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Quorum Machinery: Effect of the *las* System in *rhl* Regulation of *P. aeruginosa*

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Abstract. Quorum sensing (QS) describes a communication mechanism via signal molecules that allows colonies of bacteria to coordinate gene expression. The nature of the QS system depends very much on the species. *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen that has a highly complex QS system. The QS signalling system of *P. aeruginosa* is known to involve multiple control components, notably *las*, *rhl* and *pqs* systems. The QS signalling system of *P. aeruginosa* is responsive to two chemically different signal molecules, based on Homoserine-Lactones (HSLs) and 4-quinilines (4Qs). This paper focuses on the relation of the first two systems that manage Homoserine-Lactone (HSL) production since this is the main chemical signalling in *P. aeruginosa* that regulates many activities of bacteria, including symbiosis, virulence factors, motility, production of antibiotics, and formation of biofilm. The *las* and *rhl* system of *P. aeruginosa* do not act independently. Some gene expression that is regulated by *rhl* system is also controlled by the *las* system. Previous research has demonstrated that the *las* system can give rise to excitable pulse generation of signal molecule production. This paper examines the mechanism of how *las* and *rhl* systems act in tandem to generate sophisticated control on QS system of *P. aeruginosa*. Using analytic methods, it is shown how dynamics of the *rhl* system is affected by dynamics of the *las* system.

Keywords: *Pseudomonas aeruginosa*, Quorum sensing, *las* system, *rhl* system.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen, well known for causing a variety of diseases such as wound infections, and lung infections in cystic fibrosis patients. *P. aeruginosa* has been used as an interesting research object because of the frequency which is involved in human disease, and can infect any part of the body, including the liver, brain, bones and sinuses. It is also known for its innate resistance to many antibiotics. It has efflux pump systems, which are responsible for transporting compounds, including toxic substances and antibiotics, from within cells into the external environment [1].

In this organism, the QS signalling system is regulated by two hierarchical LuxI/LuxR homologue circuits consisting of LasI/LasR and RhlI/RhlR [2]. The first LuxI/LuxR homologue regulates expression of the elastase LasB, and is therefore named the *las* system. Enzyme elastase LasA and LasB are responsible for elastolytic activity, which destroys elastin-containing human lung tissue and causes pulmonary haemorrhages associated with *P. aeruginosa* infection [3]. The second LuxI/LuxR homologue is able to control the production of rhamnolipid, and is therefore named the *rhl* system. Rhamnolipid is a biosurfactant product that also functions as a wetting agent and affects the motility of bacteria in colonies of *P. aeruginosa*. Rhamnolipids are particularly important in swarming motility in that they are postulated to lower the surface tension of the surface through their surfactant properties, allowing the bacterial colony to swarm [4]. As a human pathogen, rhamnolipid has a glycolipid (detergent-like) structure; that serves to degrade lung surfactant and thus inhibits the function of the human respiratory system [5, 6].

The first *P. aeruginosa* QS subsystem, *las* system, is composed of synthase LasI, inhibitor RsaL, autoinducer 3O-C12-HSL, and regulator LasR that binds autoinducer to form complex of LasR/3O-C12-HSL. Meanwhile, the

second *P. aeruginosa* QS subsystem, *rhl* system, is composed of synthase RhlI, autoinducer C4-HSL, and regulator RhlR that binds autoinducer to form complex of RhlR/C4-HSL [7].

Formation of HSL autoinducer 3O-C12 HSL and C4-HSL are catalyzed by LasI and RhlI, respectively. LasI is an autoinducer synthase and LuxI homologue that synthesizes 3O-C12-HSL, whereas LasR is a LuxR homologue and the transcriptional activator for 3O-C12-HSL [7]. The LasR/3O-C12-HSL complex controls production of many virulence factors [8, 9, 10]. In addition, the LasR/3O-C12-HSL complex binds to the *lasI* gene that allows an increase in autoinducer synthesis LasI, which in turn increases the concentration of 3O-C12-HSL binding to LasR [11]. On the other side, the LasR/3O-C12-HSL complex also binds to the *rsaL* gene thus allowing an increase in transcriptional regulator RsaL, which represses expression of *lasI*. Consequently, it blocks transcription of the autoinducer synthesis LasI, which in turn decreases the concentration of 3O-C12-HSL binding to LasR [12].

In addition, RhlI is an autoinducer synthase that synthesizes C4-HSL, and RhlR is the transcriptional activator for C4-HSL [5]. The main difference between the *las* and *rhl* systems is that the *las* system has the inhibitor (*rsaL*) in the inside of the system itself, regulating the production of the synthase and hence the autoinducer production.

It should be noted, however, that the *las* and *rhl* signalling systems have their own specific autoinducers, and thus have no activating transcriptional activator protein in another system (see Fig. 1). For example, while 3O-C12-HSL activates *lasR*, it blocks the binding of C4-HSL to its transcriptional activator *rhlR* [7]. Likewise, while C4-HSL activates *rhlR*, it is unable to activate *lasR* [13, 8]. The *las* system can therefore be considered to be above the *rhl* system through the activation of *rhl* by LasR/3O-C12-HSL [7]. In other words, the *las* system controls the *rhl* system in a hierarchical signalling cascade [14, 7].

