Bacterial Growth and Siderophore Production in Bacteria: An Analytical Model

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Abstract: We have analyzed the impact of quorum sensing and resource dependency on the production of critically crucial for bacteria fitness compounds (siderophores). We have built two siderophore production models (quorum sensing and resource dependency) and linked them with Monod's growth model. As a result, siderophore accumulation is explicitly expressed through bacterial concentration N, which allows direct experimental verification. A nutrient-dependent model predicts three siderophore accumulation phases, which accompany bacterial growth: slow accumulation for $[N_0, N_{th}]$, fast accumulation for $[N_{th}, K/2]$, and slow or no accumulation for $[K/2, K)$. Here N_0 is the initial bacterial concentration, K is the carrying capacity. A quorum-sensing model predicts two regimes of siderophore accumulation: relatively slow accumulation for $[N_0, N_{cr}]$ and much faster non-linear accumulation for $[N_{cr}, K)$. N_{cr} and N_{th} are model parameters. N_{cr} has an "absolute" value. It is dependent on bacterial strain only. N_{th} has a "relative" value. In addition to the bacterial strain, it also depends on inoculums concentration and the initial nutrient concentration. Such as models predict entirely different behavior, experimental data may help differentiate between these mechanisms.

1 INTRODUCTION

Bacterial growth kinetics is a well-established research area, which can be traced back to classical growth models by Gompertz (Gompertz, 1825) and Verhulst (Verhulst, 1845). The significant conceptual step in developing bacterial growth models was introducing the concept of a limiting nutrient by Monod (Monod, 1941, 1949, 1950). Since then, multiple models have emerged (Richards, 1959), modified, or reparametrized (Zwietering, 1990). In particular, Gompertz, Baranyi, Richards, logistic, and three-phase linear models are the most widely used (Pla, 2015). These models are empirical and used mostly in the food safety industry. In Pia et al. (Pla, 2015), it was found that all these five models provided relatively high goodness of fit (R^2>0.93) for all growth curves for three different microorganisms (*Bacillus cereus, Listeria monocytogenes*, and *Escherichia coli*). Such as all models provide a good fit for experimental growth curves, the choice of a particular model is entirely subjective. Thus, the biological justification of model(s) can help with further selection and development of growth models. Some attempts were made to justify Monod's model (Lobry, 1992). Such as metabolism is described by the chain of reactions; some of each are enzymatic; it is not surprising that the Monod's growth factor is characterized by the Michaelis-Menten equation (Michaelis, 1913). However, further insights into biological justification would be of importance.

The related question to bacterial growth is the production of biomolecules, which are essential for bacteria fitness. Iron availability is a significant factor limiting the *in vivo* growth of bacteria (Ratledge, 2000). Bacteria developed multiple pathways to scavenge iron from the host. A vital pathway is to use siderophores, biomolecules used by some microorganisms to obtain iron from the environment. Siderophores' biosynthesis is iron-regulated. In response to iron limitation in their environment, genes involved in bacteria siderophore production and uptake are derepressed, leading to siderophores' production. The relationship between siderophore production and bacterial growth rates supports the hypothesis that siderophore production contributes to

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bacterial virulence. For example, mutants deficient in siderophore production have reduced virulence.

Mathematical modeling of siderophore production is concentrated mostly on bacterial cooperation and evolutionary strategies of siderophore production in bi-bacteria (Eberl, 2009) and multi-bacteria (Niehus, 2017; West, 2003) systems. In Fgaier et al. (Fgaier, 2008), the authors proposed a non-linear and non-autonomous system of four ordinary differential equations for the bacterial population, pyoverdine, dissolved iron, and chelated iron. In this model, the primary focus is on the inhibition of siderophore (pyoverdine) production in the presence of dissolved iron. In Niehus et al. (Niehus, 2017), the authors discussed a shift in the production of different siderophores in competitors' presence: downregulation of public siderophores and upregulation of partly privatized siderophores.

It is known that siderophore production is costly to bacteria (Griffin, 2004; West, 2003). It may decrease the growth rate by diverting resources (West, 2003) or increase the growth rate by making iron available (West, 2003).

The current work aims to investigate what factors can be responsible for regulating bacterial growth and the production of important biomolecules (e.g., siderophores). We have selected a quorum sensing mechanism and resource availability as two primary culprits.

We will attempt to build the bacterial growth and siderophore production models based on Monod's equations. In particular, we aim to link siderophore accumulation with experimentally measurable parameters (bacterial concentrations). Experimental verification will be provided in a separate article (Caschera, 2021).

2 METHODS

2.1 Bacterial Growth Model

It is well established that bacterial growth goes through four distinct phases: lag, log (or exponential), stationary, and death.

The bacterial growth in the case of limited resources can be described by Monod's equations (Monod, 1941, 1949, 1950):

$$\frac{dN}{dt} = N\frac{rS}{a+S} \tag{1}$$

$$\frac{dS}{dt} = \frac{-1}{\gamma}\frac{dN}{dt} \tag{2}$$

here, N is the concentration of bacteria, S is the nutrient's concentration, r is the growth rate, γ is the growth yield, a is a parameter.

