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5 **Adaptive significance of quorum sensing-dependent regulation of public goods**

6 **by integration of growth rate: a trade-off between survival and efficiency**

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Abstract

Quorum sensing (QS) is a mechanism of communication used by bacteria to monitor cell density and coordinate cooperative behaviors. An emerging framework is that the adaptive significance of QS in regulation of production of costly extracellular metabolites (public goods) is to maintain the homeostasis of cooperation. We investigated the functionality of QS-dependent regulation of rhamnolipids, extracellular surface-active glycolipids promoting the social swarming motility behavior, in *Burkholderia glumae* and found that QS is superfluous under rich nutritional conditions. In contrast, decreasing nutrient concentrations to reduce the growth rate amplifies rhamnolipid biosynthesis gene expression, revealing a system where QS-dependent regulation is triggered by the growth rate of the population rather than by its cell density. Our results provide evidence that the adaptive significance of QS in regulation of public goods is to maintain an optimized demand-driven supply of target cooperative behavior, wherein efficiency can be traded off against survival.

Introduction

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43   Quorum sensing (QS) is a cell density-dependent mechanism which enables a group of bacteria
44   to synchronize the expression of behaviors in response to the accumulation of self-produced
45   autoinducer signals in their local environment (Fuqua et al., 1994). Such auto-induction regulatory
46   systems are implicated in regulation of various cooperative phenotypes ranging from bioluminescence,
47   virulence and nutrient acquisition to multicellular swarming motility and biofilm development (Passador
48   et al., 1993, Bainton et al., 1992, Stevens and Greenberg, 1997, Daniels et al., 2004, An et al., 2014,
49   Davies et al., 1998). These behaviors are considered cooperative because they mostly involve the
50   production of costly extracellular metabolites («public goods») which, once produced, are available to
51   all members of the group to share their benefits (Platt and Bever, 2009). Examples among bacteria are
52   widespread and include production of siderophores, exoenzymes or surfactants (West et al., 2006).
53   While cooperative production of public goods enhances the fitness of the whole population, at the
54   individual level it is prone to exploitation by non-cooperative individual cells («cheaters») that, by not
55   producing the metabolite or QS signal, gain a competitive edge over cooperative cells and hence
56   threaten the homeostasis of the cooperation (Sandoz et al., 2007, Diggle et al., 2007); this brings up
57   questions about the evolutionary stability of cooperation.

58   In recent years, the adaptive functionality of QS in regulation of public goods has constituted the
59   main subject of several studies, and has been reviewed extensively (Schuster et al., 2013, Hense and
60   Schuster, 2015). The central question relates to the benefits provided by QS in the production of public
61   goods, and thus its role in the evolution and stability of cooperative behaviors (Schuster et al., 2013). In
62   this regard, few studies addressing the social function of QS in regulation of public goods have
63   demonstrated that QS can offer density-dependent fitness benefits (Pai et al., 2012, Darch et al., 2012,
64   Goo et al., 2012). QS is used to coordinate the triggering of social behaviors at high cell densities when
65   they are more efficient and will provide the greatest advantage to the community (Darch et al., 2012).
66   Moreover, the social function of QS in regulation of public goods is not only limited to providing fitness
67   benefits at higher cell densities, as it can also serve as a means to anticipate stationary phase or foresee
68   the optimum cell density of a population (Goo et al., 2012). Cooperation presents a social dilemma, as
69   cheaters should have a fitness advantage over cooperators. Still, QS can impose metabolic constraints
70   on social cheating and protect the population from a 'tragedy of the commons' by simultaneously
71   controlling the expression of private goods (Dandekar et al., 2012). Another possible solution to
72   stabilizing cooperation is policing, where cooperators actively penalize social cheats to hinder their
73   success (Wang et al., 2015).

74   While these studies offer insights on the role of QS in the evolution and stability of cooperative
75   behaviors, the adaptive significance of QS has remained debatable. In fact, it was suggested that it is
76   only through studying each specific auto-induction system in its evolutionary and ecological context that
77   we can figure out its adaptive significance (Platt and Fuqua, 2010). The general framework is that, while
78   conditions specific to each ecological context, such as population cell density and spatial diffusion
79   properties, determine whether triggering of the costly QS-regulated cooperative behaviors is beneficial
80   to the population, the integration of QS circuitry with other global regulatory pathways such as stress
81   responses, specific environmental and physiological cues ensures an optimized demand-driven supply of

82   target cooperative behaviors (Hense and Schuster, 2015). Indeed, integration of metabolic information
83   into decision-making processes governing production of public goods, called 'metabolic prudence', can
84   stabilize cooperative behaviors and reduce the cost of cooperation for the individual cell (Boyle et al.,
85   2015, Xavier et al., 2011). Thus, the emerging picture is that in order for QS to properly regulate costly
86   bacterial cooperative behaviors (when benefits outweigh costs of production) it must be integrated with
87   additional physiological cues (Hense and Schuster, 2015).