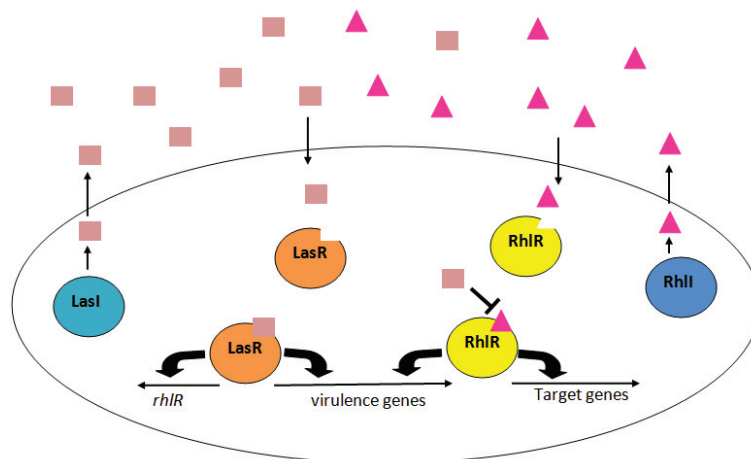


FIGURE 1. *las-rhl* signalling system in *Pseudomonas aeruginosa* consisting of LasI/LasR-RhlI/RhlR. Replotted and adapted [2]

In this article we are interested in the dynamics of the *rhl* system that is affected by the dynamics of the *las* system in *P. aeruginosa*. We begin with internal regulation Rhl system itself. In *rhl* system, RhlR operates as a transcriptional activator in the presence of autoinducer C4-HSL. RhlR would bind C4-HSL, which leads to complex chemical form of RhlR/C4-HSL. Following this process, the complex chemical form of RhlR/C4-HSL would bind and activate the *rhlI* genes in which create a positive feedback on *rhl* system [15]. Transcription and translation process on *rhlI* genes induce the production of autoinducer synthase protein RhlI, which increases the amount of C4-HSL available to bind to RhlR.

As an individual system, dynamical behaviour of *rhl* system is similar to the positive feedback in the *las* system [10]. There will be a point of loop that is classic S-shape bifurcation diagram, which is one point of low stable steady state can leap to the highest steady state that represent the production of *RhlI* protein. However, as a hierarchy system, we try to link *las* system to this (*rhl*) system (see Fig. 2).

The *las* system presents positive and negative impacts on the *rhl* system. Once the concentration of autoinducer 3O-C12-HSL on the *las* system reach the quorum, LasR would bind 3O-C12-HSL, then form chemical complex of

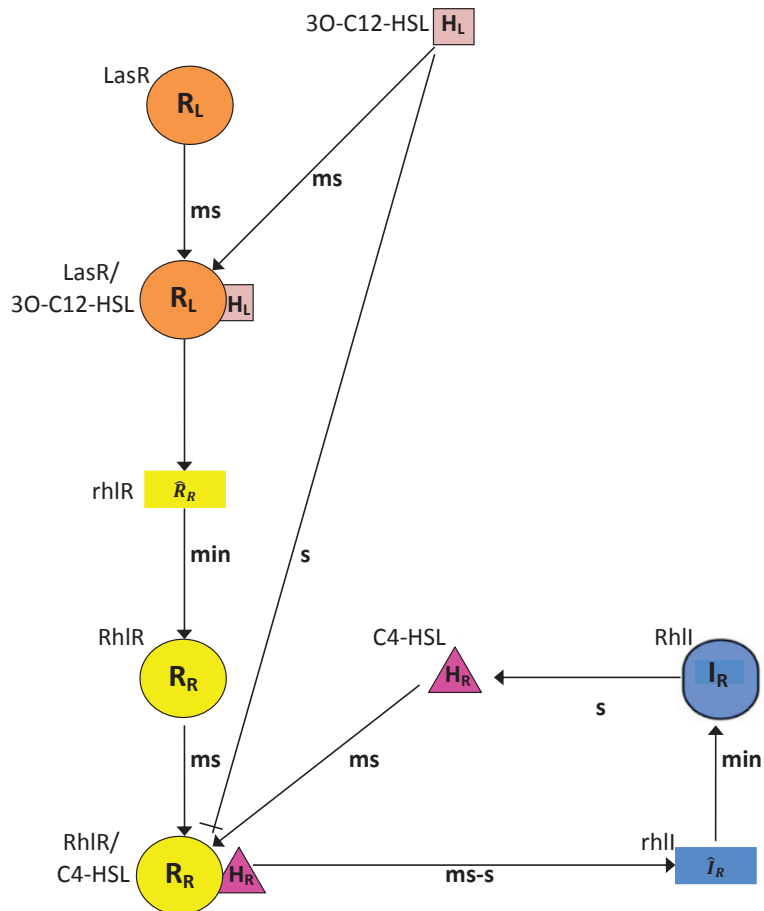


FIGURE 2. The signalling of *rhl* QS subsystem in *Pseudomonas aeruginosa*. Arrows and barred arrows indicate activating (positive) and inhibiting (negative) regulatory interactions, respectively. Shapes on the diagram depict autoregulation terminology. Letters associated with each arrow reflect the associated time scale (ms = millisecond, s = second, and min = minute). Symbols associated with each shape are detailed in Table 1

TABLE 1. Description of dimensional variables of *rhl* system.

