

Plasmid manipulation of bacterial behaviour through translational regulatory crosstalk

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1 **Abstract**

2 Beyond their role in horizontal gene transfer, conjugative plasmids commonly encode
3 homologues of bacterial regulators. Known plasmid regulator homologues have highly targeted
4 effects upon the transcription of specific bacterial traits. Here, we characterise a plasmid
5 translational regulator, RsmQ, capable of taking global regulatory control in *Pseudomonas*
6 *fluorescens* and causing a behavioural switch from motile to sessile lifestyle. RsmQ acts as a global
7 regulator controlling the host proteome through direct interaction with host mRNAs and
8 interference with the host's translational regulatory network. This mRNA interference leads to
9 largescale proteomic changes in metabolic genes, key regulators and genes involved in
10 chemotaxis, thus controlling bacterial metabolism and motility. Moreover, comparative analyses
11 found RsmQ on a large number of divergent plasmids isolated from multiple bacterial host taxa,
12 suggesting the widespread importance of RsmQ for manipulating bacterial behaviour across
13 clinical, environmental, and agricultural niches. RsmQ is a widespread plasmid global translational
14 regulator primarily evolved for host chromosomal control to manipulate bacterial behaviour and
15 lifestyle.

16

17 **Significance Statement**

18 Plasmids are recognised for their important role in bacterial evolution as drivers of horizontal gene
19 transfer. Less well understood are the effects of plasmids upon bacterial behaviours by
20 manipulating the expression of key bacterial phenotypes. Until now, examples of plasmid
21 manipulation of their bacterial hosts were limited to highly targeted transcriptional control of a
22 few related traits. In contrast, here we describe the first plasmid global translational regulator
23 evolved to control the bacterial behavioural switch from a motile to a sessile lifestyle and bacterial
24 metabolism, mediated through manipulation of the bacterial proteome. Moreover, this global
25 translational regulator is common across divergent plasmids in a wide range of bacterial host taxa,
26 suggesting that plasmids may commonly control bacterial lifestyle in the clinic, agricultural fields,
27 and beyond.

28 Introduction

29 Bacteria regulate the expression of functional traits in response to their environment, enabling
30 colonisation of diverse ecological niches. However, control over bacterial gene regulation is not
31 exclusively under the control of the bacterial genome. The mobile genetic elements which inhabit
32 bacterial hosts, such as conjugative plasmids, commonly encode homologues of bacterial
33 regulators. The introduction of plasmid-encoded regulator homologues into the bacterial cell can
34 rewire the gene regulatory networks of the bacterium, potentially altering the expression of
35 bacterial traits; a process termed plasmid-chromosome crosstalk (PCC,). However, how and why
36 plasmid-encoded regulators would manipulate the expression of bacterial traits is poorly
37 understood.

38 To date, well-characterised PCCs involve plasmid-encoded transcriptional regulators that alter the
39 expression of specific bacterial traits. For example, in *Acinetobacter baumannii* several multidrug
40 resistance plasmids encode transcriptional repressors of the bacterial type VI secretion system
41 (T6SS) (1). Plasmid-mediated repression of the T6SS enhances plasmid horizontal transmission by
42 ensuring that plasmid recipient cells are not killed by the donor's T6SS apparatus (2). Similarly,
43 plasmid encoded transcriptional regulators alter the expression of several chromosomal
44 regulators of virulence associated traits in *Rhodococcus equi*, thus enhancing survival of both the
45 bacterium and the plasmid in macrophages by stalling phagosomal maturation (3). Together these
46 examples suggest that plasmid encoded regulatory homologues may have important fitness
47 consequences for the plasmid, either through horizontal replication, through conjugation to new
48 host cells or through vertical replication within the current host cell (4).

49 The molecular mechanisms of known PCC involve plasmid-encoded transcriptional regulators
50 causing targeted changes to the expression of small numbers of chromosomal genes. Although
51 transcriptional regulation is important for bacterial survival and adaptation, bacteria also rely on
52 translational regulation to respond to changes in their environment (5). Bacteria are able to exert
53 this control by deploying second messenger signals (6), directly altering the ribosome (7) or
54 impacting mRNA stability and accessibility via pathways such as Gac-Rsm (8, 9). It is currently
55 unknown whether conjugative plasmids are able to manipulate translational regulatory pathways.

56 The Gac-Rsm pathway is one of the best characterised translational regulatory systems in
57 pseudomonads (10–14) and controls a wide variety of traits including biofilm formation (15),
58 motility (16), quorum sensing (17), siderophore production (18) and virulence (8, 19). Gac-Rsm is
59 highly conserved within the *Pseudomonas* genus and comparable systems exist in a wide range of
60 bacteria (8, 14, 18, 20, 21). Rsms are small (9 kDa) proteins that are able to interact directly with
61 the bases AnGGA around the ribosome binding site (RBS) of their target transcript (21–24). Rsm
62 proteins can both activate and repress bound mRNA transcripts, either by opening up the mRNA
63 to allow ribosomal access to the RBS, or by making the RBS inaccessible (24–26). This allows Rsm
64 proteins to exert tight translational control over a wide range of targets to impact bacterial
65 phenotypes (27). The activation of Rsm is regulated by the activation of the GacA/S two-
66 component system (TCS) which is activated by a complex but largely uncharacterised set of
67 environmental cues. Upon activation, GacA promotes transcription of the small-regulatory RNAs
68 RsmY and RsmZ, which leads to the sequestering of regulatory Rsm proteins away from their
69 mRNA targets through competition for binding (24, 28). The number of Rsm proteins encoded by
70 individual *Pseudomonas* species varies, with each protein having both unique and overlapping
71 regulons with other Rsm proteins (29). The large number of traits regulated by the Gac-Rsm
72 system suggests that there could be significant effects on bacterial behaviour caused by PCC
73 manipulating this system.

74 In this study, we investigate the role of translational regulation in mediating PCC between
75 *Pseudomonas fluorescens* SBW25 and the 425 kb conjugative plasmid pQBR103. *P. fluorescens* is
76 a common, soil-dwelling, plant growth promoting bacterium that is capable of accepting diverse
77 plasmids, including those from the pQBR family of large conjugative plasmids (30, 31). Both
78 SBW25 and the pQBR plasmids were first isolated in the 1990s from the sugar beet rhizosphere
79 at Wytham Woods in the United Kingdom (30, 32). The ability of several of the pQBR plasmids to
80 persist within *P. fluorescens* strains across a range of environments including in soil, on plants,
81 and in lab media has been well documented (30, 31, 33, 34). Moreover, acquisition of pQBR103
82 by *P. fluorescens* SBW25 alters the expression of ~440 chromosomal genes (34, 35). The large-
83 scale regulatory disruption caused by pQBR103 can be negated by a range of compensatory
84 mutations restoring wild-type (WT) expression levels, including loss of function mutations
85 affecting the bacterial TCS *gacA/S*. Notably, while the genetic sequence of pQBR103 encodes a

86 range of accessory functions including mercury resistance and UV resistance, it also encodes a
87 homologue of the widespread *rsmA* bacterial translational regulator gene, which we identify here
88 as *rsmQ*.

89 To understand the function of *rsmQ*, we explored the distribution of *rsm* genes on plasmids, and
90 the effects of *rsmQ* on the transcriptome and proteome of SBW25, as well as on the expression
91 of key bacterial ecological traits. Further, we biochemically characterised the interactions of RsmQ
92 with a close structural proxy for RNA (single stranded DNA (ssDNA)) and with the bacterial Rsm
93 proteins. Our findings show that *rsm* genes are widespread on *Pseudomonas* plasmids, and that
94 RsmQ interacts with the resident Gac-Rsm system and the host RNA pool, binding to specific
95 nucleotide motifs in order to post-transcriptionally regulate translation. RsmQ extensively
96 remodelled the SBW25 proteome including altering production of metabolism, nutrient uptake
97 and chemotaxis pathways, despite having only a limited impact on the SBW25 transcriptome. In
98 turn, RsmQ translational regulation altered the expression of ecologically important bacterial
99 behaviours, most notably motility and carbon source metabolism. Together our findings expand
100 the known molecular mechanisms causing plasmid-chromosome crosstalk to include translational
101 regulator homologues, which act in this case to extensively manipulate bacterial behaviour by
102 altering the expression of a range of ecologically important bacterial traits. These findings have
103 broad implications for understanding the role of plasmids in microbial communities.

104

105 **Results**

106 **Plasmids encode regulatory protein homologues**

107 The ORF *PQBR443*, hereafter *rsmQ*, was identified on pQBR103 as a homologue of the
108 chromosomal *csrA/rsmA* genes found widely within proteobacteria. We hypothesised that this
109 gene could act as a mediator of PCC (36). To identify whether carriage of an *rsm* homologue is
110 peculiar to pQBR103 or is a general phenomenon across plasmids, we investigated the distribution
111 of *rsmQ* homologues in the 12,084 plasmids of the COMPASS database (37). Within this set, and
112 consistent with previous studies (38), we detected 106 putative *rsmQ* homologues on 98 plasmids
113 (0.8%), mostly isolated (92/98) from proteobacteria, particularly Pseudomonadaceae and
114 Legionellaceae (Figure 1a). The distribution of *rsm*-containing plasmids was not uniform across

115 taxa (Fisher's Exact Test, $p < 0.0005$): approximately 20% of Pseudomonadaceae (41/196) and
116 Piscirickettsiaceae (14/72) plasmids, and over 50% of Legionellaceae (18/30) plasmids contained
117 *rsm* homologues, while no *rsm* homologues were detected on any of the 3621 Enterobacteriaceae
118 plasmids. *rsm*-containing plasmids were relatively large, with the smallest at 32.4 kb, sitting at the
119 larger end of the size distribution for each taxon (Figure S1a). There was no general association
120 between *rsm*-carriage and plasmid mobility across taxa, although within Legionellaceae, *rsm*-
121 encoding plasmids tended to be conjugative (Fisher's Exact Test Bonferroni-adjusted p -value =
122 0.02). Within Pseudomonadaceae, *rsm*-encoding plasmids tended to have proportionally more
123 genes with predicted *rsm* binding sites (Kolmogorov-Smirnov test, $p = 0.012$, Figure S1b). Overall,
124 these patterns suggest that plasmid carriage of *rsm* is not uncommon, but is taxon-specific,
125 indicating a functional role that is associated with particular groups of microorganisms.

126 It is possible that *rsm*-encoding plasmids have recently spread horizontally across different
127 species. If this was the case, we would expect *rsm*-encoding plasmids to be more similar to one
128 another than to non-*rsm*-encoding plasmids within each taxon. To investigate the diversity of *rsm*-
129 encoding plasmids relative to the other plasmids in COMPASS, we performed UMAP non-metric
130 multidimensional scaling (NMDS) on pairwise MASH distances between plasmids (39–41). Within
131 the diversity of plasmids in COMPASS, *rsm*-containing plasmids are diverse and often cluster close
132 to non-*rsm*-containing plasmids isolated from the same taxa (Figure 1b), suggesting that carriage
133 of *rsm* regulators by plasmids is a convergent trait that has emerged several times over.

134 Global regulatory genes may be frequently (re)acquired by plasmids from the bacterial
135 chromosome. Alternatively, these genes may have a prolonged association with plasmids and
136 evolve distinctly to chromosomal genes. To investigate these possibilities, we built a phylogenetic
137 tree of the *csrA/rsmA* homologues from all *Pseudomonas* plasmids and their associated
138 chromosomes (where available), alongside the *rsm* genes from seven diverse *Pseudomonas*
139 strains: *Pseudomonas protegens* CHA0, *P. fluorescens* Pf0-1, *P. protegens* Pf-5, *P. fluorescens*
140 SBW25, *Pseudomonas putida* KT2440, *Pseudomonas aeruginosa* PAO1, and *P. aeruginosa* PA14
141 (Figure 1c). Chromosomal homologues of *csrA/rsmA* formed several distinct clusters (bootstrap
142 support >80%), with one cluster including *P. fluorescens* SBW25 *rsmA* and the *E. coli* homologue
143 *csrA*. However, plasmid-borne *csrA/rsmA* homologues were more divergent than those that were
144 chromosomally encoded (Figure 1c). Additionally, chromosomal homologues (including the *P.*

145 *fluorescens* SBW25 genes *rsmE* and *rsmI*) formed a distinct cluster. Consistent with the
146 phylogenetic analysis, sequence variation among chromosomal *rsm* homologues was significantly
147 lower than when comparing chromosomal- with plasmid-borne *rsm* homologues (Wilcoxon test,
148 Bonferroni-adjusted $p < 0.0001$), or when comparing plasmid-borne *rsm* homologues with one
149 another (Wilcoxon test, $p < 0.0001$). The principal exception to this pattern was a cluster of closely
150 related plasmid and chromosomal *rsm* genes from plant pathogenic *Pseudomonas* (green
151 highlighted, figure 1c). However, it is possible that some of these chromosomal variants are
152 associated with chromosomally located mobile genetic elements, as at least one of these
153 homologues is located on an annotated integrative conjugative element (42).

154 Overall, our comparative sequence analysis suggests that diverse plasmids have independently
155 acquired *rsm* homologues, which then evolve and diversify as part of the plasmid mobile gene
156 pool, distinct from their chromosomal counterparts. Although plasmid-encoded *rsm* homologues
157 are widespread among plasmids (38), very little is currently known about their role in PCC or how
158 they might impact bacterial behaviour.

159 RsmQ binds to specific RNA targets

160 Despite a high degree of sequence similarity, it was unknown if RsmQ would be functionally
161 similar to the chromosomally encoded SBW25 Rsm proteins (RsmA/E/I). Rsm proteins from
162 *Pseudomonas* species interact with a conserved RNA sequence (AnGGA), with these bases
163 interacting directly with the proteins' conserved binding site (VHRE/D) (23, 24). To confirm
164 whether RsmQ acts similarly, we designed a high throughput method to examine the nucleotide
165 binding properties of RsmQ *in vitro* using the ReDCaT surface plasmon resonance (SPR) system
166 (43), which is primarily designed for examining dsDNA interactions. Because Rsm proteins only
167 interact with the nucleotide bases of RNA molecules, protein-nucleic acid interactions can be
168 effectively examined using ssDNA probes. ssDNA probes containing the predicted RNA target
169 sequence (ACGGA) and a non-specific scrambled sequence were synthesised with the ReDCaT
170 linker at the 3' end, with either a linear or a hairpin secondary structure with the potential binding
171 site at the top of the hairpin.