88   Here, we demonstrate that QS-dependent regulation of a public good in the Gram-negative
89   bacterium *Burkholderia glumae*, a pathogen of rice, is dependent on growth rate, a direct measure of
90   the carrying capacity of a population through prevailing environmental conditions. *B. glumae* uses the
91   LuxI/LuxR-type QS system TofR/TofI to regulate protein secretion, oxalate production and expression of
92   various virulence determinants such as toxoflavin, lipase, KatG and flagella (Kim et al., 2007, Goo et al.,
93   2010, Goo et al., 2012, Chun et al., 2009). The TofR QS transcriptional regulator is activated by its
94   cognate *N*-octanoyl homoserine lactone (C_8-HSL) autoinducer, the product of the TofI *N*-acyl-HSL
95   synthase, to control the expression of target genes (Kim et al., 2004). Similarly to what is seen in
96   *Pseudomonas aeruginosa* (Ochsner and Reiser, 1995, Köhler et al., 2000), we have previously
97   demonstrated that in *B. glumae* as well, QS positively regulates the expression of the *rhlA* gene,
98   encoding the enzyme catalyzing the first step in biosynthesis of rhamnolipids. These extracellular
99   surface-active glycolipids promote the social surface behavior called swarming motility (Nickzad et al.,
100   2015). Now, we show that, in contrast to *P. aeruginosa*, this QS regulation is dispensable in *B. glumae*, as
101   rhamnolipid production is unaffected in a QS-negative mutant under unrestricted, rich nutritional
102   conditions. This unexpected observation led us to investigate the interplay between QS and nutrition-
103   based regulation of rhamnolipids in *B. glumae*. We found that growth rate, and not cell density itself, is
104   the key factor triggering the QS-dependent regulation of rhamnolipid biosynthesis. Since rhamnolipids
105   act as public goods promoting social swarming motility, integration of growth rate with QS-dependent
106   regulation of rhamnolipids in *B. glumae* supports the concept that the adaptive significance of QS lies in
107   providing a regulatory mechanism for trade-off between survival and efficiency.

Results

Quorum sensing regulation of rhamnolipid production in *B. glumae* is nutritionally conditional

111   To strengthen our understanding on the specific role of QS regulation in the production of
112   rhamnolipids in *B. glumae* we studied the kinetics of rhamnolipid production in wild-type strain and *tofI⁻*
113   mutant using a minimal (mineral salts medium; MSM) typically used to promote production of
114   rhamnolipids and a complex (nutrient broth; NB) culture media, both supplemented with non-limiting
115   glycerol as carbon source (thus MSMG and NBG). As expected, the *tofI⁻* mutant that lacks the production
116   of C_8-HSL does not produce rhamnolipids in the minimal medium and this production is restored by
117   adding C_8-HSL (Fig. 1a). On the other hand, unexpectedly, the *tofI⁻* mutant presents no defect in
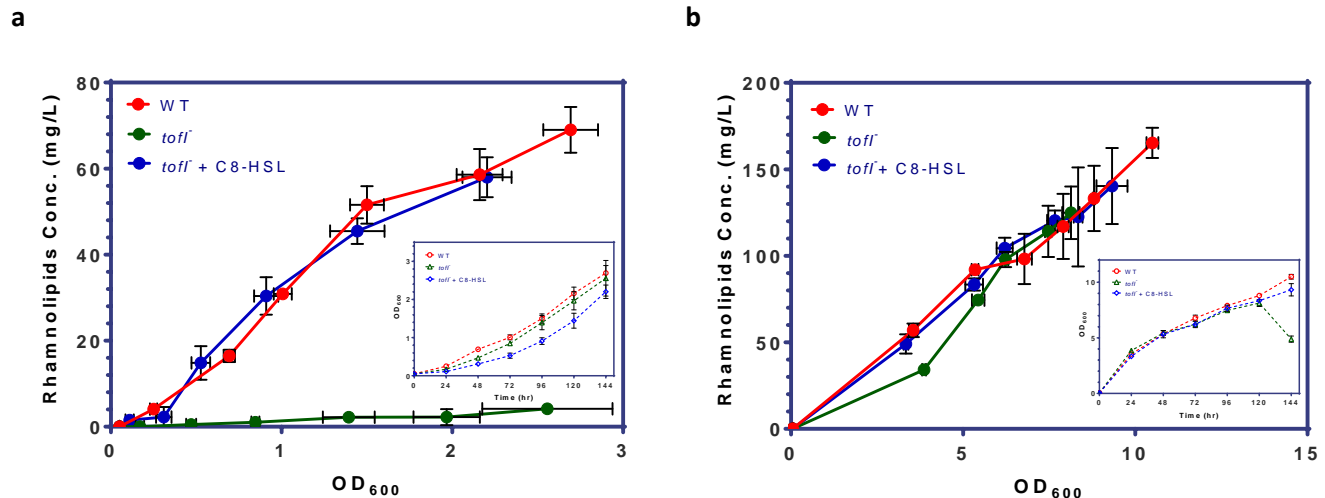118   production of rhamnolipids in rich NB medium (Fig. 1b).