Variable	Description	Unit
H_L	3O-C12-HSL	nM
R_R	RhlR	nM
H_R	C4-HSL	nM
R_{RH}	RhlR/C4-HSL complex	nM
\hat{R}_R	rhlR mRNA	nM
I_R	RhII	nM
\hat{I}_R	rhlI mRNA	nM

LasR/3O-C12-HSL. The LasR/3O-C12-HSL complex would not activate the *lasI* and *rsaL* genes on the *las* system only, but it also binds and activates *rhlR* genes on the *rhl* system in which induce the production of RhlR regulator [7]. On the other hand, QS signal molecule 3O-C12-HSL that is synthesized by LasI synthase in the *las* system would not bind LasR regulator only, but some of those signal molecules also bind RhlR as a regulator in the *rhl* system. Consequently, the signal molecule 3O-C12-HSL blocks the binding of C4-HSL to its regulator protein (RhlR) in which 3O-C12-HSL works as an inhibitor of the *rhl* system [7].

This paper is organised as follows. In next section, we describe in greater detail the biological system as well as the mathematical approach. Then we conduct a numerical exploration of our model. In the last section, we conclude by discussing our findings and suggest future works.

METHODS

There are a considerable number of published models about modelling of *rhl* subsystem only [e.g., 16] or both *las* and *rhl* subsystem [e.g., 17] for *P. aeruginosa*. However, to the best knowledge of the authors, none of these studies connect the impact of the *las* to the *rhl* subsystem modelling, even though all of studies about QS in *P. aeruginosa* demonstrate that the placing of the *las* subsystem in a cell signalling hierarchy is above the *rhl* subsystem. Thus we try to rebuild the existing *rhl* model by involving the role of the *las* system.

We construct the governing equations using mass action kinetics, guided by the literature. First, consider the RhlR regulator (R_R), its binding activator, the autoinducer C4-HSL (H_R) that compete with the binding of the autoinducer 3O-C12-HSL to its regulator, and the complex RhlR/C4-HSL (R_{RH}). In the *rhl* system, we are interested in the downstream feedback of autoinducer 3O-C12-HSL. In this regard, we can assume the input of the RhlR/C4-HSL complex from the *las* system is constant, $R_R := R$ (constant). Basically, in the *las-rhl* systems of QS for *P. aeruginosa*, LasR (R_L) activates several genes' expressions including *Rhlr* gene that encodes RhlR (R_R) production. Here, we have assumed R_R to be constant in order to simplify the model equations and focus on the impact of the autoinducer 3O-C12-HSL on the *rhl* system without involving the whole *las* system. We consider H_L as a parameter in this model rather than a variable. We take an arbitrary value ($H_L = 50$ nM) for modelling purposes that generates high production of autoinducer 3O-C12-HSL.

In order to keep the number of equations low, we considered the *las* autoinducer signal inhibition of the complex RhlR/C4-HSL (R_{RH}) in a manner akin to transcription inhibition rather than treating the *las* autoinducer signal as an antagonist that binds the complex RhlR/C4-HSL (R_{RH}). We represent this using mass action kinetics in a similar manner to [15]. If the RhlR/C4-HSL complex is formed at α_{RH} through all of those processes and degrades at rate γ_{RR} , then we may write

$$\frac{dR_{RH}}{dt} = \alpha_{RH}(R - R_{RH}) \frac{H_R}{K_R \left(1 + \frac{H_L}{K_{HR}}\right) + H_R} - \gamma_{RR}R_{RH}. \quad (1)$$

where γ_{RR} is positive constant.

The autoinducer C4-HSL (H_R) is created in the system via the activity of the RhII synthase (I_R), which we take to be at rate β_{HR} , and is naturally lost from the system at rate γ_{HR} . The most significant loss of the autoinducer from the cell is via diffusion through the cell membrane. Taking a simplified description of diffusion we can express the diffusive term as being proportional to the concentration difference across the membrane of H_R . Therefore, D_{HR}

represents an additional loss rate, which is multiplied by the concentration H_R yielding

$$\frac{dH_R}{dt} = \beta_{HR}I_R - \gamma_{HR}H_R - D_{HR}H_R. \quad (2)$$

TABLE 2. Parameters employed in the model of *rhl* system.