172 RsmQ interacted strongly with both the minimal (GGA) and full length (ACGGA) binding sites when
173 these were at the top of a hairpin loop. When the binding sequence was presented in a linear

174 oligo RsmQ could interact but quickly dissociated, suggesting that the preferred binding site is
175 open at the top of a hairpin loop (Figure S2). No interaction was seen between RsmQ and a
176 scrambled binding site confirming that the binding is specific to the target GGA/ACGGA sequence
177 (Figure 2).

178 Next, we tested if the RNA binding interaction was co-ordinated by the conserved VHRD/E motif
179 at the C-terminus of RsmQ by examining the binding of two RsmQ mutants (H43A and R44A) to
180 the ssDNA probes. The alteration of these residues significantly reduced the efficiency of RsmQ
181 binding to the target sequence (Figure 2a). Finally, to confirm the minimum RNA binding sequence
182 a series of near-identical ssDNA nucleotides were tested containing the simple and full binding
183 site sequences with a single base pair change in each case. RsmQ preferentially bound to the
184 known binding sites GGA and A(N)GGA with a markedly higher affinity than to any of the alternate
185 sequences tested and with a slight preference for ATGGA/AGGGA sequences, further supporting
186 the hypothesis that RsmQ is a specific RNA binding protein that functions similarly to the
187 chromosomal Rsm proteins (Figure 2b).

188 RsmQ post-transcriptionally regulates the abundance of metabolism, nutrient
189 transport, and chemotaxis proteins

190 To examine the impact of RsmQ on SBW25 regulation, a plasmid lacking *rsmQ* was created by
191 allelic exchange in a kanamycin resistance gene-containing variant of pQBR103 (pQBR103^{km}) and
192 freshly conjugated into SBW25. Expression of the chromosomal *rsm* genes generally peaks as
193 growing cells make the switch from exponential to stationary phase growth (44). Therefore, *P.*
194 *fluorescens* SBW25 cells carrying pQBR103^{km} or pQBR103^{km}- Δ *rsmQ* or no plasmid were grown to
195 late exponential phase ($OD_{600} = 1.4$) in KB liquid medium and a combination of RNA-seq and TMT
196 quantitative proteomics were used to examine changes in mRNA and protein abundances. Five
197 independent transconjugants for each plasmid as well as five independent colonies of the
198 plasmid-free strain were used for these experiments.

199 Contrary to previous studies of exponentially growing cultures where carriage of pQBR103 caused
200 large-scale transcriptional changes (35, 36, 45), we observed only modest transcriptional changes
201 associated with plasmid carriage under our growth conditions, with only 54 genes upregulated
202 more than two-fold and 33 downregulated by pQBR103^{km} carriage, and similar numbers seen for

203 pQBR103^{Km} - Δ *rsmQ*. Genes whose transcription was upregulated predominantly encoded
204 transport-related membrane proteins, such as ABC and glutamine high-affinity transporter
205 components. In addition, *PFLU1887* and *PFLU1888* encoding components of a putative
206 transposase, were upregulated by pQBR103^{Km} carriage, consistent with previous studies (46).
207 Genes downregulated by plasmid acquisition included several cytochrome C oxidases and
208 metabolic enzymes such as L-lactate and shikimate dehydrogenases. pQBR103^{Km} and pQBR103^{Km}-
209 Δ *rsmQ* had highly similar transcriptional effects, suggesting that that RsmQ had little impact on
210 the transcription of either chromosomal or plasmid genes.

211 In contrast, we observed major differences between the proteomes of SBW25 (pQBR103^{Km}) and
212 SBW25 (pQBR103^{Km}- Δ *rsmQ*), confirming that RsmQ is indeed a post-transcriptionally acting
213 translational regulator. Specifically, 581 SBW25 proteins showed at least two-fold increased
214 abundance in the absence of *rsmQ* (i.e. their translation is suppressed by RsmQ) and 203 showed
215 at least two-fold decreased abundance (Figure 3a and Table S1). Intriguingly, RsmQ regulation
216 predominantly affected the host proteome, with the abundances of only a small fraction of
217 plasmid-encoded proteins (16/733) altered by the presence of *rsmQ*.

218 We next determined the COG functional categories of SBW25 proteins whose abundances were
219 altered by the presence of RsmQ (Figure 3b). The 581 proteins downregulated by RsmQ were
220 disproportionately associated with amino acid, coenzyme and carbohydrate transport and
221 metabolism, as well as proteins involved in mRNA translation and ribosome stability. Among the
222 50 most strongly downregulated proteins we observed multiple inorganic ion transporters and
223 receptor proteins, for example pyoverdine receptors and iron transporters (*PFLU3378*, *PFLU2545*
224 (*FpvA*), *PFLU0295*) (47–50), a copper transport outer-membrane porin (*PFLU0595* (*OprC*
225 homolog)) and the PhoU phosphate ABC transporter known to repress the Pho operon (*PFLU6044*
226 (51)), alongside proteins involved in amino-acid transport (e.g. ABC transporters *PFLU0827* &
227 *PFLU0332* and metabolism (e.g. *GlyA* – *PFLU565*). Together these data suggest a role for RsmQ in
228 the control of SBW25 nutrient acquisition and metabolism.

229 Conversely, proteins upregulated by RsmQ included a large number of motility proteins and DNA
230 recombination and repair systems, alongside a larger fraction of uncharacterised proteins than in
231 the RsmQ downregulated group of proteins. The most strongly upregulated proteins included a

232 striking number of chemotaxis pathway components. In addition to CheA (PFLU4414) we
233 identified 5 putative methyl-accepting chemotaxis proteins (e.g. PFLU2358, PFLU3427 &
234 PFLU2486). RsmQ upregulated proteins also included the master-regulator of motility FleQ
235 (PFLU4443 (52, 53)) and an uncharacterised RpiR family transcriptional regulator (PFLU257). This
236 suggests that RsmQ modifies bacterial motility through altering cellular perception of the
237 environment and the availability of local nutrient sources, as well as by directly controlling
238 production of motility apparatus. Interestingly, the Gac-Rsm TCS repressor protein RetS
239 (PFLU0610 (54)) is also upregulated by RsmQ, supporting a further regulatory linkage between
240 pQBR103^{Km} carriage, RsmQ function and the Gac/Rsm pathway.

241 The global regulator Hfq (PFLU0520) was shown to be downregulated in a $\Delta rsmQ$ background,
242 suggesting an additional level of post-transcriptional regulatory control. A fraction of the
243 published SBW25 Hfq regulon (55) was shown to be up/down-regulated in the $\Delta rsmQ$
244 background. However, only relatively modest regulatory overlap was observed between the two
245 systems. Intriguingly, one of the few plasmid-encoded proteins that was significantly affected by
246 RsmQ was an Hfq homologue (pQBR0137), whose abundance increased upon *rsmQ* deletion
247 (Table S1). Only the chromosomally encoded Hfq possesses a predicted RsmQ binding site
248 however, suggesting that the plasmid-borne protein may be compensating for reduced
249 chromosomal Hfq levels under conditions where RsmQ is not functional.

250 Sequence analysis suggests that around 50% of the genes encoding proteins whose abundance is
251 differentially regulated by RsmQ contained an AnGGA binding site upstream, or within the first
252 100 bp of the ORF, with an additional 25% of all genes containing the simpler GGA motif. This
253 pattern is consistent with RsmQ binding to these mRNAs to regulate their translation. To test this,
254 we next designed ssDNA probes to examine if RsmQ indeed targeted the binding sites of the genes
255 predicted to be directly regulated. Binding site probes were designed to be 30 bp long with the
256 predicted binding site in the centre of the oligo with the ReDCaT linker on the 3' end. As previously
257 described SPR was performed to determine if an interaction occurred between the sequence and
258 RsmQ *in vitro*. Using the hairpin AnGGA synthetic binding site oligos as a guide, five of the
259 potential binding site oligos showed a %R_{max} of greater than 50% (Figure 4). These binding sites
260 were the upstream regions of PFLU0923 (ATGGA), PFLU3378 (AGGGA), PFLU1516 (AGGGA),
261 PFLU4443 (AGGGA, FleQ) and PFLU4726 (ATGGA) with the highest binding seen with PFLU3378.

262 Despite an apparent preference for ATGGA/AGGGA binding sites, it is likely that the secondary
263 structure was the overriding predictor of binding. Secondary structure predictions suggested that
264 PFLU3378 was the only oligo with the binding site fully open at the top of the stem loop, with the
265 rest showing partial occlusion of the ssDNA binding site by incorporation into a stem loop. These
266 data confirm direct interaction between RsmQ and at least some of its predicted targets and
267 further support the importance of mRNA secondary structure for successful RsmQ binding.

268 RsmQ interacts with the host Rsm system

269 Notwithstanding the evidence for direct regulation of translation by RsmQ binding to mRNA, the
270 large remainder of differentially regulated proteins without predicted Rsm binding sites suggests
271 an indirect mechanism by which RsmQ regulates the abundance of these proteins. Given that
272 RsmQ closely mimics the RNA binding characteristics of host Rsm proteins (Figure 2), we next
273 investigated whether RsmQ interacts with other elements of the host Rsm regulatory pathway.

274 The activity of host Rsm proteins is controlled by the ncRNAs RsmY/Z, which act as protein
275 sponges, sequestering Rsm proteins away from their target mRNAs. To test RsmQ binding to the
276 ncRNAs RsmY and RsmZ, we copied the individual stem loops of each ncRNA into ssDNA oligos of
277 approximately 25bp in length and attached them to the ReDCaT linker. The oligos were modelled
278 to determine the location of the binding site in both the ncRNA and in the case of the ssDNA
279 sequence, to determine if this was located at the top of a stem loop. Strong binding to several
280 ssDNA probes was observed, in each case contingent on the presence of at least a GGA sequence
281 at the top of a stem loop, with RsmY 1-25 and RsmZ 26-50 having AnGGA motifs present (Figure
282 5a). These data suggest that RsmQ interacts with the Gac-Rsm regulatory system by binding to
283 the host ncRNAs RsmZ and RsmY. This would lead to either an increase in RsmQ target translation
284 as RsmQ is titrated away from its targets, or an increase in RsmA/I/E binding to target mRNAs due
285 to a reduction in available RsmZ/Y binding sites.

286 Rsm proteins have been seen to homodimerize and are regularly found as homodimers within the
287 cell (21, 22, 56). With the exception of RsmN from *P. aeruginosa*, they generally have a conserved
288 tertiary structure. Furthermore, an AlphaFold (57) model of RsmQ was shown to be highly similar
289 to the crystal structures of SBW25 host Rsm proteins (Figure 5b)(56). We hypothesised therefore
290 that RsmQ may also interfere with regulation by forming heterodimers with host Rsm proteins.

291 To test this, we expressed *rsmQ* and the SBW25 host *rsm* genes heterologously in *E. coli* using the
292 BACTH system. Interestingly, with the exception of RsmI, we saw evidence of homo- and
293 heterodimerisation within and between the Rsm proteins. Both RsmE and RsmQ homodimerised,
294 and heterodimerisation was observed between all pairwise combinations of RsmA, RsmE and
295 RsmQ (Figure 5c and d). These results therefore support two indirect mechanisms for RsmQ
296 regulation of the SBW25 proteome in addition to direct mRNA binding: either by sequestering
297 ncRNAs, or directly interfering with the activity of host Rsm regulators.

298 RsmQ causes phenotypic changes in SBW25

299 Given the largescale changes that RsmQ caused to the SBW25 proteome, we hypothesised that
300 these altered protein abundances would in turn affect bacterial behaviour. To test this, we
301 quantified the impact of RsmQ on ecologically important traits normally controlled by the Gac-
302 Rsm regulatory system. Specifically, we initially quantified swarming motility and production of
303 exopolysaccharide/adhesin (measured using an indirect Congo red binding assay (58)) by SBW25
304 in the presence and absence of *rsmQ*. To examine the direct impact of *rsmQ* on chromosomally
305 encoded genes, *rsmQ* was expressed under an inducible promoter on a multicopy plasmid, in the
306 absence of pQBR103^{Km}. Overexpression of *rsmQ* led to a complete loss of swarming motility and
307 a significant increase in Congo red binding (Figure 6a and c). This suggests that *rsmQ* shifts SBW25
308 towards a more sessile lifestyle as characterised by reduced flagellar motility and increased
309 production of attachment factors and/or extracellular polysaccharides associated with biofilm
310 formation.

311 To test if *rsmQ* had similar effects on SBW25 behaviour when encoded on pQBR103, we repeated
312 the experiments using SBW25 with or without pQBR103^{Km} ± *rsmQ*. Acquisition of pQBR103^{Km}
313 caused reduced swarming motility and Congo red binding relative to plasmid-free SBW25.
314 However, deletion of *rsmQ* only partially ameliorated the reduction in swarming motility (Figure
315 6b) and had no effect on Congo red binding (Figure 6c). The expression of Rsm proteins is normally
316 tightly controlled by the cell, these results suggest that at high concentrations RsmQ is able to
317 override the native cellular control to cause drastic phenotypic changes that are not observed at
318 the native level. This is also consistent with our proteomic data (Figure 3b), which showed little
319 or no impact of RsmQ on the abundance of structural biofilm or motility proteins such as flagella
320 and adhesins, but a significant impact on chemotaxis pathways.

321 It was therefore considered that the role of RsmQ may be in the perception and uptake of specific
322 nutrients, and therefore any phenotypic changes may be carbon source dependent. We tested
323 the effect of the nutrient environment on swarming motility phenotypes and observed that
324 pQBR103 carriage strongly effected swarming motility on poorer carbon sources (Figure S3), with
325 the loss of *rsmQ* leading to a small restoration of swarming, again suggesting *rsmQ* is involved in
326 manipulating the cellular perception of the environment.