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Figure 1. Rhamnolipid production of *B. glumae* strains in minimal vs. complex media. (a) While the wild-type strain produces rhamnolipids during culture in MSMG medium, the *tofI⁻* mutant is not capable of producing rhamnolipids. The wild-type production level is restored upon exogenous addition of 8 μM C$_8$-HSL to *tofI⁻* mutant culture medium. (b) In contrast, in NBG medium the *tofI⁻* mutant is capable of producing rhamnolipids at wild-type levels. The error bars indicate the standard deviation of the mean for three independent cultures. Insert graphs show the growth data plotted against time for the all the strains.

Quorum sensing-dependent regulation of rhamnolipids is coupled to growth rate

Since a key difference between the two culture conditions is slower growth in MSMG vs NBG (Fig. 1), we hypothesized the impact of QS on biosynthesis of rhamnolipids is influenced by the nutritional conditions. We thus compared the rhamnolipid yield per biomass in wild-type and *tofI⁻* mutant in increasing dilutions of NB medium, while maintaining the glycerol at 4%, in order to have a decreasing gradient of nutrients.

By calculating the ratio of specific production yields of the wild-type over the *tofI⁻* mutant, we found that the contribution of QS on rhamnolipid production rises linearly with increasing NB medium dilution (Fig. 2). This shows that the regulation of rhamnolipid production relies more and more on QS upon increasing the nutrient limitation, supporting the hypothesis.
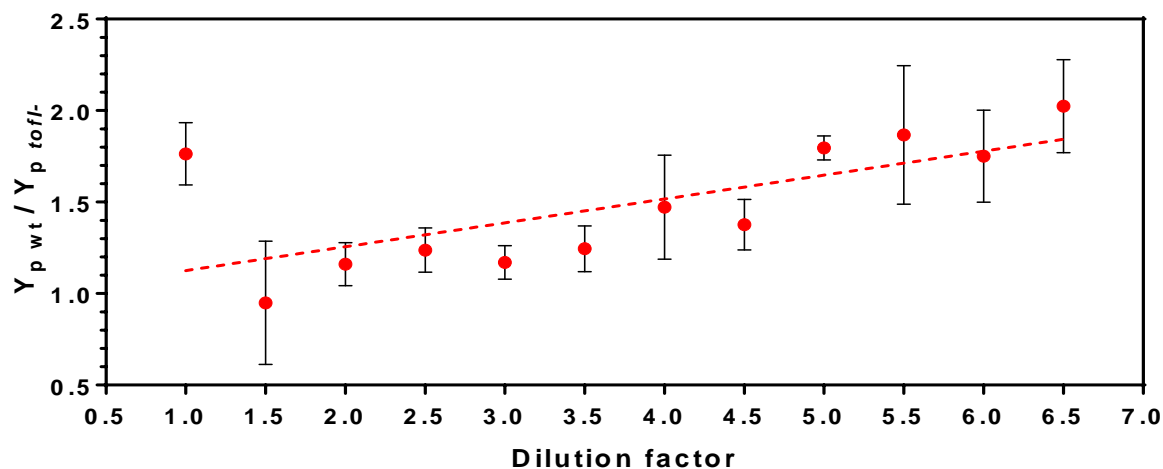
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Figure 2. **Contribution of quorum sensing in production of rhamnolipids by _B. glumae_ in different dilutions of complex NB culture medium.** Cultures were performed in increasing dilutions of NB (glycerol maintain stable) medium for 48 h of incubation, then bacterial growth and rhamnolipids production were measured and specific production yield was calculated. The error bars indicate the standard deviation of the mean for three independent experiments.