Par	Description	Standard value	Unit	Value/Range	Comments (Based on)/Ref
α_{RH}	rate at which RhIR/C4-HSL produced by RhIR, then inhibited by 3O-C12-HSL	0.5	min^{-1}	0.5 - 0.8	estimate
α_I	rate at which RhII produced by rhII mRNA	0.5	min^{-1}	0.5	2 min to translate protein, [18]
β_{HR}	rate at which C4-HSL produced by RhII	8×10^2	min^{-1}	8×10^2	[19]
β_I	max. production rate of RhII at which rhII mRNA is activated by RhIR/C4-HSL	1	nM min^{-1}	1	Estimate
β_{R0}	basal production rate of rhIR mRNA	0.1	nM min^{-1}	0.1	basal transcription rate of a protein, [18]
β_{I0}	basal production rate of rhII mRNA	0.1	nM min^{-1}	0.1	basal transcription rate of a protein, [18]
K_R	affinity constant between <i>las</i> system and rhIR mRNA	250	nM	1-1000	[18]
K_I	affinity constant between RhIR/C4-HSL and rhII mRNA	250	nM	1-1000	[18]
K_{HR}	dissociation constant of inhibitor 3O-C12-HSL to <i>rhl</i> system	250	nM	1-1000	[18]
γ_I	degradation rate of RhII	0.01	min^{-1}	0.01	[18]
γ_{HR}	degradation rate of C4-HSL	0.01	min^{-1}	0.01	[18]
γ_{RR}	degradation rate of RhIR/C4-HSL	0.01	min^{-1}	0.01 - 0.1	estimate
γ_{mI}	degradation rate of rhII mRNA	0.14	min^{-1}	0.2	2 min lifetime of RNA, [18]
D_{HR}	diffusion constant of C4-HSL	200	min^{-1}	$0 - 10^4$	[20]

The enzyme RhII (I_R) is produced by the *rhII* gene through a transcription and translation process of *rhII*-mRNA (\hat{I}_R) at rate α_I and degrades at rate γ_I , such that

$$\frac{dI_R}{dt} = \alpha_I \hat{I}_R - \gamma_I I_R. \quad (3)$$

Transcription at the *rhII* promoter site (\hat{I}_R) is activated by the RhIR/C4-HSL complex (R_{RH}). The production process is assumed to follow a Hill form with a Hill number m . The *rhl* system has not been explored as massive as the *las* system. Many of researchers are interested to investigate rhamnolipid as a product of the *rhl* system, which is

produced by *rhlAB* gene and activated by RhlR/C4-HSL complex rather than the system per se. Biochemically there is insufficient evidence to determine the value of the Hill number, m . For simplicity of the system, we adopt $m = 2$ as adopted from [21]. With basal expression of β_{I0} and a loss rate of γ_{ml} this leads to the following expression for *rhlI*:

$$\frac{d\hat{I}_R}{dt} = \beta_I \frac{R_{RH}^2}{K_I^2 + R_{RH}^2} - \gamma_{ml}\hat{I}_R + \beta_{I0}. \quad (4)$$

In a similar way to the modelling of *las* system, we are able to make the same assumptions regarding the timescales to simplify the system. We assume that the dominant, slowest processes are protein production from mRNA via translation and folding. Therefore, other processes, namely the liganding of regulators, DNA binding, synthetase operation, and the transcription of DNA are much faster and we can assume that the differential equations for *rhlI* genes in the *rhl* system are at a quasi-steady state, such that

$$\hat{I}_R = \frac{\beta_I R_{RH}^2}{\gamma_{ml}(K_I^2 + R_{RH}^2)} + \frac{\beta_{I0}}{\gamma_{ml}}. \quad (5)$$

In addition, we make the simplifying assumption that HSL diffusion is rapid and $\gamma_{HR} \approx 0$ and, therefore, that the equation for C4-HSL, H_R can also be written in a quasi-steady state, providing

$$H_R = \frac{\beta_{HR} I_R}{D_{HR}}. \quad (6)$$

From the system (see Fig. 2), we know that all processes involving binding activator C4-HSL, inhibitor 3O-C12-HSL, and transmission process from *las* to *rhl* system happen very fast. Thus we can assume that differential equation for the complex RhlR/C4-HSL (R_{RH}) in the *rhl* system is at a quasi-steady state, such that

$$\alpha_{RH}(R - R_{RH}) \frac{H_R}{K_R \left(1 + \frac{H_L}{K_{HR}}\right) + H_R} - \gamma_{RR} R_{RH} = 0. \quad (7)$$

Thus

$$\begin{aligned} R_{RH} &= \frac{\alpha_{RH} R \frac{H_R}{K + H_R}}{\frac{\alpha_{RH} H_R}{K + H_R} + \gamma_{RR}} \\ &= \frac{\alpha_{RH} R H_R}{H_R (\alpha_{RH} + \gamma_{RR}) + K \gamma_{RR}}, \end{aligned} \quad (8)$$

where $K = K_R \left(1 + \frac{H_L}{K_{HR}}\right)$.