327 Carbon source sensing by RsmQ

328 Rsm proteins were first characterised by their involvement in carbon storage and metabolism (e.g.
329 CsrA in *E. coli*) and the regulation of secondary metabolism (59, 60). Although small phenotypic
330 changes were observed on solid media (Figure S3), it remained unclear the extent to which RsmQ
331 was involved in the sensing of specific carbon sources. The proteomic and phenotypic data
332 suggested a role for RsmQ in sensing and responding to a variety of carbon sources.

333 Carbon source utilisation was compared between plasmid free SBW25 cells and cells carrying
334 pQBR103^{Km} ±*rsmQ* using BioLog PM1 and PM2A plates, with NADH production being used as a
335 readout. Cells were grown on 190 different carbon sources to determine the metabolic changes
336 that occur with plasmid carriage. After 24 hours several differences were observed, with the
337 majority of changes relating to amino acid utilisation (Table S2 and Figure 7). The pQBR103^{Km}-
338 Δ *rsmQ* containing-strain showed decreased metabolism of L-alanine, L-aspartic acid and L-
339 arginine, each of which has also been found within root exudates (61) compared to SBW25
340 pQBR103^{Km}. Proteomic data suggests that RsmQ represses amino acid transportation and
341 metabolism in rich media conditions. However, when these are available as the sole carbon
342 source, they are more easily metabolised by cells that have RsmQ present, supporting the idea
343 that RsmQ is involved in the regulation of amino acid uptake and metabolism.

344 As well as this, the metabolism of other carbon sources typically found in plants, such as Hydroxyl-
345 L-Proline (62) and m-Tartaric acid (63) were impacted by the loss of RsmQ (Figure 7a). This, along
346 with the increase in D,L-malic acid (malate) metabolism suggest that there may be a link between
347 the carbon sources utilised by plasmid carriers and their plant host species (Figure 7a). This is
348 particularly interesting as malate is a major component of root exudates (64, 65), suggesting that
349 plasmid carriage may increase growth rate when in close proximity to plant roots.

350 To interrogate this further we selected a subset of carbon sources shown to be metabolised
351 differently by SBW25 containing pQBR103^{Km} \pm *rsmQ*. Both itaconate and malate were chosen as
352 interesting and biologically relevant candidates that are found within root exudates (66). In M9
353 minimal media all tested cells were able to metabolise itaconate, in contrast to the BioLog assays,
354 which was possibly a consequence of the different media bases used. While our M9 growth data
355 did not simply replicate the BioLog results, itaconate and malate metabolism were both affected
356 by *rsmQ* deletion. Curiously, RsmQ appeared to exert a biphasic effect on growth in the presence
357 of either carbon source (Figure 7b and c). Multiple growth curves showed a difference in growth
358 rate between pQBR103^{Km} and pQBR103^{Km}- Δ *rsmQ*. However, which of these genotypes had a
359 growth advantage (or penalty) constantly varied between different biological replicates (Figure
360 7b and c), suggesting that RsmQ may function to stabilise a bistable metabolic system, consistent
361 with its chromosomal counterpart (67).

362

363 Discussion

364 By encoding homologues of bacterial regulators plasmids can manipulate the expression of
365 chromosomal genes and thereby alter the behaviour and phenotype of their host bacterial cells
366 (4) . Previous studies of PCC have identified plasmid-encoded transcriptional regulators with
367 limited and specific regulons (1). Here we expand the known molecular mechanisms mediating
368 PCC to include a global post-transcriptional regulator, RsmQ. We show that RsmQ is a homologue
369 of the widespread Csr/Rsm family of translational regulators. RsmQ has only a minimal effect on
370 the *P. fluorescens* transcriptome but a large impact on the proteome both directly, through
371 binding mRNAs to control their translation, and indirectly, through interactions with components
372 of the cellular Rsm regulatory system. RsmQ alters the abundance of chemotaxis/motility and
373 metabolism related proteins, leading to observable growth differences in distinct carbon sources.

374 The soil surrounding plant roots is a complex and intensely competitive environment.
375 Rhizosphere-dwelling bacteria respond to their surroundings at an individual level using networks
376 of signalling proteins (68) that control bacterial behaviour, enabling effective colonisation and
377 environmental adaptation. Simultaneously, the distribution of genes in rhizosphere
378 metagenomes are under intense selection to best fit the prevailing environment. In recent years,
379 substantial progress has been made towards understanding both the regulatory pathways that

380 control bacterial rhizosphere colonisation (69), and the effect of environmental inputs on
381 microbiome composition and species' metagenomes (70–72). Horizontal gene transfer, for
382 example by conjugative plasmids, is well understood as an important driver of genetic adaptation
383 ((35, 73, 74)). However, the influence of plasmid encoded regulatory genes in bacterial signalling
384 and behaviour, and the importance of this process to bacterial fitness and evolution in the
385 rhizosphere is much less clear.

386 To address these questions, we first examined the distribution of *rsm*-family genes on plasmids in
387 the PROKKA database. Numerous plasmids were found to encode *rsm* homologs, although these
388 were not evenly distributed. Plasmids associated with some taxa, including Pseudomonadaceae,
389 frequently contained *rsm* genes, while those associated with others had none. This distribution
390 suggests that plasmid encoded Rsm proteins may fulfil important functional roles that are
391 associated with particular groups of microbes. Plasmid carriage of *rsm* regulators appears to be a
392 convergent trait that has emerged many times, potentially in response to similar environmental
393 pressures affecting different plasmids. Overall, our analysis suggests that diverse plasmids have
394 acquired *rsm* regulator genes from their bacterial hosts over time, and that these genes now
395 appear to be evolving distinctly from their chromosomally located ancestors. Interestingly, *rsm*
396 genes have also been identified on bacteriophage (38), suggesting that diverse mobile genetic
397 elements may exploit host post-transcriptional regulation to ultimately serve the fitness interests
398 of mobile genetic elements.

399 Next, to determine the functional role of plasmid borne Rsm regulators we examined the
400 pQBR103-encoded protein RsmQ, in the plant-associated rhizobacterium *P. fluorescens* SBW25.
401 Our data shows that RsmQ functions as a global, post-transcriptional regulator of metabolism,
402 nutrient transport and chemotaxis pathways. In this respect RsmQ-linked PCC differs markedly
403 from most recently described PCC systems, which involve altered transcription of a small number
404 of genes upon plasmid carriage, typically relating to one or two bacterial phenotypes (1). By
405 contrast, RsmQ-driven regulation occurs predominantly at a post-transcriptional level and is
406 global in scope, affecting the abundance of hundreds of proteins and extensively subverting
407 bacterial motility and metabolic pathways to the benefit of the plasmid. Our results implicate
408 RsmQ and by extension other, similar plasmid-borne regulators, in considerably more extensive
409 control of bacterial behaviour than has previously been observed.

410 In contrast to earlier studies (45), we saw relatively little effect of plasmid carriage or *rsmQ* on
411 SBW25 gene transcription. In itself, this finding is not particularly surprising as our test conditions
412 were chosen to interrogate RsmQ function and differed markedly from those used previously.
413 Nonetheless, this does suggest that the nature and extent of pQBR103 control of SBW25
414 transcription is highly dependent on the surrounding environment. Overall, this suggests that
415 RsmQ appears to reprogram chemotaxis and metabolism towards a more sessile, biofilm forming
416 lifestyle. This is consistent with the phenotypic data for pQBR103 carriage, where a reduction in
417 swarming motility is partially recovered by *rsmQ* deletion, as well as changes in both bacterial
418 growth in a carbon source dependent manner.

419 RsmQ functions in SBW25 by interacting with the host Gac/Rsm pathway in several different ways.
420 Firstly, RsmQ binds to specific RNA target sequences at conserved binding motifs in a similar
421 manner to chromosomally encoded Rsm proteins. RsmQ has been shown to have a binding
422 preference for both short (GGA) and extended (AnGGA) motifs, especially where these are
423 presented on the loops of ssDNA hairpins. To some extent, this RNA binding effect contributes to
424 RsmQ-mediated PCC by directly regulating gene targets through chromosomal mRNA binding. In
425 support of this, we confirmed direct RsmQ binding to ssDNA sequences corresponding to the
426 upstream regions of several RsmQ-regulated genes. Furthermore, the AnGGA RsmQ binding motif
427 is only found upstream of around half of RsmQ-affected SBW25 genes. Thus, direct mRNA binding
428 probably only accounts for a fraction of the observed RsmQ regulon.

429 At least part of the observed RsmQ regulon is likely the result of indirect proteomic adaptation in
430 response to changes induced by RsmQ. This has been shown previously for the translational
431 regulator Hfq in SBW25, where the direct targets of translational control do not correspond neatly
432 to the observed proteomic regulon (5, 55). Another, potentially direct route for RsmQ function is
433 through interaction with the host ncRNAs RsmZ and RsmY. RsmQ binds strongly to several RsmY/Z
434 motifs as shown by SPR, suggesting that similar binding takes place in the SBW25 cell. This binding
435 interaction could in-turn mediate pleiotropic changes in gene translation, either through RsmY/Z
436 titration of RsmQ away from its mRNA binding targets, or alternatively via a reduction in the
437 amount of RsmY/Z available to modulate the activity of the host Rsm proteins. Both of these
438 alternative, and potentially antagonistic mechanisms may function simultaneously to some

439 extent, with the abundance of RsmQ and RsmY/Z in the cell determining their relative importance
440 to cellular Rsm function.

441 In addition to its interaction with host RNAs, we also saw evidence for extensive Rsm protein
442 homodimerisation, as well as heterodimerisation between RsmQ and the host proteins RsmA and
443 RsmE. Rsm protein homodimerisation is a well-characterised trait, with structural evidence
444 supporting widespread homodimer formation in *Pseudomonas* spp. (21, 56, 75). However, to our
445 knowledge Rsm proteins have not previously been shown to form heterodimers. The mechanistic
446 consequences of RsmQ/A/I heterodimerisation are currently unknown. The simplest explanation
447 is that the three Rsm proteins are functionally and mechanistically interchangeable, and Rsm
448 heterodimers have no *in vivo* function, however this seems unlikely given their distinct regulons
449 (18). Alternatively, RsmQ may have an agonistic or antagonistic effect on the host Rsm proteins,
450 increasing or reducing their RNA binding affinity. Heterodimerisation may also influence mRNA
451 binding preference, shifting the global Rsm regulatory response towards an outcome that benefits
452 horizontal or vertical plasmid transmission, although this is yet to be determined.

453 The presence of multiple, interconnected layers of regulation make signalling phenotypes
454 challenging to interrogate in a laboratory setting. For example, Hfq is global master regulator of
455 translation (7, 55) and has an overlapping regulon with the Rsm proteins (76) as well as
456 circumstances in which Hfq and RsmA directly interact (77), adding further regulatory complexity.
457 Hfq controls multiple phenotypes in SBW25 (7), however the effects of Hfq dysregulation upon
458 *rsmQ* deletion appeared to be modest, particularly when compared to secondary Hfq control by
459 the RimABK system, for example (7, 78). An intriguing possibility is that the plasmid-borne Hfq
460 homologue pQBR0137, whose abundance increases in the absence of *rsmQ*, may compensate for
461 reduced chromosomal Hfq activity. The reasons behind any potential compensatory regulation
462 are unknown and the subject of active investigation, although it is striking that multiple plasmid
463 encoded regulators may in fact be functioning in a coordinated manner in SBW25, modulating
464 cellular responses to the environment at a global level.

465 Carriage of pQBR103^{Km} leads to an increase in biofilm formation and reduced motility, two Gac-
466 system associated traits. From the perspective of the plasmid this makes sense; tightly packed
467 biofilms are more likely to support plasmid transmission (4). These key phenotypes associated

468 with plasmid carriage were also seen upon *rsmQ* overexpression. However, while *rsmQ* deletion
469 from pQBR103 partially ameliorated the plasmid-induced loss of motility, we saw no effect on
470 biofilm, suggesting that this *rsmQ* overexpression phenotype may be non-specific. This could
471 potentially be explained by excess RsmQ binding to the *fleQ* mRNA, as FleQ is a master regulator
472 of *Pseudomonas* behaviour that controls both motility and biofilm formation (16, 79). Gac/Rsm is
473 a tightly controlled global regulatory system, with multiple links to other signalling pathways and
474 extensive in-built functional redundancy. Therefore, the relatively subtle phenotypic differences
475 seen upon *rsmQ* deletion under lab conditions are unsurprising.

476 The effects of RsmQ on SBW25 metabolism and growth were strikingly inconsistent. Across
477 repeated, independent assays *rsmQ* conferred either a growth advantage or a penalty, apparently
478 at random. This suggests that the outcome of assay was defined stochastically, and implies a
479 degree of bistability within the system. Regulatory bistability has been shown for several bacterial
480 systems (80, 81) and has been linked to Gac/Rsm (67, 76). It is possible therefore that the loss of
481 regulatory control by RsmQ has knock-on effects within the wider regulatory network, inducing
482 stochastic, bistable growth phenotypes compared to WT pQBR103^{Km}.

483 In conclusion, we propose that RsmQ functions as a global PCC regulator that both directly
484 controls host mRNA translation and interferes with the host Gac/Rsm pathway and the master
485 regulator Hfq. RsmQ activity remodels host metabolism and suppresses motility as part of a wider
486 PCC programme that directs *P. fluorescens* towards biofilm formation and a sessile lifestyle, where
487 horizontal plasmid transmission is likely to be favoured. We hypothesise that core elements of
488 this regulatory paradigm will be shared between diverse plasmid-borne Rsm proteins, with the
489 PCC regulon tuned in each case to best support plasmid fitness in the host environment. More
490 broadly, whereas plasmid accessory genes are often conceptualised as directly providing distinct,
491 novel functions such as toxin efflux or enzymatic degradation of metabolic substrates, our work
492 shows that plasmids might also find success, and indeed exert ecologically important effects, by
493 manipulating and tuning the expression of functions encoded by genes already resident within
494 the cell.