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149   Diluting the concentration of available nutrients should decrease the growth rate. Thus, to investigate the effect of growth rate on the production of rhamnolipids upon increasing nutrient limitation, the specific growth rate for each NB dilution was determined (Fig. 3). Interestingly, upon decreasing nutrient availability and consequently the specific growth rate (μ), the specific rhamnolipid production yields of the wild-type and _tofl-_ mutant were not significantly different until about $\mu = 0.08$ (1/h), mirroring the absence of contribution of QS under unrestricted growth conditions (Fig. 1b). However, from this point on to lower μ values, and in contrast with the QS mutant, we observed a sudden increase in rhamnolipid production yield by the wild-type strain, indicating the initiation of an activating contribution by QS as the growth rate decreases. This reveals a situation where QS-dependent regulation of rhamnolipids is triggered by the growth rate of the population rather than by its cell density.

160   To better understand this mechanism, we measured the yield of C_8-HSLs in wild-type strain cultures and uncovered a gradual increase in signal specific concentration as the growth rate decreases (Fig. 3). This means that the number of molecule of C_8-HSL per unit of cell decreases as the growth rate augments. In addition, we saw that the wild-type C_8-HSL and rhamnolipid yield curves are never parallel, which further substantiates that additional regulatory elements control the expression of QS-modulated factors such as rhamnolipids.
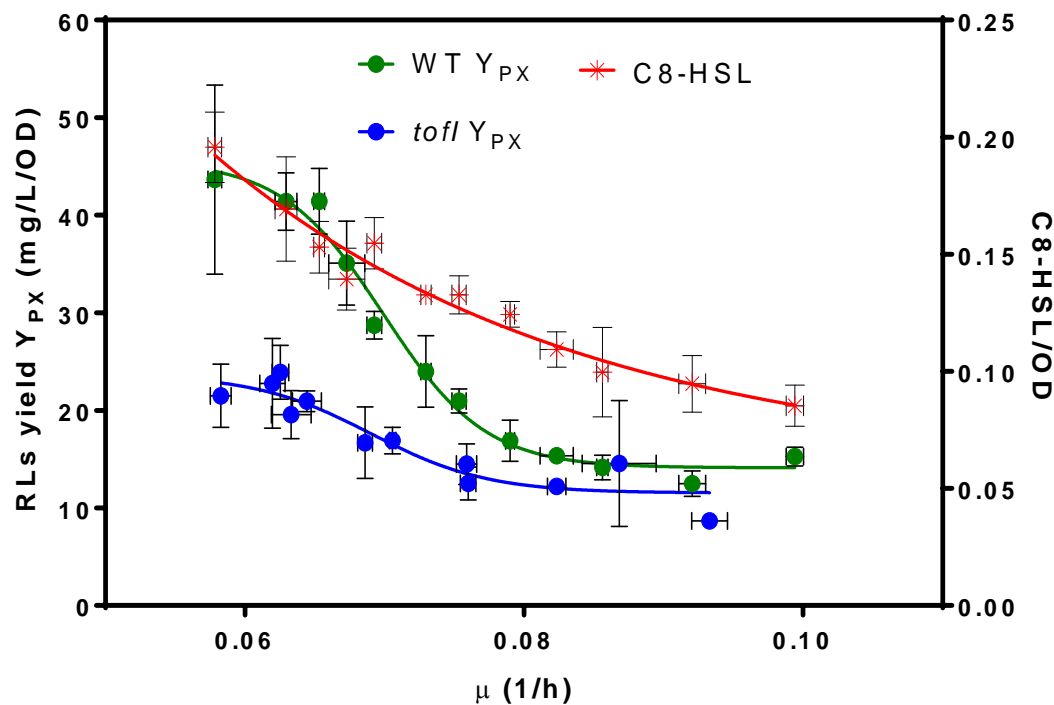
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Figure 3. Effect of growth rate on specific yield of rhamnolipids and C$_8$-HSL concentration. After 48 h of incubation, rhamnolipids specific production yield in wild-type and *tofI⁻* mutant and specific yield of C$_8$-HSLs in wild-type in different dilutions of NB medium were determined for a range of specific growth rates, determined as described in Materials and methods. The error bars indicate the standard deviation of the mean for three independent experiments.