With the simplification above, the system of equations for the *rhl* system become just one differential equation. By assuming the basal production of *rhlI* genes is negligible ($\beta_{I0} = 0$), the governing equation becomes

$$\frac{dI_R}{dt} = \frac{\alpha_I \beta_I}{\gamma_{ml}} \frac{\alpha_{RH}^2 \beta_{HR}^2 R^2 I_R^2}{K_I^2 (\beta_{HR} I_R (\alpha_{RH} + \gamma_{RR}) + K \gamma_{RR} D_{HR})^2 + \alpha_{RH}^2 \beta_{HR}^2 R^2 I_R^2} - \gamma_I I_R. \quad (9)$$

We nondimensionalize this model by writing

$$I_R^* = \frac{I_R}{I_0}, \quad \text{and} \quad t^* = \frac{t}{t_0} \quad (10)$$

so the equation 9 becomes

$$\frac{dI_R^*}{dt^*} = \frac{b_1 I_R^{*2}}{b_2 (I_R^* + b_3)^2 + I_R^{*2}} - b_4 I_R^*, \quad (11)$$

where

$$\begin{aligned} b_1 &= \frac{\alpha_I \beta_I}{\gamma_{ml}} \frac{t_0}{I_0}, & b_2 &= \frac{K_I^2 (\alpha_{RH} + \gamma_{RR})^2}{\alpha_{RH}^2 R^2}, \\ b_3 &= \frac{K}{I_0} \frac{\gamma_{RR} D_{HR}}{\beta_{HR} (\alpha_{RH} + \gamma_{RR})}, & b_4 &= \gamma_I t_0. \end{aligned} \quad (12)$$

Here b_1, b_2, b_3 and b_4 are positive constants. The biological interpretation of this model is that 3O-C12-HSL inhibits the binding process of C4-HSL, which is represented by parameter $b_3 \propto K$ (definition of K can be seen in Eqs. 8). The activation of *rhl* system that is affected by *las* system describes positive connection. This is reflected by parameter $b_2 \propto \frac{1}{R^2}$. In addition, I_R^* degrade exponentially.

TABLE 3. Non-dimensional Parameters involved in the model of *rhl* system.

Name	Description	Standard value	Range
b_1	the production of signal molecules C4-HSL	3.51	2.45 – 4.9
b_2	The control of concentration of RhlR that is affected by the <i>las</i> system	1.69	$1.1 \times 10^{-5} - 8.42 \times 10$
b_3	The control binding of 3O-C12-HSL to RhlR/C4-HSL	1.47	0 – 735.29
b_4	The degradation of RhlI relative to the time	0.3	

RESULTS

The non-dimensional differential Eq. 11 has been investigated analytically with the assistance of Maple (18; MapleSoft) and solved numerically using MATLAB (R2016a; MathWorks).

Table 3 lists parameters that have either been adopted from the literature based on experimental evidence or estimated, as stated. Moreover, some parameters are chosen for the following reasons. The basal production rate of genes-mRNA can be considered as similar to the basal transcription rate of a protein. This is because the transcriptional regulator protein activates genes-mRNA in a very fast process before encoding the protein. We take typical values of total concentration of RhlR and RhlR/C4-HSL to be 200 nM, as for the concentration of QseB in *E. coli* [22].

The dimensionless variable b_1 is proportional to β_I , the maximum production rate of RhlI at which rhlI mRNA is activated by RhlR/C4-HSL. Parameter b_2 is inversely proportional to R^2 , depends on K_I and represents the relative binding strength of the RhlR dimer. b_2 is important factor to explore the effect of *las* system to the dynamical *rhl* system. We shall see that b_2 influences the location of the key bifurcation.

Parameter b_3 is inversely proportional to β_{HR} , the HSL production rate, an important factor in controlling both the intracellular HSL production and cell-cell communication. We shall see that the parameters b_2 and b_3 provide quorum memory explanation on *QS* system of *P. aeruginosa*. Meanwhile, parameter b_4 describes degradation rate of RhlI.

Downstream Impact of *las* to *rhl* System

The nullclines for concentration RhlI is a solution of $\frac{dI_R^*}{dt^*}$ equals zero. It can be seen clearly that Eq. 8 might have one, two or three fixed points. Those are $I_R^* = 0$ and two other fixed points that can be derived from

$$(b_2 b_4 + b_4) I_R^{*2} + (2b_2 b_3 b_4 - b_1) I_R^* + b_2 b_3^2 b_4 = 0. \quad (13)$$

The stability of those three points can be seen in Fig. 3.

The *rhl* system that is represented by Eq. 11 yields fold bifurcation if the discriminant Eqs. 13 is greater than or equal to zero. At first the system has stable steady state and nothing else, then there will be a point of loop that is a classic S-shape bifurcation diagram. From that diagram, we can get the creation from low stable states that leap to the high stable state by passing the unstable state, or conversely.