495 **Materials and Methods**

496 Strains and growth conditions

497 Strains and plasmids are listed in Table S3 and primers are listed in Table S4. Unless otherwise
498 stated *P. fluorescens* SBW25 were grown at 28°C and *E. coli* strains at 37°C in Lysogeny broth (LB
499 (82) solidified with 1.5% agar where appropriate. Liquid cultures were grown in 10 mL microcosms
500 at 28°C for *P. fluorescens* and 37°C for *E. coli* at 250 rpm unless otherwise stated. Minimal media
501 was made using M9 salts supplemented with 2 mM MgSO₄ and 0.1 mM CaCl₂ and each carbon
502 source present at 0.4%. For motility assays plates were solidified with 0.5% agar. Gentamicin
503 (Gent) was used at 25 µg ml⁻¹, Streptomycin (Strep) at 250 µg ml⁻¹, Kanamycin (Kan) at 50 µg ml⁻¹,
504 Carbenicillin (Carb) at 100 µg ml⁻¹, Tetracycline (Tet) at 12.5 µg ml⁻¹, IPTG at 1 mM and X-gal at 40
505 µg ml⁻¹.

506 Molecular biology procedures

507 Cloning was carried out in accordance with standard molecular biology techniques. All pTS1
508 plasmid inserts were synthesised and cloned into pTS1 by Twist Bioscience. The ORF of *rsmQ* was
509 amplified by PCR with primers RsmQ_EcoRI_F and RsmQ_XhoI_R and ligated between the EcoRI
510 and XhoI sites of pME6032. The ORF of *rsmQ* with a TEV cleavage site and a hexahistidine tag at
511 the C-terminus was synthesised by Twist Bioscience. The ORFs of each Rsm protein were amplified
512 by PCR using the primers indicated in Table S3. The fragment in each case was cloned between
513 the NdeI and XhoI sites of pET28a. Bacterial-2-hybrid plasmids were made by Gibson assembly
514 (RsmE/I) and restriction cloning into the BamHI and EcoRI sites of pKNT25 and pUT18C.

515 Transformation of *Pseudomonas* strains

516 Overnight cultures of each strain were grown in LB media at 28°C, 250 rpm shaking then harvested
517 at 6000 *xg* for 8 minutes. Cell pellets were washed twice with 0.3 M sucrose, then the pellet was
518 resuspended in a final volume of 150 µL and placed in a 2 mm electroporation cuvette with either
519 2 µL of replicative plasmids or 5 µL of integrative plasmids (60-100 ng/µL concentration) and
520 incubated at RT for 2 minutes. Cells were electroporated at 2.5 kV and recovered in 3 mL LB
521 medium at for 3 hours before being plated onto LB agar containing the appropriate antibiotic.
522 Plates were incubated for 24-48 hours at 28°C and transformed colonies re-streaked onto fresh
523 selective media.

524 Conjugations of pQBR103^{Km}

525 Donor (*P. fluorescens* SBW25 Ω StrepR-LacZ + pQBR103^{Km} and *P. fluorescens* SBW25 Ω StrepR-LacZ
526 + pQBR103^{Km} Δ rsmQ) and recipient strains (*P. fluorescens* SBW25 Ω GentR WT) were plated onto
527 their respective selective antibiotics. Overnight cultures were set up in LB medium for each of the
528 strains and grown overnight. 10 mL glass microcosms of Kings Broth (KB) medium were inoculated
529 with 20 μ L of the donor strain and 80 μ L of the recipient strain and incubated overnight without
530 shaking. 50 μ L of this overnight culture was plated onto LB medium supplemented with Gent and
531 Kan.

532 Allelic Exchange

533 Deletion constructs were created by Twist bioscience and extracted from *E. coli* DH5- α cells. *P.*
534 *fluorescens* cells were transformed as above and incubated for 48 hours until colonies appeared.
535 Colonies were re-streaked to single colonies on fresh LB-Tet agar and incubated for 24 hours. A
536 single colony was picked and grown overnight in 50 mL of LB medium (containing Kanamycin for
537 pQBR103^{Km} allelic exchange). The culture was serially diluted, and the 10⁻⁵ to 10⁻⁸ dilutions were
538 plated onto LB agar plates containing 10% sucrose (and kanamycin for pQBR103^{Km} allelic
539 exchange). Single colonies on sucrose plates were checked for tetracycline sensitivity and
540 confirmed as mutants by PCR.

541 Swarming motility assays

542 Motility plates were made with 20 mL of sterile 0.5% agar in M9 GC (M9 minimal media
543 supplemented with 0.4% Glucose and 0.4% Casein amino acids) media unless otherwise indicated
544 and dried for 1 hour in a laminar flow hood, rotated 180 degrees after 30 minutes. 3 μ L of an
545 overnight culture adjusted to an OD₆₀₀ = 1 was spotted onto the centre of the plate and the lid
546 replaced. Plates were incubated face up at room temperature for 72 hours undisturbed and then
547 imaged. For overexpression strains, filter sterilised 0.5 mM IPTG and tetracycline were added to
548 the induced samples.

549 Congo red binding assays

550 Cultures of each strain were grown overnight in LB microcosms with selection. 10 μ l spots were
551 placed onto 20 mL KB agar plates and incubated for 72 hours. For overexpression strains, filter

552 sterilised 0.5 mM IPTG and tetracycline were added to the induced samples. Colonies were
553 removed from the plate and placed into 1 mL of 0.003% sterile Congo red solution and incubated
554 at 37°C, 200 rpm shaking for 2 hours. Cell material was removed by centrifugation (13,000 xg for
555 5 minutes) and absorbance was measured at 490 nm using a SPECTROstar nano plate reader
556 (BMG).

557 BioLog carbon source screening

558 Two colonies were picked from freshly streaked LB plates and resuspended into IF-0 inoculating
559 fluid as per the manufacturer's instructions. PM1 and PM2A plates were inoculated with 100 µl
560 of inoculum and incubated at 28°C for 24 hours. Plates were imaged and the absorbance at 590
561 nm was read on an EON microplate reader.

562 Growth rate assays

563 Cultures of each strain were grown overnight in LB microcosms with selection. Cells were
564 harvested at 8,000 xg and washed in M9 media without a carbon source twice. Cells were then
565 resuspended in M9 media with each carbon source (0.4% w/v) at a starting OD₆₀₀ of 0.01 in a 96-
566 well plate. Measurements were taken every 30 minutes for 40 hours on a FLUOstar nano plate
567 reader (BMG) with the plate being incubated at 28°C and shaken for 2 seconds before each
568 reading.

569 Bacterial 2 hybrid assays

570 The ORF of RsmA/E/I/Q were cloned into pKNT25 and pUT18C using either conventional
571 restriction enzyme cloning or Gibson assembly using standard manufacturers protocols as
572 indicated in Table S4. Chemically competent BTH101 cells were co-transformed with both a pUT18
573 and a pKNT25 plasmid containing the ORF of the protein of interest using the heat shock method.
574 Briefly, cells were incubated on ice with the plasmids for 30 minutes, followed by a 45s incubation
575 at 42°C followed by 5 minutes on ice. Cells were recovered in 6 volumes of SOC media for one
576 hour and plated onto LB agar supplemented with Carb, Kan and 0.5 mM IPTG. 5 mL LB+Carb+kan
577 microcosms were grown overnight at 28°C. 100 µL of this overnight culture was used for the beta-
578 gal assay and 5 µL spots were placed onto LB+Carb+Kan+X-gal+IPTG plates and incubated
579 overnight.

580 β -galactosidase assays

581 *E. coli* BTH101, 5 mL microcosms of cells carrying both pUT18C and pKNT25 plasmids were grown
582 at 28°C. 100 μ L of this was taken and incubated with 900 μ L lysis buffer (60 mM Na₂HPO₄·7H₂O,
583 40 mM NaH₂PO₄·H₂O, 10 mM KCl, 1 mM MgSO₄, 7.7 mM β -mercaptoethanol, 0.001% SDS) and
584 20 μ L chloroform at 28°C for >10 minutes until cells are lysed. 200 μ L of 4 mg mL⁻¹ ONPG was
585 added, and samples monitored until the substrate had turned yellow. To stop the reaction 500 μ L
586 of 1M Na₂CO₃ was added, and the absorbance was taken at 420 and 550 nm using a FLUOstar
587 plate reader (BMG) and OD₆₀₀ of each sample was measured using a spectrophotometer. The
588 Miller units were calculated using the standard calculation (82).

589 Protein purification

590 The ORF of *rsmQ* was synthesised with a C-terminal TEV cleavage site extension and a 6x Histidine
591 tag (Twist bioscience) and cloned into pET29a between the NdeI and XhoI sites. The plasmid was
592 transformed into BL21(DE3) pLysS (Promega) by heat shock. 2.5 L of culture was inoculated at
593 1:50 from an overnight culture and grown until mid-log phase (OD₆₀₀ = ~0.6). Cultures were
594 induced with 1 mM IPTG and grown for 16 hours at 37°C. Cells were harvested at 6000 xg , 4°C for
595 15 minutes and resuspended in binding buffer (20 mM Tris-HCl, 500 mM NaCl, 10 mM imidazole,
596 5% glycerol, pH 7.5) containing 1 mg ml⁻¹ lysozyme (sigma), 1 complete protease inhibitor tablet
597 EDTA-free and 5 μ L DNaseI (Promega), lysed using a cell disruptor and the insoluble fraction
598 removed by centrifugation (15,000 xg , 25 minutes, 4°C). The soluble fraction was loaded onto a
599 HisTrap HP 5 mL column (Cytivia) and washed with binding buffer (20 mM Tris-HCl, 500 mM NaCl,
600 2.5% glycerol, pH 7.5) with 50 mM Imidazole to remove non-specific contaminants. Proteins were
601 eluted over a gradient of 50-500 mM Imidazole and fractions analysed by SDS-PAGE.

602 Fractions containing RsmQ were dialysed overnight at 4°C into SEC buffer (50 mM Tris-HCl, 200
603 mM NaCl, 2.5% Glycerol, pH 7.5) and further purified by size exclusion chromatography using an
604 Superdex S75 column (Cytivia). Fractions were analysed by SDS-PAGE and pure fractions were
605 concentrated to 3 mg ml⁻¹ and stored at -80°C until needed.

606 For H43A and R44A plasmids were created by site-directed mutagenesis PCR using overlapping
607 primers (RsmQ_H43A_F, RsmQ_H43A_R, RsmQ_R44A_F, RsmQ_R44A_R), confirmed by
608 sequencing and purified as for the WT.

609 Surface plasmon resonance

610 Single stranded DNA oligos (ssDNA) with the ReDCaT linker region at the 3' end were synthesised
611 by IDT and diluted to a final concentration of 1 mM in HBSEP+ buffer (10 mM HEPES, 150 mM
612 NaCl, 3 mM EDTA and 0.05% v/v Tween 20, pH 7.4). All primer sequences can be found in Table
613 S4 (ReDCaT). RsmQ, RsmQ H43A and RsmQ R44A were diluted to 1000 and 100 nM concentrations
614 in HBSEP+ buffer. ssDNA oligos were synthesised (IDT) and diluted to a final concentration of 1 μ M
615 in HBSEP+. SPR measurements were recorded at 20 °C using a Biacore 8k system using a ReDCaT
616 SA sensor chip (GE Healthcare) with 8 immobilised channels as described in (43). RsmQ interaction
617 was first analysed using an affinity method to examine presence and absence of binding to each
618 of the oligos.

619 RNAseq

620 5 independent conjugations were set up for each biological replicate as described above. A single
621 colony from each conjugation event was picked and grown overnight in 10 mL KB medium with
622 Gent and Kan at 28°C, 230 rpm. The OD₆₀₀ of these cultures was measured and 60 mL KB cultures
623 were set up at OD₆₀₀ = 0.01. Cultures were grown at 28°C, 230 rpm until OD₆₀₀ = 1.4. 2 mL of this
624 culture was harvested for RNA extraction and 50 mL was taken for whole proteome analysis.
625 Pellets were collected at 8000 *xg*, 10 minutes at 4°C and flash frozen in liquid nitrogen before
626 storage at -80°C.

627 For RNAseq the pellets were resuspended in 150 μ L 10 mM Tris-HCl pH 8 and mixed with 700 μ L
628 of ice cold RLT+BME (RLT buffer (Qiagen) supplemented with 1% β -mercaptoethanol) and cells
629 were lysed using a Fastprep (MP Bio) using Lysis matrix B beads (MP Bio). Lysis matrix was
630 removed by centrifugation (13,000 *xg*, 3 minutes) and the supernatant was added to 450 μ L of
631 ethanol. The supernatant was applied to a RNeasy column and RNA extraction was performed as
632 per the manufacturer's instruction including the on-column DNA digest. After extraction a Turbo
633 DNase (Promega) digest was performed as per the manufacturer's instruction and total RNA yield
634 was quantified using a Qubit RNA broad spectrum assay kit as per the manufacturer's instructions.
635 Library construction, rRNA depletion and paired-end Illumina sequencing (Novaseq 6000, 2x150
636 bp configuration) was performed by Novogene. Reads provided by Novogene (as fastq.gz files)
637 were mapped to the genome of *Pseudomonas fluorescens* (NCBI accession AM181176.4) and the

638 plasmid pQBR103 (NCBI accession AM235768.1), using the "subread-align" command of the
639 Subread package (83). The resulting .bam files were then sorted and indexed using the
640 appropriate functions from the Samtools package (84). A custom Perl script was used to make a
641 saf file for all the gene in the genome and the plasmid. The "featureCounts" tool of the Subread
642 package was used to count the reads mapping to every gene. The counts were read into a DGEList
643 object of the Bioconductor package edgeR and a quasi-likelihood negative binomial generalized
644 log-linear model was fitted to the data using the "glmQLFit" function of edgeR(85). Genewise
645 statistical tests were conducted using the "glmQLFTest" function of edgeR. Finally, the "topTags".
646 Processed data is deposited in ArrayExpress (E-MTAB-11868).