Nutritional cues amplify rhamnolipid biosynthesis gene expression through QS-dependent regulation

Finally, to look more closely at the regulatory level where QS is acting, we wanted to know if nutrient-depleted conditions directly influence the expression of QS-dependent rhamnolipid biosynthesis genes. To do so, we monitored *rhlA* transcription in wild-type and *tofI⁻* strains using a *rhlA-lux* reporter gene fusion in different dilutions of NB medium (always with the same glycerol concentration). This allowed us to investigate the direct effect of growth rate on QS-dependent regulation of *rhlA* gene. Hence, we plotted the *rhlA* expression levels in wild-type and *tofI⁻* strains at a range of specific growth rates. As shown in figure 4, as the growth rate decreases we can observe a sudden increase in *rhlA* gene expression in wild-type strain. However, the expression level of *rhlA* gene in the *tofI⁻* mutant remains mostly unchanged. Taken together, these data suggests that induction of *rhlA* transcription by TofR occurs at a specific growth rate, instead of specific cell density or AHL concentration.
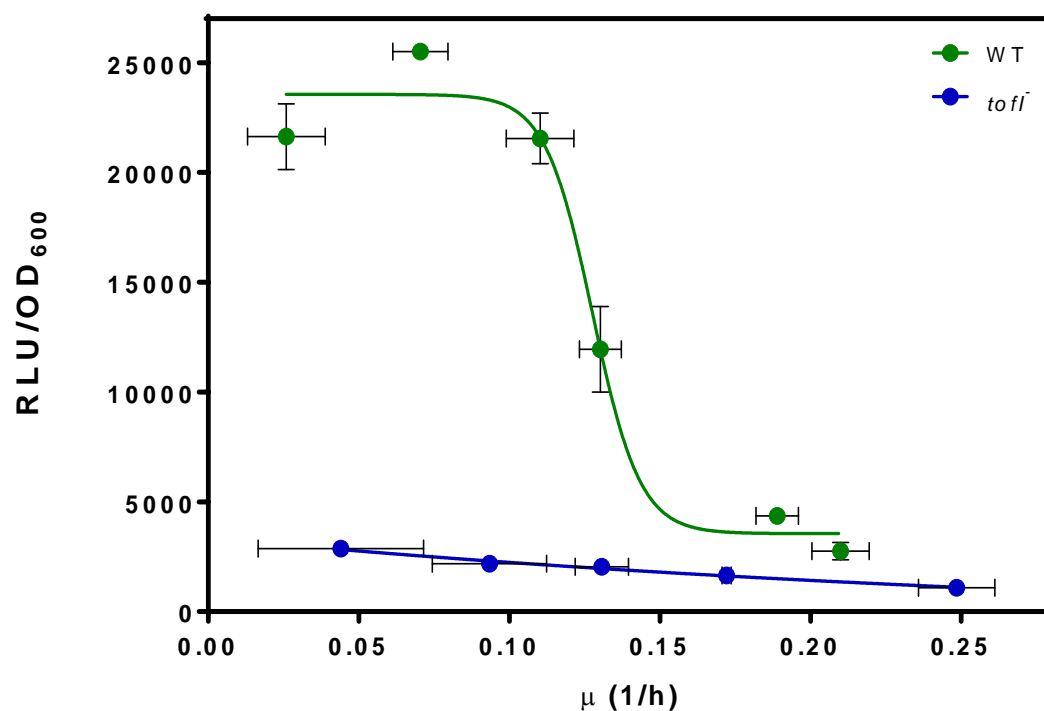
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Figure 4. The effect of growth rate on expression from the *rhl* operon promoter in wild-type and *tofI*⁻ **strains carrying a chromosomal *rhlA'-lux* transcriptional reporter**. The *rhlA* promoter activity in wild-type and *tofI*⁻ mutant plotted against growth rates achieved by different dilutions of NB medium. The error bars indicate the standard deviation of the mean for three independent experiments.

