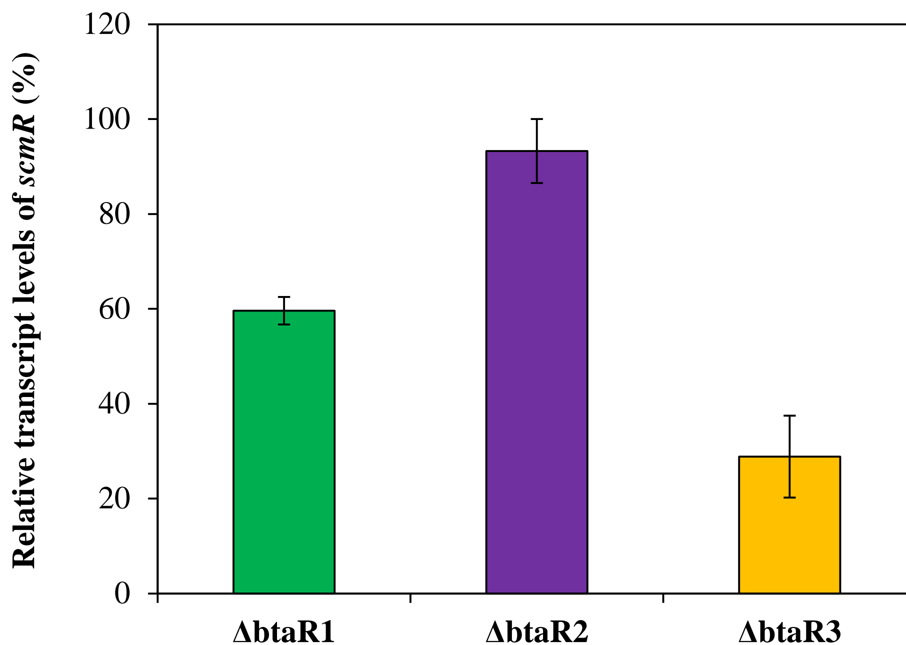


Fig. 8. The QS-1 and QS-3 systems activate the transcription of *scmR*. The relative transcript levels of *scmR* were assessed by qRT-PCR in cultures of the wild-type and of the Δ*btaR1*, Δ*btaR2*, and Δ*btaR3* mutant strains of *B. thailandensis* E264. The results are presented as relative quantification of transcription of the gene compared to the wild-type strain, which was set at 100%. The error bars represent the standard deviations of the averages for three replicates.

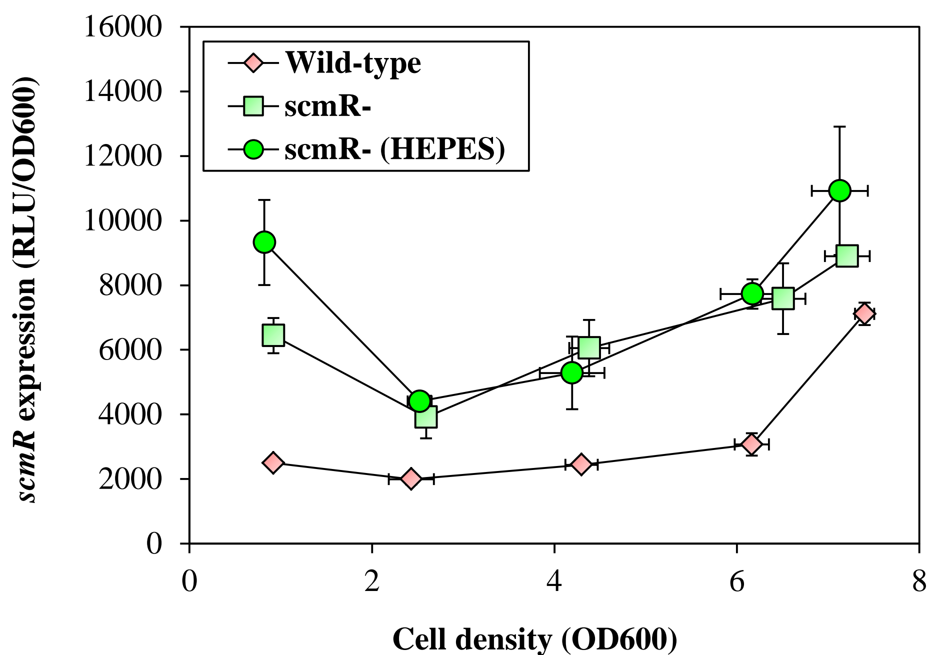


Fig. 9. The *scmR* gene is negatively autoregulated. The luciferase activity of the chromosomal *scmR-lux* transcriptional fusion was monitored at various times during growth in cultures of the *B. thailandensis* E264 wild-type strain and the *scmR*- mutant strain. Cultures were buffered with 100 mM HEPES. Water only was added to the controls. The error bars represent the standard deviations of the averages for three replicates. The luminescence is expressed in relative light units per optical density of the culture (RLU/OD_{600}).

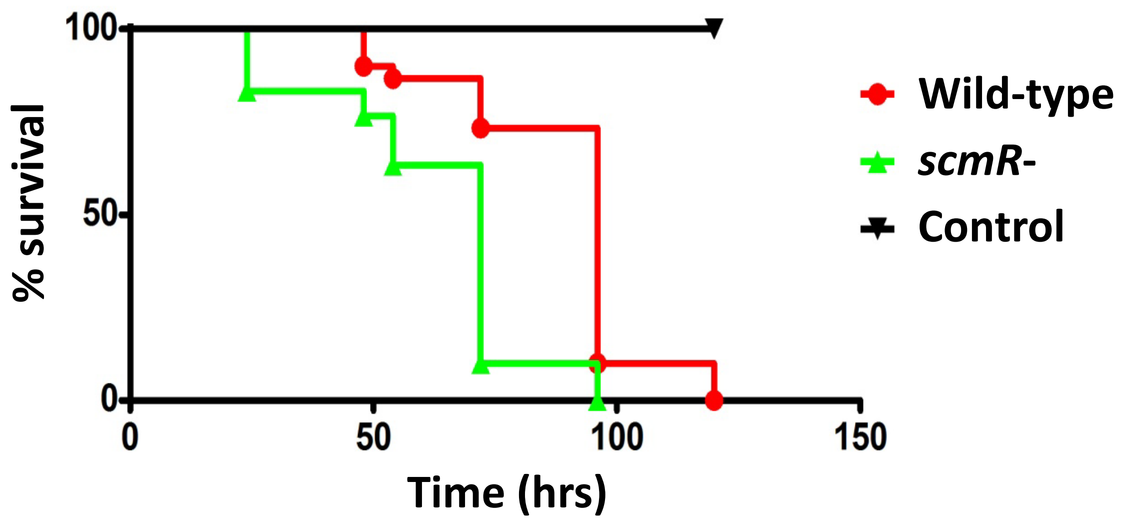


Fig. 10. Virulence of the wild-type strain and of the *scmR*- mutant strain of *B. thailandensis* E264 toward the fruit fly *D. melanogaster*.