647 Whole proteome analysis

648 Cells were grown as above and stored at -80°C until the proteome was extracted. Three samples
649 were thawed on ice and resuspended in ice cold 500 µL Lysis buffer (20 mM Tris, 0.1 M NaCl, pH
650 8 + 1 complete protease inhibitor tablet). Cells were lysed by sonication at 12 mA (1 second on, 3
651 seconds off for 20 cycles). Insoluble material was removed by centrifugation at 4°C, 4000 xg, 10
652 minutes. The supernatant was taken and the proteome precipitated for 10 minutes with the
653 addition of 8 volumes of acetone at RT. The proteome was pelleted by centrifugation at 7000 xg
654 for 10 minutes and washed once more with acetone. Protein pellets were resuspended in 400 µl
655 of 2.5% sodium deoxycholate (SDC; Merck) in 0.2 M EPPS-buffer (Merck), pH 8.5, and vortexed
656 under heating for a total of three cycles. Protein concentration was estimated using a BCA assay
657 and approx. 200 µg of protein per sample was reduced, alkylated, and digested with trypsin in the
658 SDC buffer according to standard procedures. After the digest, the SDC was precipitated by
659 adjusting to 0.2% TFA, and the clear supernatant subjected to C18 SPE (OMIX tips; Agilent).
660 Peptide concentration was further estimated by running an aliquot of the digests on LCMS (see
661 below). TMT labelling was performed using a TMTpro™ 16plex kit (Lot WB314804, Thermofisher
662 Scientific) according to the manufacturer's instructions with slight modifications; approx. 100 µg
663 of the dried peptides were dissolved in 90 µl of 0.2 M EPPS buffer (MERCK)/10% acetonitrile, and
664 250 µg TMT reagent dissolved in 22 µl of acetonitrile was added. Samples were assigned to the
665 TMT channels.

666 After labelling, aliquots of 1.5 µl from each sample were combined in 500 µl 0.2% TFA, desalted,
667 and analysed on the mass spectrometer (see below) to check labelling efficiency and estimate

668 total sample abundances. The main sample aliquots were then combined correspondingly to
669 roughly level abundances and desalted using a C18 Sep-Pak cartridge (200 mg, Waters). The eluted
670 peptides were dissolved in 300 μ l 0.1% TFA and fractionated with the Pierce™ High pH Reversed-
671 Phase Peptide Fractionation Kit (Thermo) according to the manufacturer. Fractions for the mass
672 spectrometry analysis were eluted sequentially with the following concentrations of acetonitrile:
673 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, 30%, 40%, 50% and dried down and resuspended in 0.1 %
674 TFA, 3% acetonitrile.

675 Aliquots were analysed by nanoLC-MS/MS on an Orbitrap Eclipse™ Tribrid™ mass spectrometer
676 coupled to an UltiMate® 3000 RSLCnano LC system (Thermo Fisher Scientific, Hemel Hempstead,
677 UK). The samples were loaded onto a trap cartridge (PepMap 100, C18, 5 μ m, 0.3x5mm, Thermo)
678 with 0.1% TFA at 15 μ l min⁻¹ for 3 min. The trap column was then switched in-line with the
679 analytical column (nanoEase M/Z column, HSS C18 T3, 1.8 μ m, 100 Å, 250 mm x 0.75 μ m, Waters)
680 for separation using the following gradient of solvents A (water, 0.1% formic acid) and B (80%
681 acetonitrile, 0.1% formic acid) at a flow rate of 0.2 μ l min⁻¹ : 0-3 min 3% B (parallel to trapping); 3-
682 10 min linear increase B to 8 %; 10-90 min increase B to 37%; 90-105 min linear increase B to 50
683 %; followed by a ramp to 99% B and re-equilibration to 3% B. Data were acquired with the
684 following parameters in positive ion mode: MS1/OT: resolution 120K, profile mode, mass range
685 m/z 400-1800, AGC target 100%, max inject time 50 ms; MS2/IT: data dependent analysis with
686 the following parameters: top10 in IT Rapid mode, centroid mode, quadrupole isolation window
687 0.7 Da, charge states 2-5, threshold 1.9e4, CE = 30, AGC target 1e4, max. inject time 50 ms,
688 dynamic exclusion 1 count for 15 sec mass tolerance of 7 ppm; MS3 synchronous precursor
689 selection (SPS): 10 SPS precursors, isolation window 0.7 Da, HCD fragmentation with CE=50,
690 Orbitrap Turbo TMT and TMTpro resolution 30k, AGC target 1e5, max inject time 105 ms, Real
691 Time Search (RTS): protein database *Pseudomonas fluorescens* SBW25 (uniprot.org, 02/2016,
692 6388 entries), enzyme trypsin, 1 missed cleavage, oxidation (M) as variable, carbamidomethyl (C)
693 and TMTpro as fixed modifications, Xcorr = 1, dCn = 0.05.

694 The acquired raw data were processed and quantified in Proteome Discoverer 2.4.1.15 (Thermo)
695 using the incorporated search engine Sequest HT and the Mascot search engine (Matrix Science,
696 London, UK; Mascot version 2.8.0). The processing workflow included recalibration of MS1
697 spectra (RC), reporter ion quantification by most confident centroid (20 ppm), fasta databases P.

698 fluorescens SBW25 (as for RTS) and common contaminants, precursor/fragment tolerance 6
699 ppm/0.6 Da, enzyme trypsin with 1 missed cleavage, variable modification was oxidation (M),
700 fixed were carbamidomethyl (C) and TMTpro 16plex. The consensus workflow included the
701 following parameters: unique peptides (protein groups), intensity-based abundance, TMT channel
702 correction values applied (WB314804), co-isolation/SPS matches thresholds 50%/70%,
703 normalisation on total peptide abundances, protein abundance-based ratio calculation, missing
704 values imputation by low abundance resampling, two or three replicates per sample (non-nested),
705 hypothesis testing by t-test (background based), adjusted p-value calculation by BH-method.
706 Experimental data is deposited in ProteomeXChange (PXD033640).

707 Bioinformatics and sequence analysis

708 The COMPASS database (37) was downloaded from <https://github.com/itsmeludo/COMPASS> and
709 annotated using PROKKA 1.14.6 (86) using the default settings. PROKKA uses BLAST+ matches with
710 the curated UniProtKB (SwissProt) databases to annotate proteins, followed by hidden Markov
711 model (HMM)-based searches. To compare chromosomal and plasmid *csrA/rsmA* genes,
712 chromosomal sequences associated with the COMPASS plasmids were identified and downloaded
713 using NCBI **elink** and **efetch** tools and were reannotated using PROKKA to ensure comparability
714 between plasmid and chromosomal sequences. *csrA/rsmA* sequences which were identical at the
715 nucleic acid level were removed before conducting analyses (there were no identical sequences
716 between chromosomes and plasmids). Start codons were unified across CsrA/RsmA homologues
717 by manual examination and sequence editing. Sequences were aligned by codon alignment in
718 PRANK v.170427 (87) using the default settings. Initial alignments had a high proportion (>70%)
719 of gaps owing to sequence divergence towards the 3' end of the gene, which interfered with
720 phylogenetic analysis. Trees were therefore built using information from conserved sites, by
721 removing columns from the alignment that consisted of majority (>60%) gaps. Duplicate
722 sequences were removed. Trees were estimated using RAxML 8.2.12 (88) with the settings **-f a -**
723 **m GTRCAT -p 12345 -x 12345 -# 100**. Qualitatively similar outcomes were obtained if gap columns
724 and/or duplicate sequences were retained. Jukes-Cantor distance matrices were extracted from
725 the alignments for analysis using the EMBOSS 6.6.0.0 and distances were compared using
726 Bonferroni-corrected Wilcoxon tests. The script CSRA_TARGET.pl (89) was adapted to predict
727 binding sites for *csrA/rsmA* in 5' untranslated regions of the PROKKA-predicted plasmid genes,

728 and differences in distributions of number of sites/CDS were compared between *csrA/rsmA*-
729 encoding and non-encoding plasmids using two-sample Kolmogorov-Smirnov tests. Analyses were
730 performed in R (4.1.2, R Core Team, Vienna, Austria), bash, and Python 3.6 within the RStudio IDE
731 (RStudio Team, Boston, USA) with the assistance of tidyverse (90) and ggtree (91) packages.
732 Example scripts and analyses can be found at www.github.com/jpjh/PLASMAN_RsmQ

733

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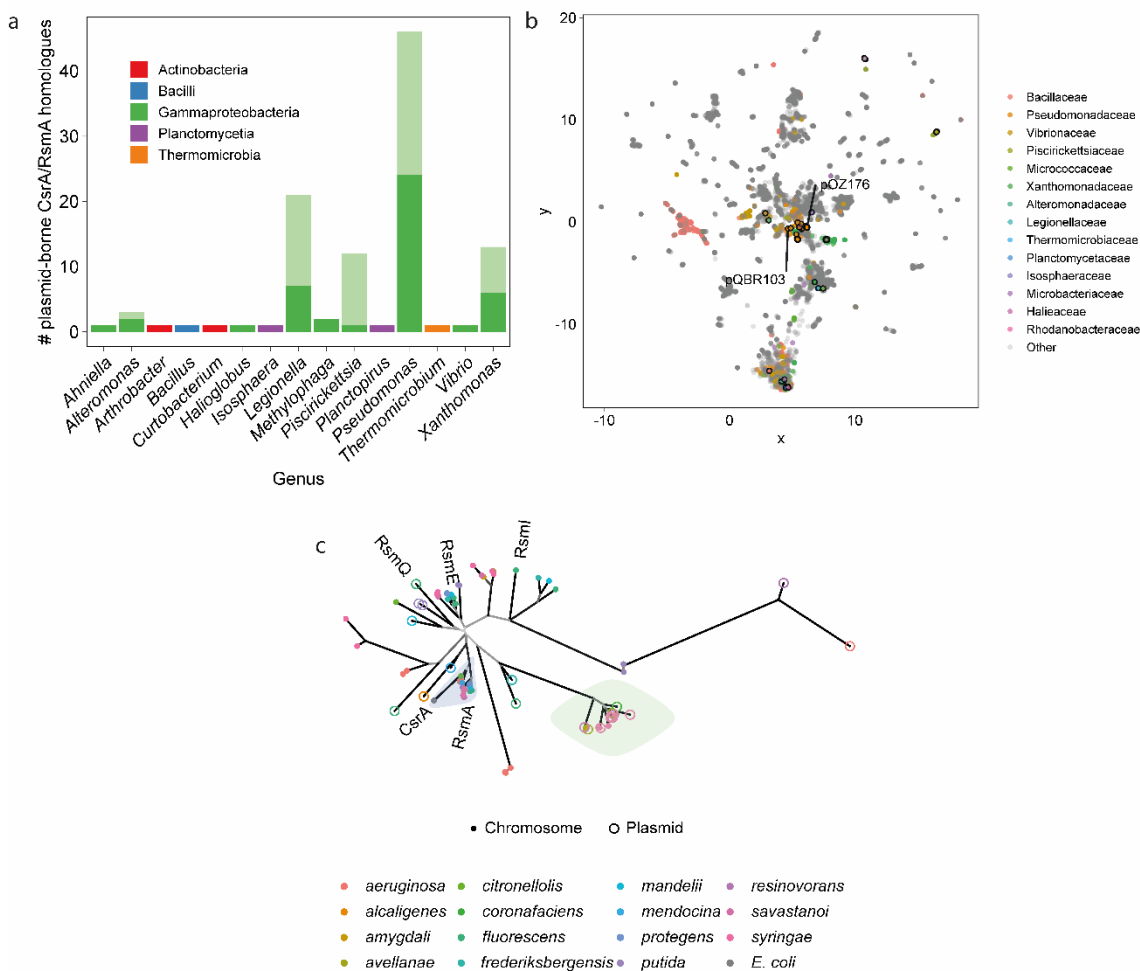
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968 **Figures and Tables**



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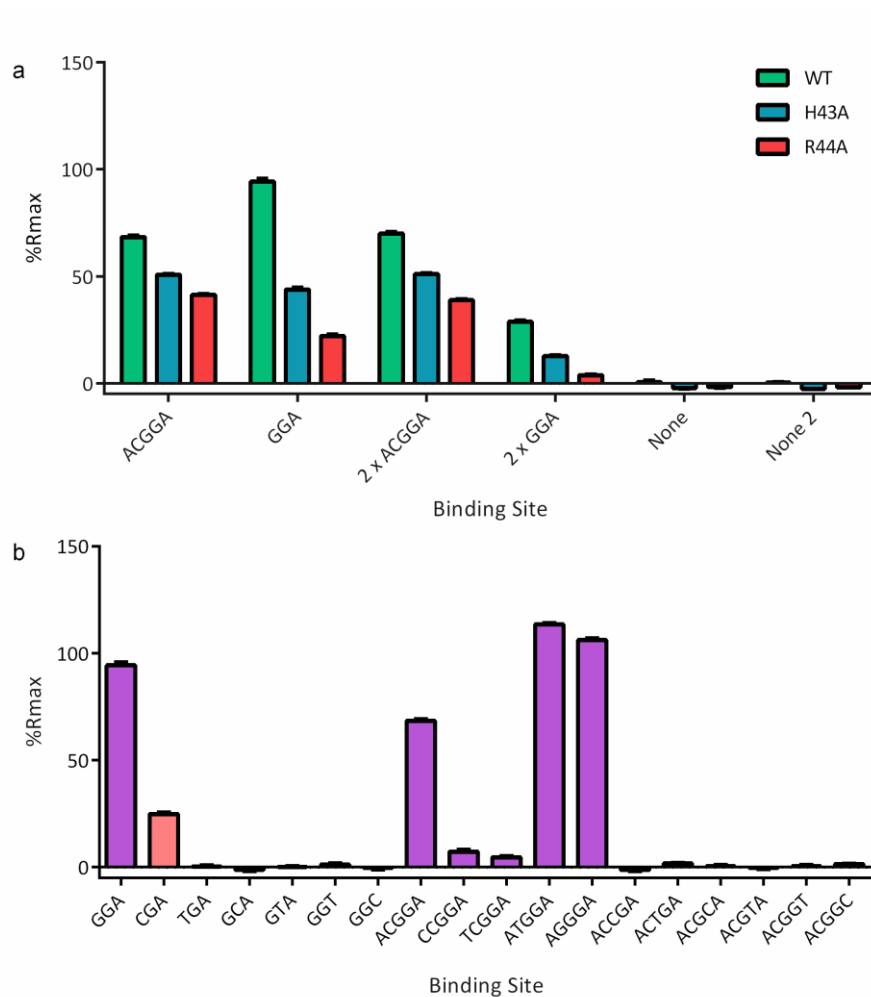
970 **Figure 1. RsmQ is found on a wide range of conjugative plasmids** a) Taxonomic distribution
 971 of plasmid borne *csrA/rsmA* homologues identified in COMPASS. The paler part of each stacked
 972 bar indicates genes that were identical at the nucleotide level to other identified homologues. b)
 973 COMPASS plasmid diversity represented by non-metric multidimensional scaling of MASH
 974 sequence distances. Families with ≥ 1 plasmid with a *csrA/rsmA* homologue are coloured according
 975 to the legend. Plasmids encoding *csrA/rsmA* homologues are outlined in black. Selected plasmids
 976 from various taxa are annotated. c) Unrooted phylogenetic tree of *Pseudomonas csrA/rsmA*
 977 homologues from COMPASS, with corresponding chromosomal homologues (where available)
 978 and genes from selected reference strains. Branches leading to nodes with $>80\%$ bootstrap
 979 support are coloured black, with decreasing support indicated with increasingly pale grey. *P.*
 980 *fluorescens* SBW25 *csrA/rsmA* homologues are labelled, as is pQBR103 *rsmQ*, and *E. coli* *csrA*. The

981 blue highlight indicates a well-supported (bootstrap support 0.84) group of *rsmA*-like
982 homologues. The green highlight indicates the group of related plasmid and chromosomal genes
983 from plant pathogen *Pseudomonas* discussed in the text.