Discussion

We present conclusive evidence for a functional role of QS in providing an optimized demand-driven supply of target cooperative behavior, wherein QS-dependent regulation of rhamnolipids is tightly coupled to specific growth rate. Our results support the proposed model that the adaptive functionality of QS in regulation of public goods is to maintain the homeostasis of cooperation where the production of costly public goods is conditioned by assessment of demand and efficiency (Hense and Schuster, 2015).

We have previously demonstrated that in the rice pathogen *B. glumae*, QS positively regulates the biosynthesis of rhamnolipids, extracellular surface-active glycolipids which are necessary for promoting the social surface behavior called swarming motility (Nickzad et al., 2015). Here, we found that a signal-negative mutant that does not produce C_8-HSL maintains the ability to produce rhamnolipids in a rich nutrient broth medium, in contrast with growth in a minimal medium where rhamnolipid production is absent (Fig. 1). This led us to hypothesize that QS-dependent regulation of rhamnolipids in *B. glumae* is nutritionally conditional. Comparison of the yield of rhamnolipids per biomass in the wild-type and *tofI*⁻ mutant strains in a decreasing gradient of nutrients revealed that the more we dilute the growth medium the more QS regulation contributes to the production of

rhamnolipids (Fig. 2). We increasingly realize that the QS circuitry in various bacteria is highly integrated with other global regulatory pathways, stress responses and specific environmental and physiological cues (Mellbye and Schuster, 2011, Williams and Camara, 2009). Although up-regulation or down-regulation of QS-controlled factors such as rhamnolipids biosynthesis genes *rhlAB* in *P. aeruginosa* by elements other than cell-to-cell communications such as nutritional status is well known (Deziel et al. 2003; Guerra-Santos et al. 1986), this modulation always necessitates the presence of a functional QS system. The identification of a QS-dispensable regulation of rhamnolipids in *B. glumae* adds these metabolites to the list of *B. glumae* factors whose dependence on QS is conditional (Chen et al., 2012, Jang et al., 2014, Kato et al., 2014, Kim et al., 2007).

The dispensability of QS-regulated virulence factors and other costly metabolites, such as rhamnolipids, upon prevailing conditions and environmental cues provides insights into how these regulatory pathways have evolved to optimize the functionality of target genes they regulate. For instance, in the case of rhamnolipids biosynthesis in *B. glumae*, we found that once unrestricted nutrient resources are available, production of rhamnolipids is carried out independently of QS regulation, obviously through still-unidentified regulatory mechanisms. However, under nutrient-limited conditions with excess carbon source, QS becomes the main regulating mechanism significantly enhancing the specific rhamnolipids yield. Under nutrient-limited conditions, which *B. glumae* presumably meets in its natural habitat such as plant tissue environment, it might be more cost-effective for rhamnolipids biosynthesis to be carried out *via* a decision-making mechanism such as QS which places more restrictive controls over nutrient utilization. Notably, QS in *B. glumae* directly controls nutrient acquisition of individual cells in crowded populations (An et al., 2014).

Since growth rate is a direct function of nutrient availability, we hypothesized that growth rate is the key triggering factor that induces QS-dependent regulation of rhamnolipids. Our results indicate there is a growth rate threshold below which QS engages in the regulation of rhamnolipids (Fig. 3). In *P. aeruginosa*, slow growth is one key condition inducing regulation of QS-controlled public good genes in a metabolic prudent manner (Mellbye and Schuster, 2014). For instance, prudent regulation of rhamnolipid production ensures that cells only invest carbon into rhamnolipids biosynthesis when growth is limited by another growth-limiting nutrient, such as nitrogen (Xavier et al., 2011). In fact, presence of excess carbon in different dilutions of NB medium in our experiments, also suggest that in *B. glumae*, QS-dependent regulation of rhamnolipids is coupled to growth rate in a metabolic prudent manner. Furthermore, a gradual increase in QS signal specific concentration upon decrease of specific growth rate suggests a reduction in activation signal threshold, which reflects an increase in cellular demand for production of QS-dependent target gene product at such low density populations. Importantly, our gene expression analyses demonstrate the direct influence of the specific growth rate on QS-dependent induction of *rhlA* expression in the wild-type background, in agreement with a previous report showing that *rhlAB* expression in *P. aeruginosa* in the presence of excess carbon is tightly coupled to the growth rate, and not just cell density (Xavier et al., 2011). Therefore, the role of QS in regulation of public goods is not limited to stationary phase situations at higher cell densities, but also upon encountering restrictive environmental conditions, an early induction of QS by slowed growth rate could provide some inclusive fitness benefits for the survival of an individual cell and could act as a

247 trade-off mechanism between survival and efficiency. Our results are also in accordance with the
248 regulation of QS gene expression by starvation in *P. aeruginosa* in which stringent response can lower
249 the quorum threshold *via* triggering an increased signal synthesis (van Delden et al., 2001, Schuster et
250 al., 2013).