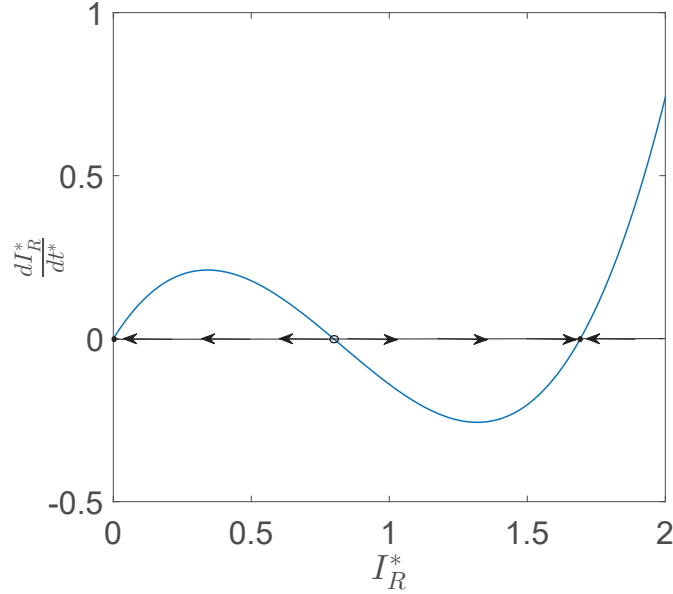


FIGURE 3. Three solutions are found at $\frac{dI_R^*}{dt^*}$ equals zero. Solid (open) circles denote stable (unstable) nodes. All parameters are in Table 3.

Initially we investigated the dynamics of the *rhl* system with a one-parameter bifurcation, b_2 . Non-dimensional parameter b_2 consist of R , which give information how the input from the *las* system affects the dynamical behaviour of the *rhl* system. We used continuation methods to track the evolution of solutions for I_R^* versus b_2 .

Figure 4 depicts a *reversible* bistability diagram and is also referred as *hysteresis*. The concentration of RhlI (I_R^*) is low until b_2 level exceeds the critical value, very small value that can be said it equals zero. This critical point is labelled BP (Branch Point), which is a permanent saddle-node. Any changes in parameter values of the model will not shift the position of the BP point, at which point the concentration of RhlI increases abruptly to a high value. In a similar manner, starting with b_2 is very low, the concentration of RhlI does not drop significantly until b_2 reaches the high critical value, SN (see Fig. 4, $b_2 = 1.77$).

Furthermore, the non-dimensional parameter b_3 is also supposed to be an essential parameter value in the *rhl* system. Once there is a change in the parameter value of b_3 , the critical value (SN) in Fig. 4 moves to another value. Moreover, we can recognize that one of the component of b_3 is the K -parameter, which represents inhibition of 3O-C12-HSL to the activator of C4-HSL. Thus b_2 and b_3 have different roles to determine the behaviour of the *rhl* system. Non-dimensional parameters b_2 and b_3 provide positive and negative effects, respectively, to the concentration of RhlI (Eqs. 11). For a clear picture of the relation between b_2 and b_3 , refer to Fig. 5.

Figure 5 demonstrates the downstream impact of *las* on the *rhl* system. We start from region A (K and R are small values) which represents low level of both 3O-C12-HSL and RhlR. Then, there is pulse generation on the *las* system that generates pulse production of 3O-C12-HSL. In the region B, the concentration of 3O-C12-HSL is increased and reaches the quorum level but the concentration of RhlR is still low. Once the quorum of 3O-C12-HSL is reached, then it increases the concentration of the LasR/3O-C12-HSL complex. This process triggers the transcription of genes in the *las* system (*rsaL* gene, *lasI* gene) and in the *rhl* system (*rhlR* gene). In the case of *rhl* system, the transcription of the *rhlR* gene increases the concentration of RhlR activator, R . Meanwhile, the consequent increase of 3O-C12-HSL levels also prevents the activation of RhlR by C4-HSL. However, the effect of pulses is still to increase the concentration of RhlR. This stage is referred to as ‘handbraked acceleration’, which represents the concentration of RhlR and 3O-C12-HSL in the high level (region C). Then, when the concentration of 3O-C12-HSL decreases as explained in [23], the handbrake of 3O-C12-HSL is removed and consequently increases the activation of the *rhl* system. Thus in the region D, the concentration of 3O-C12-HSL is low, while RhlR is high which leads to production of rhamnolipid. Following that, cells lose memory of their experience in the local environment with high concentration of 3O-C12-HSL. A decrease in the level of 3O-C12-HSL concentration over extended periods is followed by a decrease in the level of RhlR (a return to the region A).

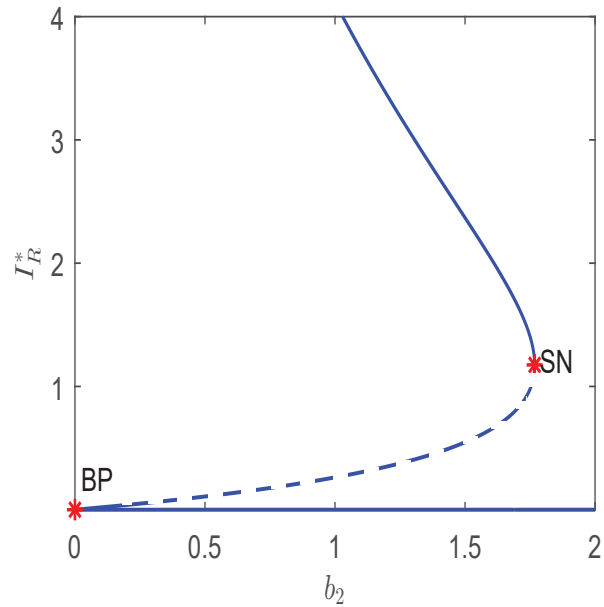


FIGURE 4. Bifurcation diagram for autoregulation of the *rhl* system with respect to b_2 , which represents the concentration of activator as the outcome of the *las* system. Solid (dashed) lines depict stable (unstable) steady states. Co-dimension-1 singular points marked as BP (Branch point) as permanent solution, and SN indicate a saddle-node point. All parameters are in Table 3.