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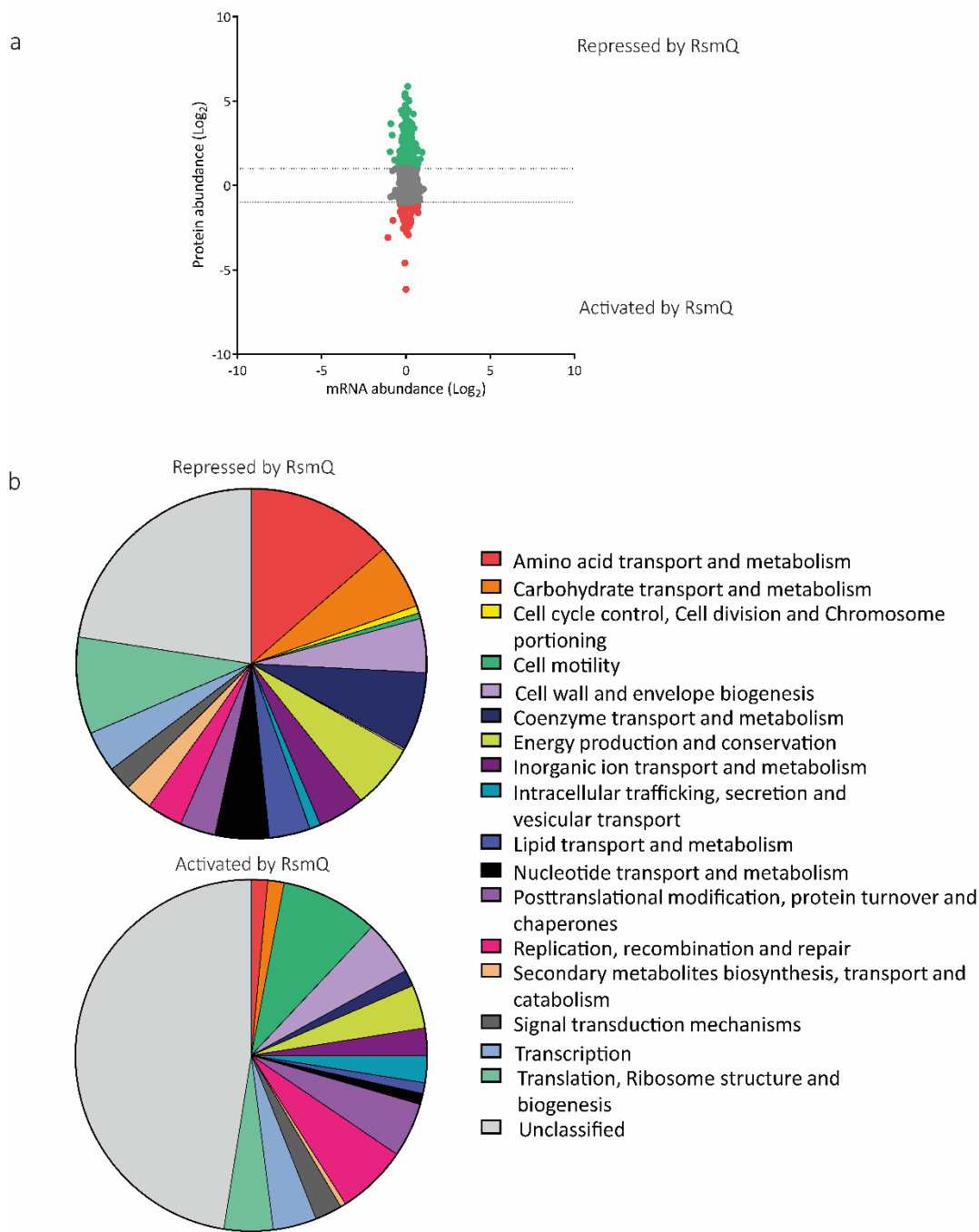
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989 **Figure 2: RsmQ interacts with its preferred binding sequence (GGA/AnGGA) and this interaction**
 990 **is mediated by the VHRE/D binding site.** a) Percentage R_{max} values for RsmQ WT (green) H43A
 991 (blue) and R44A (red) binding to ssDNA containing the binding sites shown above. b) Percentage
 992 R_{max} values for WT RsmQ binding to ssDNAs containing the above binding site. Binding sites that
 993 are predicted to bind RsmQ are shown in purple. All oligos are designed as hairpins and results
 994 shown are for RsmQ at a concentration of 100 nm.

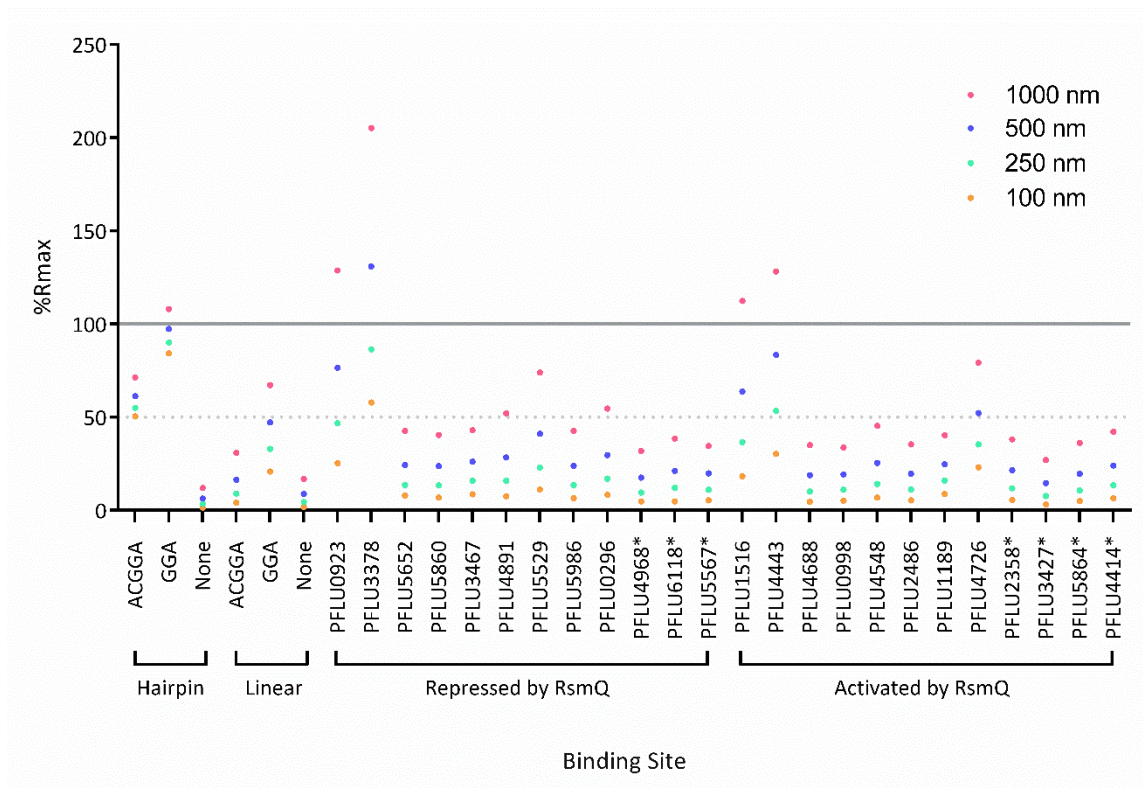
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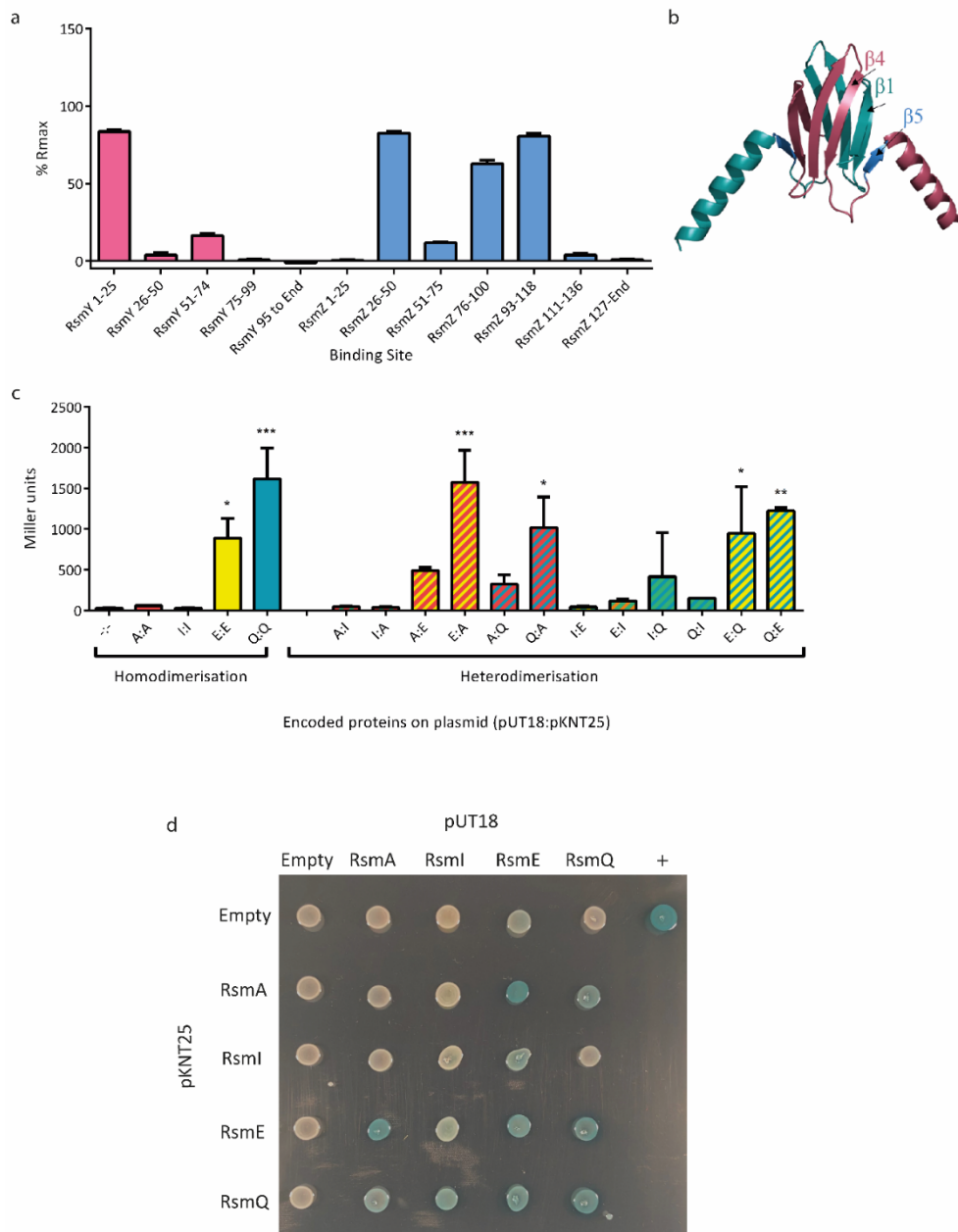
998 **Figure 3: The loss of RsmQ causes widescale proteomic changes.** a) Comparative scatter plot
999 comparing log_2 -fold mRNA abundance changes from RNAseq ($n=5$) to protein abundance changes
1000 seen by TMT quantitative proteomics ($n=3$). b) COG categories of proteins that showed a greater
1001 than log_2 -fold change when *rsmQ* is lost (Repressed by RsmQ = 581, Activated by RsmQ = 203).



1002

1003 **Figure 4: RsmQ binds to the upstream regions of predicted mRNA targets.** Purified RsmQ was
 1004 tested against ssDNA oligos with synthetic oligos run as a control. Oligos are labelled as in table
 1005 S4, with the genetic identifiers used to indicate the binding sites associated with those ORFs.
 1006 Genes annotated with a * indicate ORFs that contain a simplified GGA binding site but are not
 1007 predicted to have a full Rsm (AnGGA) binding site within 500 bp upstream or the first 100 bp of
 1008 the ORF. Percentage R_{max} values are shown for four concentrations. A solid line indicates 100%
 1009 R_{max} and a dotted line for 50% R_{max}. 100% R_{max} suggests 1:1 ligand:protein binding as the
 1010 experimentally acquired response is equal to the predicted response, with a 50% R_{max} suggesting
 1011 a weaker interaction or a 2:1/1:2 response.