251 From an ecological significance perspective, induction of QS-dependent regulation of
252 rhamnolipids at low specific growth rates could reflect two distinct scenarios for which such integrated
253 regulation has evolved: (i) restricted nutritional conditions with low cell density (free living bacteria
254 exposed to nutritional stress during establishment of host infection), and (ii) exhausted nutritional
255 environment with a high cell density (bacteria living in biofilms). In both instances, since population
256 growth is limited by the carrying capacity of the direct environment, it is conceivable that slow growth
257 can serve as a predictive factor of the population carrying capacity (de Vargas Roditi et al., 2013).
258 Accordingly, in *B. glumae*, QS is used for anticipation of a population reaching the carrying capacity of an
259 habitat to avoid lethal ammonia-induced alkalization by activating oxalate production, and thus
260 promoting survival in the stationary phase (Goo et al., 2012).

261 An effective colonization of surface niches necessitates a balance between biofilm and motility
262 (van Ditmarsch et al., 2013). In this regard, there is an inverse regulation of biofilm formation and
263 swarming motility (Caiazza et al., 2007). Since production of surfactants is as crucial for biofilm
264 development as for swarming motility (Nickzad and Déziel, 2014), it seems that the induction of QS-
265 dependent rhamnolipids biosynthesis upon reduced growth rate is a key evolved strategy to modulate
266 swarming motility and promote dissemination of bacterial cells to find new surface niches to colonize
267 (Déziel et al., 2003, Tremblay and Déziel, 2010). Thus, integration of slowed growth rate with QS as a
268 decision-making mechanism for biosynthesis of costly rhamnolipids could serve to assess the demand
269 and timing for expanding the carrying capacity of a population through spatial expansion mechanisms
270 such as swarming motility, thus promoting the chances of survival. In conclusion, we propose that the
271 adaptive significance of growth rate-dependent functionality of QS in rhamnolipids biosynthesis lies
272 within providing a regulatory mechanism for selecting the optimal trade-off between survival and
273 efficiency.

Materials and methods

Bacterial strains, plasmids, media and growth conditions

276 All *B. glumae* strains used are derivatives of the wild-type strain BGR1; the bacterial strains and
277 plasmids used in this study are listed in Table 1. Unless otherwise specified, the strains were routinely
278 regrown from frozen stocks by culturing at 37°C in tryptic soy broth (TSB) (BD) and 240 rpm in a TC-7
279 roller drum (New Brunswick, Canada), or on TSB agar plates. Antibiotics were used at the following
280 concentrations: tetracycline (Tc), 10 μg ml^{-1}, gentamycin (Gm), 20 μg ml^{-1} and triclosan 25 μg ml^{-1}.
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Table 1. Bacterial strains and plasmids

Bacteria	Relevant characteristics	Source or reference
E. coli		
DH5α	F-, φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA*1, *endA*1, *hsdR*17(rk-, mk+), *phoA*, *supE*44, λ-, *thi*-1, *gyrA*96, *relA*1	
SM10 λ*pir*	thi-1 *thr leu tonA lacY supE recA*::RP4-2-Tc::Mu Km^r λpir	(Simon et al., 1983)
B. glumae		
BGR1	Wild-type, Rif^R	(Jeong et al., 2003)
BGS2	*tofI*::Ω in BGR1	(Kim et al., 2004)
ED2123	BGR1::pAN2	(Nickzad et al., 2015)
ED2124	BGS2::pAN2	(Nickzad et al., 2015)
Plasmids		
mini-CTX-*lux*	Integration vector with promoterless *luxCDABE*	(Becher and Schweizer, 2000)
pAN2	*rhlA* promoter fused to *luxCDABE* in mini-CTX-lux	(Nickzad et al., 2015)

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287   For rhamnolipid production, complex nutrient broth (NB) medium (BD Difco, Mississauga, ON,

288 Canada) supplemented with 4% (w/v) glycerol or mineral salt medium supplemented with 2% (w/v)

289 glycerol and 0.1 M urea was used. The composition of the latter medium was (g/l): KH_2PO_4, 2.5; K_2HPO_4,

290 1.5; $MgSO_4$, 0.1; $CaCl_2.2H_2O$, 0.1. For NB dilution cultures, glycerol concentration was kept constant at

291 4% (w/v). To determine whether the rhamnolipid production could be restored by exogenous addition

292 of signal molecules, culture media were supplemented with 8 µM C_8-HSL. Cultures were grown at 34°C

293 with shaking (240 rpm) in a TC-7 roller drum (New Brunswick, Canada).