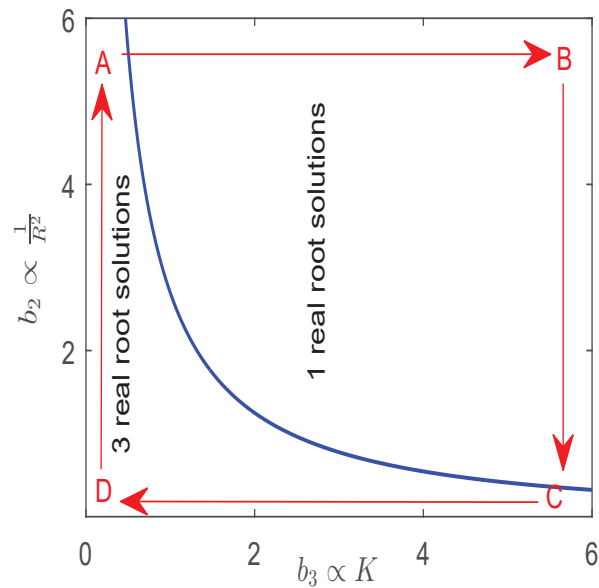


FIGURE 5. Two-dimensional bifurcation diagram for (b_3, b_2) . The *bifurcation lines* divide the parameter regions. Two different regions that explain how binding inhibition 3O-C12-HSL to the C4-HS (K) affects the concentration of RhlR (R). All parameters are in Table 3.

DISCUSSION

The autoinducer based QS system is a form of cell-cell communication, which is common among Gram-negative bacteria. Although QS of *P. aeruginosa* has been intensively studied, it is still unclear how the QS system acts as a global regulator of gene expression in the cell. Mathematical modelling has gained attention as a research tool to identify the process and key parameters for the system being studied. The *rhl* system model developed here are based on *las* system model of [6] and [21], as the *las* system can be considered to be above the *rhl* system through the activation of *rhlR* by LasR/3O-C12-HSL. Van Delden and Iglewski [5] demonstrated the *las* system is coupled to the *rhl* system by the LasR transcription factor and 3O-C12-HSL signal molecules. The LasR transcription factor promotes the transcription of the *rhlR* gene that induces the production of RhlR in the *rhl* system. Furthermore, Alfiniyah et al. [21] demonstrated the potential for the *las* system to act as a pulse generator on 3O-C12-HSL production.

We have described a model of the *rhl* QS system in *P. aeruginosa* by considering the positive feedback loop associated with the production of the synthase RhlI. Dimensionless equations describe the behaviour of the system, where we have taken parameters from the biological literature. Continuation methods were employed for the reduced system in the b_2 - b_3 plane to track two-dimensional diagram of the system. The parameters b_2 and b_3 represent the information inside and outside the cell, respectively. We demonstrated how the *las* system affects the dynamical behaviour of the *rhl* system. By showing that the *rhl* system follows the same dynamics as the *las* system, the *las* system, through the complex LasR/3O-C12-HSL, the production of RhlR is increased even though it is also handbraked by 3O-C12-HSL, inhibiting the activation of the *rhlR* gene and its downstream consequences. This demonstrates the inherent competition between production of the two signal molecules, 3O-C12-HSL and C4-HSL, which are produced by the *las* and *rhl* system, respectively, in terms of generating quorum memory in the *rhl* system.