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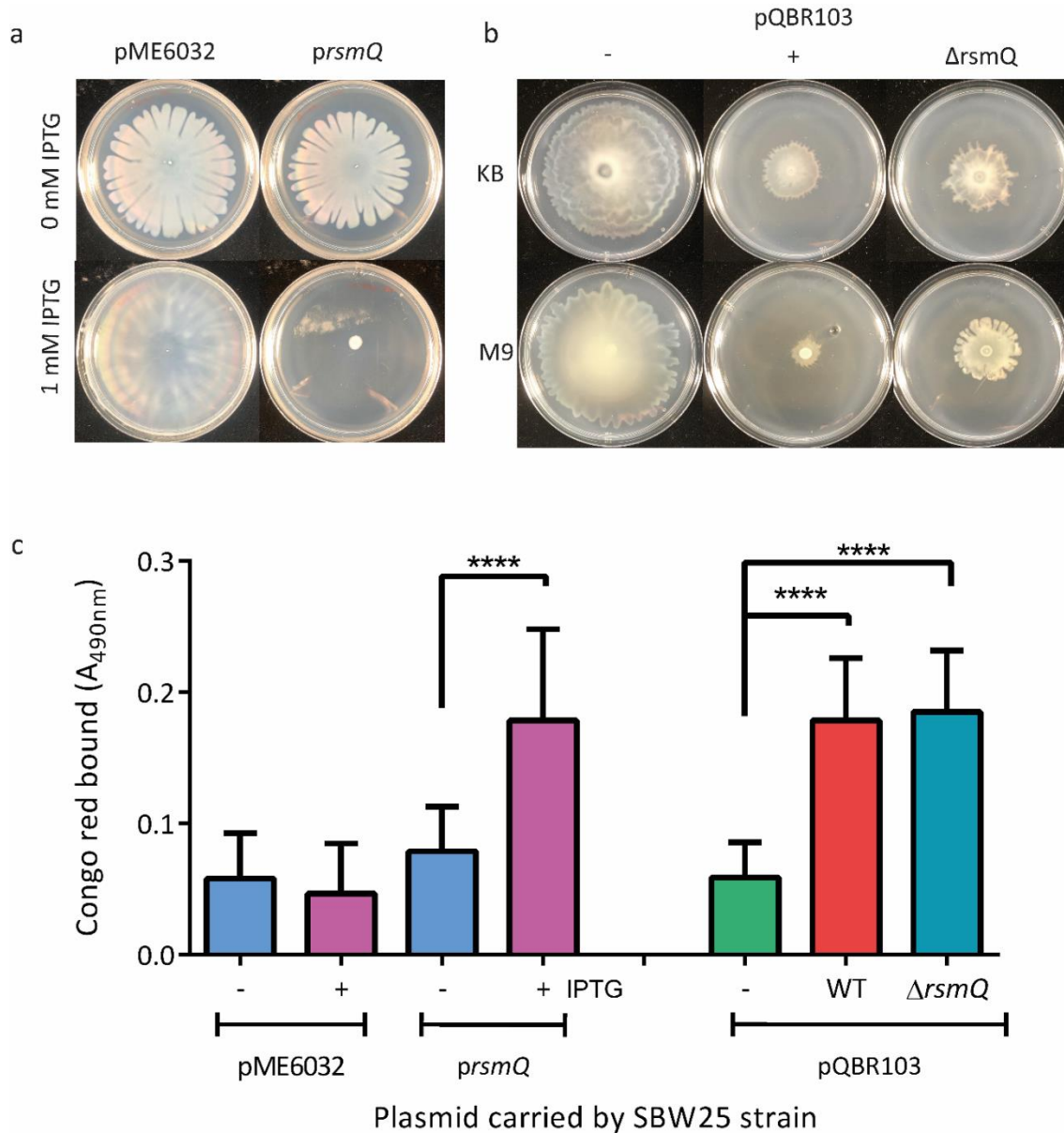


1013

1014 **Figure 5: RsmQ can both homo- and heterodimerise.** a) Percentage R_{max} values for RsmQ binding
 1015 to portions of the ncRNAs RsmY (pink) and RsmZ (blue) showing preferential binding to ssDNAs
 1016 that contained the binding site in a hairpin loop. b) AlphaFold model of RsmQ suggests that it
 1017 forms homodimers (monomers shown in contrasting colours), with the RNA-binding region
 1018 highlighted in marine (B5). c) Quantitative bacterial-2-hybrid β -galactosidase assays showing
 1019 interactions between pUT18c and pKNT25 fusions are shown for RsmA (A), RsmE (E), RsmI (I) and
 1020 RsmQ (Q). Results were analysed by a one-way ANOVA ($p < 0.0001$) with comparisons against the

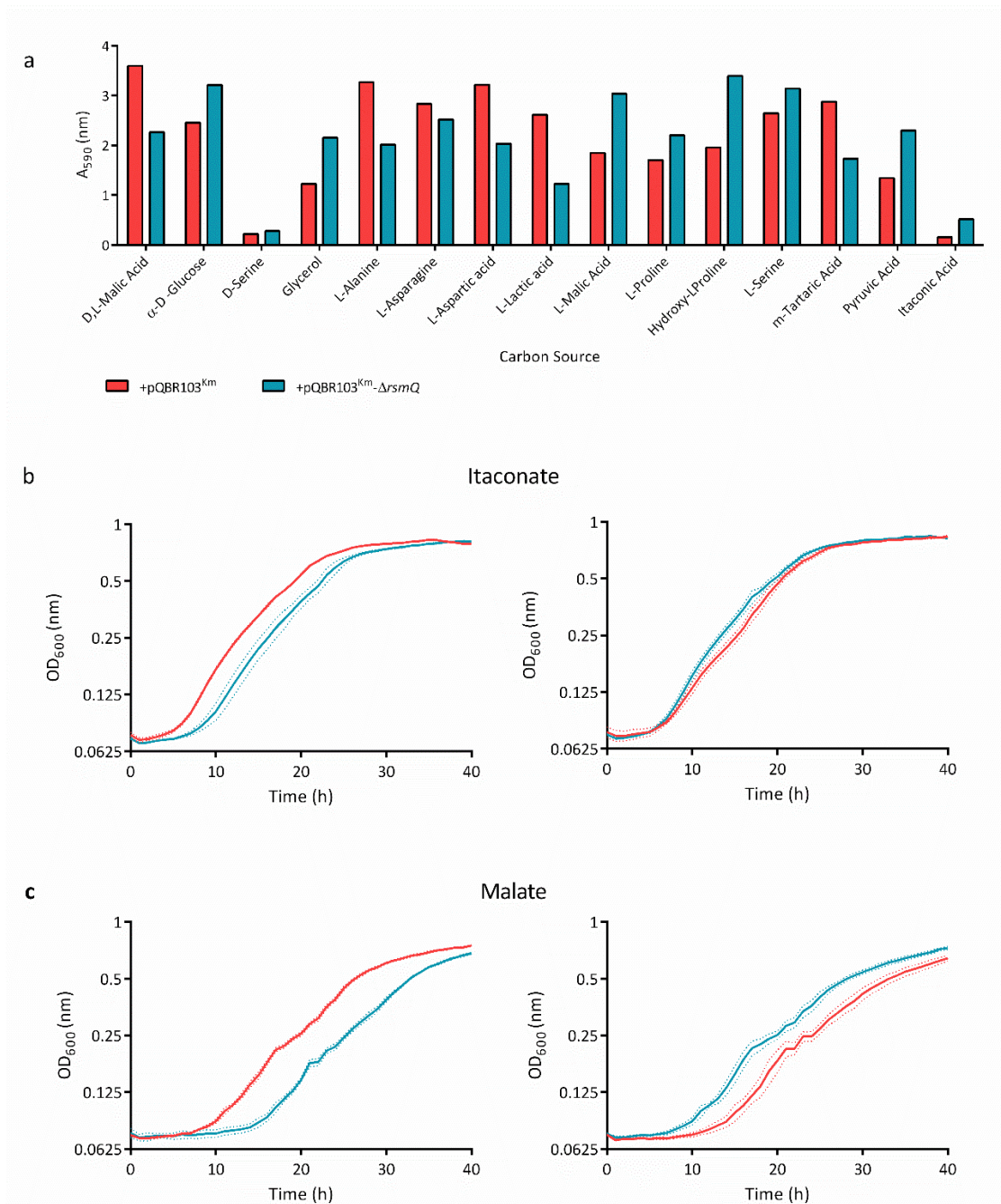
1021 Empty plasmid control (-:-) indicated (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Additional
1022 controls are shown in Figure S4. d) Representative image of qualitative β -galactosidase assays on
1023 agar plates. pKT25 fusions are shown in rows and pUT18c fusions in columns, with the indicated
1024 protein/empty vector present in each case.

1025



1026

1027 Figure 6: **Motility and biofilm formation are impacted by RsmQ.** A) 48h swarming motility assays
 1028 for SBW25 containing pME6032 +/- *rsmQ*. B) 72h swarming motility assays for SBW25 cells either
 1029 plasmid free (-), or carrying pQBR103^{Km} (+) or pQBR103^{Km}- $\Delta rsmQ$ grown on 0.5% agar plates with
 1030 media as indicated. C) Congo red absorbance (A_{490}) of SBW25 strains after 48 hours (light blue,
 1031 pink bars) or 72 hours (green, red, dark blue bars). ANOVA results show statistically significant
 1032 differences for both overexpression ($p < 0.0001$) and deletion ($p < 0.0001$). Statistical significance
 1033 from multiple comparisons is indicated ($p < 0.0001$, ****).

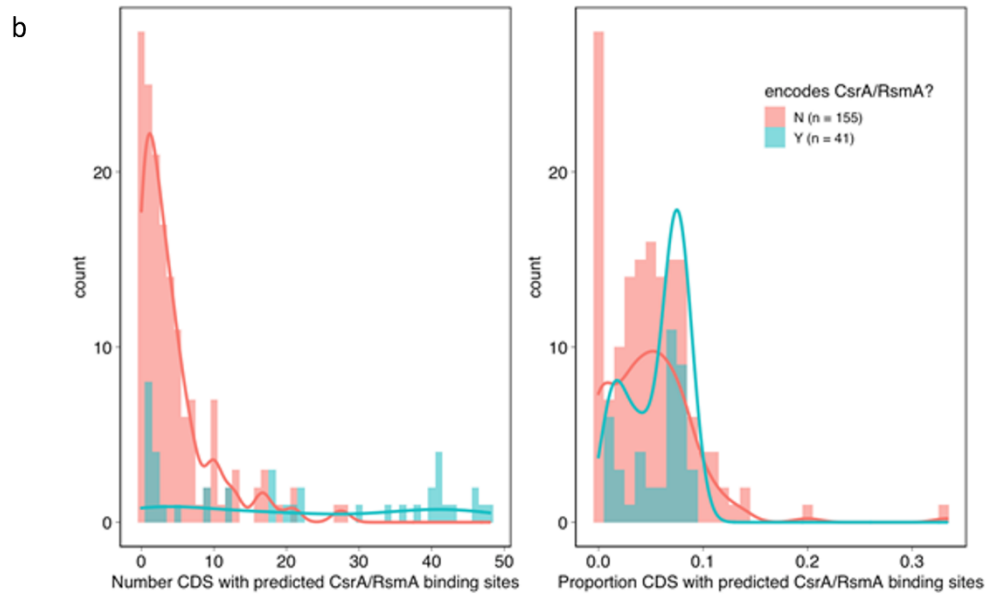
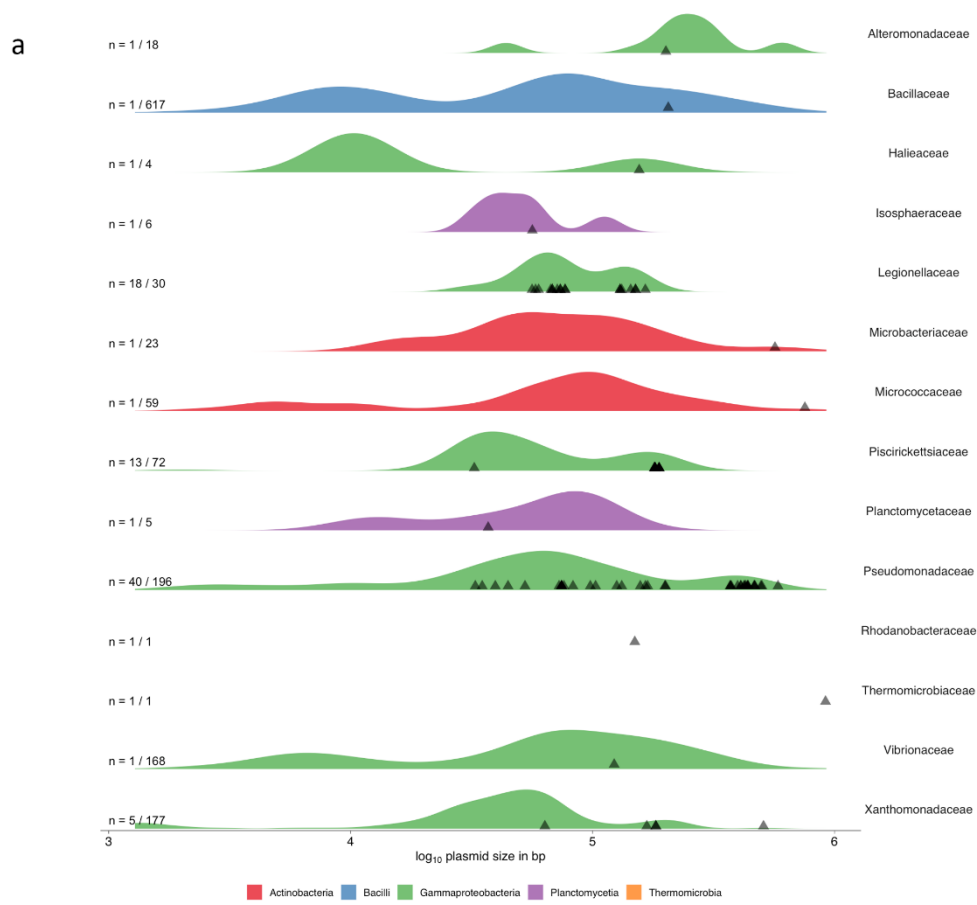