Growth rate measurement

295 The specific growth rate for wild-type and *tofI*$^-$ mutant in each dilution of NB medium was determined

296 using the following equation: $\mu = \frac{Ln(X_{t2}) - Ln(X_{t1})}{(t2 - t1)}$ where X_{t2} refers to optical cell density at time t_2, X_{t1} is

297 the optical cell density at time t_1 and μ represents specific growth rate.

Reporter construction

299   Transcriptional fusion strains of *B. glumae* were constructed by PCR amplification of a 923-bp

300 fragment containing the promoter region of *rhlA* from BGR1 genome using primers promrhlAF: 5'-

301 ACGTAGAATTCGGGAAAGCAGGCAGGGTAG and promrhlAR: 5'-

302 ACGTAAAGCTTTTTCGATAGGCATGGCGTACTC. The PCR product was digested with *EcoR*I and *Hind*III and

303 ligated within the corresponding sites in mini-CTX-*lux* (Becher and Schweizer, 2000). The resulting

304 construct was integrated into BGR1 and *tofI*$^-$ mutant chromosome at the *attB* site through conjugation.

305 Successful chromosomal integration of construct in BGR1 and *tofI*$^-$ mutant was confirmed by PCR.

LC/MS rhamnolipid analysis

307   The rhamnolipid concentration in the various bacterial cultures was determined by LC∕MS

308 (Abdel-Mawgoud et al., 2014). During a period of six days, 400 µl culture samples were retrieved at

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309   regular time intervals and the OD_{600} was measured (Nanodrop ND-1000, Thermo Fisher Scientific). Then
310   the samples were centrifuged at 16,000 × g for 10 min. to remove the bacteria. To 300 µl of supernatant
311   were added 300 µl of acetonitrile and 10 mg/L 5,6,7,8-tetradeutero-4-hydroxy-2-heptylquinoline (HHQ-
312   d4) as an internal standard (Dubeau et al., 2009). Samples were analyzed by high-performance liquid
313   chromatography (HPLC; Waters 2795, Mississauga, ON, Canada) equipped with a C8 reverse-phase
314   column (Kinetex, Phenomenex) using a water/acetonitrile gradient with a constant 2 mmol l^{-1}
315   concentration of ammonium acetate (Dubeau et al., 2009). The detector was a mass spectrometer
316   (Quattro Premier XE, Waters). Analyses were carried out in the negative electrospray ionization (ESI-)
317   mode.

LC/MS-MS analyses for AHL production

319   AHL production analysis were carried out by HPLC (Waters 2795, Mississauga, ON, Canada)
320   equipped with a C8 reverse-phase column (Kinetex C8, Phenomenex), and the detector was a mass
321   spectrometer (Quattro Premier XE, Waters). Analyses were carried out in the positive electrospray
322   ionization (ESI+) mode, supplemented by the multiple reactions monitoring (MRM) mode. Samples were
323   prepared as described in (Chapalain et al., 2013).

Measurement of *rhlA'-lux* activity

325   Expression from the *rhl* operon promoter in wild-type and QS-negative *tofI*[-] strains was
326   quantified by measuring the luminescence of cultures of bacteria containing a transcriptional fusion of
327   the *rhlA* promoter with the luciferase gene integrated in the genome. Cells from overnight cultures were
328   washed twice in PBS and inoculated into test medium at an OD_{600} of 0.05 in white clear bottom 96-well
329   plates (Corning; 3632) containing three replicates of 200 µl per sample. The plates were incubated at 34
330   °C in a multimode plate reader (Cytation3, BioTek, Winooski, VT) with double orbital shaking. OD_{600} and
331   luminescence (relative light units, RLU) were measured every 15 minutes for a duration of 48 hr. The
332   luminescence (*rhlA-lux* activity) was adjusted for culture density (RLU/OD_{600}).

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