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REFERENCES

- [1] M. A. Webber and L. J. V. Piddock, "The importance of efflux pumps in bacterial antibiotic resistance," *Journal of Antimicrobial Chemotherapy*, vol. 51, no. 1, pp. 9–11, 2003.
- [2] M. B. Miller and B. L. Bassler, "Quorum sensing in bacteria," *Annual Review Microbiology*, vol. 55, pp. 165–199, 2001.
- [3] D. R. Galloway, "*Pseudomonas aeruginosa* elastase and elastolysis revisited: recent developments," *Molecular Microbiology*, vol. 5, no. 10, pp. 2315–2321, 1991.
- [4] D. Glick, S. Barth, and K. F. Macleod, "Autophagy: Cellular and molecular mechanisms," *Journal of Pathology*, vol. 221, no. 1, pp. 3–12, 2010.
- [5] C. Van Delden and B. H. Iglewski, "Cell-to-cell signaling and *Pseudomonas aeruginosa* infections," *Emerging Infectious Diseases*, vol. 4, no. 4, pp. 551–560, 1998.
- [6] J. D. Dockery and J. P. Keener, "A mathematical model for quorum sensing in *Pseudomonas aeruginosa*," *Bulletin of Mathematical Biology*, vol. 63, no. 1, pp. 95–116, 2001.
- [7] E. C. Pesci, J. P. Pearson, P. C. Seed, and B. H. Iglewski, "Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*," *Journal of Bacteriology*, vol. 179, no. 10, pp. 3127–3132, 1997.
- [8] J. P. Pearson, E. C. Pesci, and B. H. Iglewski, "Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes," *Journal of Bacteriology*, vol. 179, no. 18, pp. 5756–5767, 1997.
- [9] D. G. Davies, M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg, "The involvement of cell-to-cell signals in the development of a bacterial biofilm," *Science*, vol. 280, no. 5361, pp. 295–298, 1998.
- [10] E. C. Pesci, J. B. J. Milbank, J. P. Pearson, S. McKnight, A. S. Kende, E. P. Greenberg, and B. H. Iglewski, "Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 20, pp. 11229–11234, 1999.

- [11] P. C. Seed, L. Passador, and B. H. Iglewski, "Activation of the *Pseudomonas aeruginosa lasI* gene by lasr and the *Pseudomonas* autoinducer pai: An autoinduction regulatory hierarchy," *Journal of Bacteriology*, vol. 177, no. 3, pp. 654–659, 1995.
- [12] T. De Kievit, P. C. Seed, J. Nezezon, L. Passador, and B. H. Iglewski, "Rsal, a novel repressor of virulence gene expression in *Pseudomonas aeruginosa*," *Journal of Bacteriology*, vol. 181, no. 7, pp. 2175–2184, 1999.
- [13] A. Latifi, M. K. Winson, M. Foglino, B. W. Bycroft, G. S. Stewart, A. Lazdunski, and P. Williams, "Multiple homologues of luxr and luxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* pao1," *Molecular Microbiology*, vol. 17, no. 2, pp. 333–343, 1995.
- [14] A. Latifi, M. Foglino, K. Tanaka, P. Williams, and A. Lazdunski, "A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators lasr and rhir (vsmr) to expression of the stationary-phase sigma factor rpos," *Molecular Microbiology*, vol. 21, no. 6, pp. 1137–1146, 1996.
- [15] M. G. Fagerlind, P. Nilsson, M. Harlén, S. Karlsson, S. A. Rice, and S. Kjelleberg, "Modeling the effect of acylated homoserine lactone antagonists in *Pseudomonas aeruginosa*," *BioSystems*, vol. 80, no. 2, pp. 201–213, 2005.
- [16] F. Chen, C. C. Chen, L. Riadi, and L. K. Ju, "Modeling *rhl* quorum-sensing regulation on rhamnolipid production by *Pseudomonas aeruginosa*," *Biotechnology Progress*, vol. 20, no. 5, pp. 1325–1331, 2004.
- [17] M. G. Fagerlind, S. A. Rice, P. Nilsson, M. Harlen, S. James, T. Charlton, and S. Kjelleberg, "The role of regulators in the expression of quorum-sensing signals in *Pseudomonas aeruginosa*," *Journal of Molecular Microbiology and Biotechnology*, vol. 6, no. 2, pp. 88–100, 2003.
- [18] U. Alon, *An Introduction to System Biology: Design Principles of Biological Circuits*. Boca Raton, FL: Chapman and Hall or CRC Press, 2006.
- [19] A. Raychaudhuri, A. Jerga, and P. A. Tipton, "Chemical mechanism and substrate specificity of rhII, an acylhomoserine lactone synthase from *Pseudomonas aeruginosa*," *Biochemistry*, vol. 44, no. 8, pp. 2974–2981, 2005.
- [20] A. Pai and L. You, "Optimal tuning of bacterial sensing potential," *Molecular Systems Biology*, vol. 5, no. 1, p. 286, 2009.
- [21] C. Alfiniyah, M. A. Bees, and A. J. Wood, "Pulse generation in the quorum machinery of *Pseudomonas aeruginosa*," *Bulletin of Mathematical Biology*, vol. 79, no. 6, pp. 1360–1389, 2017.
- [22] A. Ishihama, A. Kori, E. Koshio, K. Yamada, H. Maeda, T. Shimada, H. Makinoshima, A. Iwata, and N. Fujitac, "Intracellular concentrations of 65 species of transcription factors with known regulatory functions in *Escherichia coli*," *Journal of Bacteriology*, vol. 196, no. 15, pp. 2718–2727, 2014.
- [23] G. Rampioni, M. Schuster, E. P. Greenberg, I. Bertani, M. Grasso, V. Venturi, E. Zennaro, and L. Leoni, "Rsal provides quorum sensing homeostasis and functions as a global regulator of gene expression in *Pseudomonas aeruginosa*," *Molecular Microbiology*, vol. 66, no. 6, pp. 1557–1565, 2007.