1034

1035 **Figure 7. RsmQ is involved in the control of carbon source metabolism.** a) Selected results from
1036 BioLog carbon source screens showing the differences in metabolism for relevant carbon sources
1037 between SBW25 cells carrying pQBR103^{Km} (red) and pQBR103^{Km}- Δ rsmQ (blue). Representative
1038 growth curves are shown for itaconate (b) and malate (c). Cells were incubated at 28°C for 48
1039 hours and measured at A₆₀₀ every 30 minutes for 40 hours. Cells carrying pQBR103^{Km} are shown

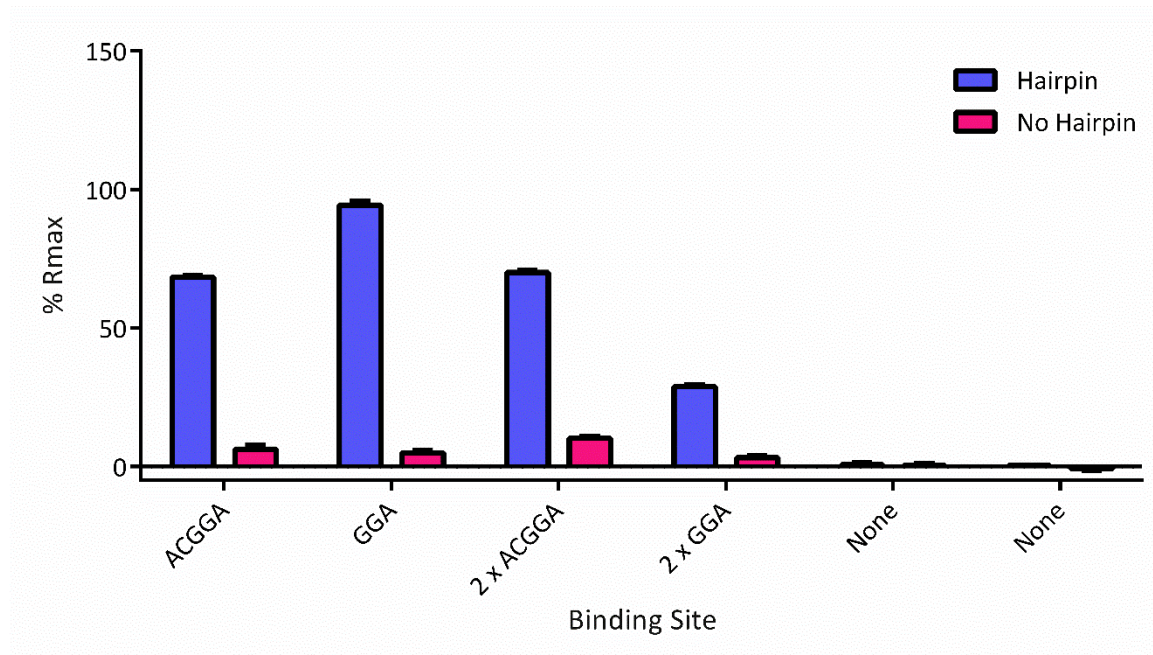
44

1040 in red and those carrying pQBR103^{Km}- *ΔrsmQ* in blue with standard deviation of three technical
1041 replicates shown as dotted lines. A minimum of six independent biological replicates were carried
1042 out for each carbon source.



1044 Figure S1: a) Across Families, CsrA/RsmA-encoding plasmids are relatively large. Size density plots
1045 for all Families with >20 plasmids and ≥ 1 plasmid-encoded CsrA/RsmA homologue. Each row
1046 describes a different Family. Semi-transparent triangles indicate the size of CsrA/RsmA-encoding
1047 plasmids. On the left, the proportion of total plasmids encoding CsrA/RsmA homologues for that
1048 Family. b) Comparison of putative CsrA/RsmA-regulated gene frequencies between CsrA/RsmA-
1049 encoding and non-encoding Pseudomonadaceae plasmids. Plots show overlaid histograms and
1050 density plots. Left hand plot shows absolute numbers of putative CsrA/RsmA-regulated genes,
1051 whereas right hand plot shows as a proportion of total CDS on that plasmid. Distributions were
1052 significantly different between plasmid types in both panels (Kolmogorov-Smirnov test, $p < 0.001$
1053 for absolute counts, $p = 0.012$ for proportions).

1054

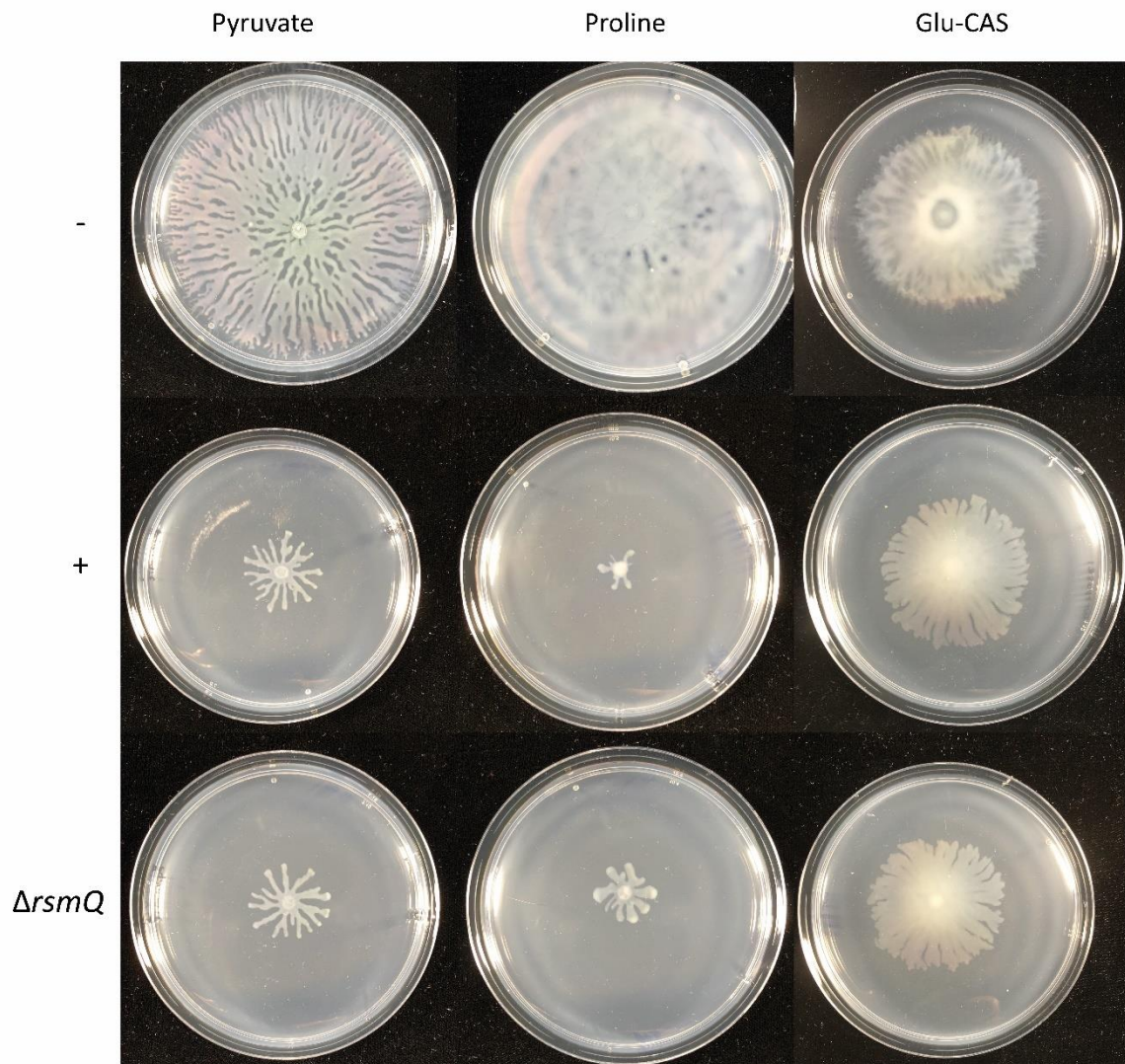


1055

1056 Figure S2: Percentage R_{max} values for RsmQ binding to ssDNAs containing the indicated binding
1057 site sequence in either a linear format (pink) or at the top of a hairpin loop (purple).

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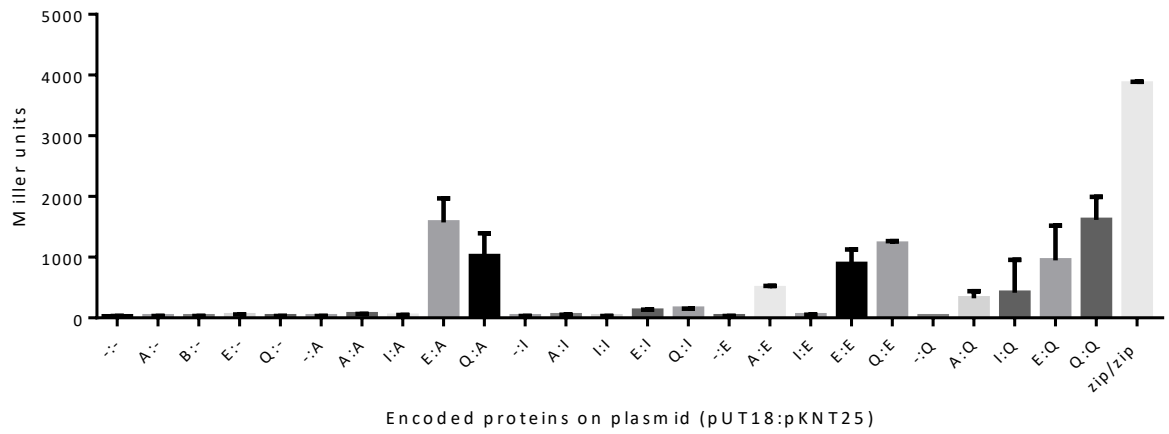


1060

1061 Figure S3: a) Swarming motility after 72 h for SBW25 cells either plasmid free (-) or carrying

1062 pQBR103^{Km} (+) or pQBR103^{Km}- $\Delta rsmQ$ grown on 0.5% M9 media with the carbon source indicated.

1063



1064

1065 Figure S4: β -galactosidase assay (as shown in figure 5) with all controls shown.

1066

1067 **Table S1.** Up and Downregulated proteins in for pQBR103^{Km}-*ΔrsmQ* / pQBR103^{Km}

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1071 **Table S2.** BioLog results for SBW25 plasmid free, SBW25 pQBR103^{Km} and pQBR103^{Km}-*ΔrsmQ*
1072 after 24 hours of growth. Absorbance measured at 590 nm.

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1076 **Table S3** Table of Strains and Plasmids

Strains	DESCRIPTION	REFERENCE
<i>P. fluorescens</i>		
SBW25	Environmental <i>P. fluorescens</i> isolate	1
SBW25Ωgentr	SBW25 with a gentamicin resistance cassette in a neutral location within the genome	This study
SBW25 ΩStrep ^f -LacZ	SBW25 with a Streptomycin resistance cassette and <i>LacZ</i> in a neutral location within the genome	This study
SBW25 ΩStrep ^f -LacZ + pQBR103 ^{km}	SBW25 ΩStrepR-LacZ carrying pQBR103 ^{km}	This study
SBW25 ΩStrep ^f -LacZ + pQBR103 ^{km} Δ <i>rsmQ</i>	SBW25 ΩStrepR-LacZ carrying pQBR103 ^{km} Δ <i>rsmQ</i>	This study
SBW25 ΩGent ^f + pQBR103 ^{km}	SBW25 ΩGentR carrying pQBR103 ^{km}	This study
SBW25 Ωgentr + pQBR103 ^{km} Δ <i>rsmQ</i>	SBW25 ΩGentR carrying pQBR103 ^{km} Δ <i>rsmQ</i>	This study
<i>E. Coli</i>		
BL21-(DE3)	Sm ^R , K12 <i>recF143 lacI^q lacZΔ.M15, xylA</i>	Novagen
DH5α	<i>endA1, hsdR17(r_K-m_K+), supE44, recA1, gyrA (Nal^f), relA1, Δ(lacIZYA-argF)U169, deoR, Φ80dlacΔ(lacZ)M15</i>	2
BTH101	F ⁻ , <i>cya-99, araD139, galE15, galK16, rpsL1 (Str^r), hsdR2, mcrA1, mcrB1.</i>	3
Plasmids		
pME6032	Tet ^R , P _K , 9.8 kb pVS1 derived shuttle vector	4
pME-rsmQ	pME6032 containing	This study
pKNT25	Plasmid for constructing N-terminal fusions to T25, Kan ^f	3
pKNT25-rsmQ	pKNT25 with the ORF of <i>rsmQ</i> cloned within the EcorI/BamHI sites	This study
pKNT25-rsmA	pKNT25 with the ORF of <i>rsmA</i> cloned within the EcorI/BamHI sites	This study
pKNT25-rsmI	pKNT25 with the ORF of <i>rsmI</i> cloned within the XbaI site using Gibson assembly	This study
pKNT25-rsmE	pKNT25 with the ORF of <i>rsmE</i> cloned within the XbaI site using Gibson assembly	This study
pUT18c	Plasmid for constructing C-terminal fusions to T18, Carb ^f	3
pUT18c – rsmQ	PUT18C with the ORF of <i>rsmQ</i> cloned within the EcorI/BamHI sites	This study
pUT18c -rsmA	PUT18C with the ORF of <i>rsmA</i> cloned within the EcorI/BamHI sites	This study

pUT18c -rsml	PUT18C with the ORF of <i>rsml</i> cloned within the XbaI site using Gibson assembly	This study
pUT18c -rsmE	PUT18C with the ORF of <i>rsmE</i> cloned within the XbaI site using Gibson assembly	This study
PKT25-ZIP	pKT25 carrying the leucine zipper of GCN4, Km ^r	3
pUT18-zip	pUT18 carrying the leucine zipper of GCN4, Km ^r	3
pQBR103 ^{km}	Environmental pQBR103 plasmid with kanamycin resistance marker placed within a neutral location	This study
pQBR103 ^{km} Δ <i>rsmQ</i>	pQBR103 ^{km} with amino acids 2-46 from the ORF of <i>rsmQ</i> removed using allelic exchange.	This study

1077

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1095 **Table S4.** Table of Primers

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