From Eq.2, we can find the nutrient's concentration S as a function of the bacterial concentration N, which can be measured experimentally (e.g., using optical density, OD)

$$S = S_0 + \frac{N_0 - N}{\gamma} \tag{3}$$

Here, S_0 is the initial nutrient concentration; N_0 is the initial bacterial concentration.

2.2 Siderophore Production Model

Siderophores are critical components for bacteria fitness and virulence. It is known that bacteria do not always produce siderophores. Bacteria employ several mechanisms to regulate siderophore production.

It is well established that multiple bacteria strains have a quorum sensing (QS) mechanism. Each bacteria a) excretes a certain amount of a specific biomolecule (autoinducer) and b) detects these molecules' concentration. Such as a concentration of autoinducers is proportional to the local number of bacteria; the bacteria can regulate their individual functions based on their overall concentrations. Certain bacteria have multiple (e.g., *P.aeruginosa* has three) QS mechanisms, typically arranged hierarchically.

Similarly, one can expect that bacteria may have a mechanism to sense the nutrient's availability, or alternatively, the accumulation of the metabolic products (e.g., through pH).

Thus, our first hypothesis is that the siderophore production is governed by quorum sensing and/or resource availability. So, let's assume that at some point in time $t=t_0$, bacteria start producing a particular compound (e.g., a siderophore), diverting a portion (ζ) of consumed nutrients to the synthesis. Then, the expression for the compound concentration C can be linked to nutrient's concentration S:

$$dC = -\xi N\frac{dS}{N} = -\xi dS \tag{4}$$

3 RESULTS

3.1 Bacterial Growth

From Eq.3, we can obtain a well-known result: a "carrying capacity," the maximum concentration of

bacteria, which can be supported by a particular media:

$$K = N_0 + \gamma S_0 \quad (5)$$

3.2 Nutrient's Availability

Siderophore's function is to increase the fitness of bacteria in a hostile environment. Thus, it is plausible to hypothesize that bacteria may start producing siderophores when the nutrient's concentration drops below a certain level, S_{th}.

If bacteria started the synthesis at nutrient's concentration S_{th}, it corresponds to a bacterial concentration N_{th} through Eq.3:

$$N_{th} = N_0 + \gamma(S_0 - S_{th}) \quad (6)$$

Then integrating Eq.4 and taking into account Eq.6, we can write

$$C = -\xi(S - S_{th}) = \frac{\xi}{\gamma}(N - N_{th}) = \frac{\xi}{\gamma}(N - N_0) - \xi(S_0 - S_{th})) \quad (7)$$

In the final expression, we substituted N_{th} from Eq.6. Thus, we obtained an explicit equation for compound accumulation as a function of bacterial concentration. It is affected by the initial bacterial concentration N_0 and the initial nutrient concentration S_0.

A more general case is that bacteria change their siderophore production rate upon reaching the threshold S_{th}. We can suppose that bacteria divert a portion (ζ_1) of resources if the nutrient's concentration is above S_{th} and ξ_2 if it is below S_{th}.

$$C = \begin{cases} \frac{\xi_1}{\gamma}(N - N_0) & if\ S > S_{th} \\ C_1 + \frac{\xi_2}{\gamma}(N - N_{th}) & if\ S < S_{th} \end{cases} \quad (8)$$

In this case, solutions can be stitched together at $S=St_h$ if $C_1 = \xi_1(S_0 - S_{th}) = \xi_1(N_{th} - N_0)/\gamma$

Finally, it has been established (Bren, 2013) that in the last generation of bacteria, before growth stops due to resource limitation, bacteria growth is accompanied by a pulse-like up-regulation of gene expression in the relevant nutrient assimilation pathways. This mechanism circumvents other uses of nutrients (including siderophore production). It allows the cells to maintain their growth rate for about one more generation in which they can utilize low levels of substrate. Thus, we can expect that the last cycle of bacteria growth will not have siderophore production or have very minimal production.

Thus, we can expect that we can have three potential siderophore accumulation scenarios with bacterial growth (see Figure 1):

Phase I: Slow siderophore accumulation with bacterial growth for $N \in [N_0, N_{th}]$

Phase II: Fast siderophore accumulation with bacterial growth for $N \in [N_{th}, K/2]$

Phase III: No or minimal siderophore accumulation with bacterial growth for $N \in [K/2, K)$

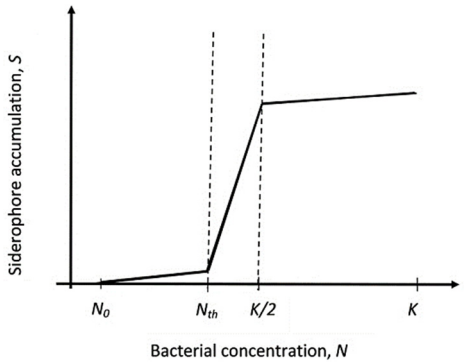

Figure 1: Three phases of siderophore production: slow accumulation, fast accumulation, and slow (or no) accumulation in nutrient-dependent case.

3.3 Quorum Sensing

Quorum sensing is another plausible mechanism of siderophore regulation. Quorum sensing works in two-phase mode (Dockery, 2001) (see Fig 2). At low densities, autoinducers' production is nominal and slowly increases with bacteria density until bacteria density reaches a critical value (N_{cr}). At this point, the production of autoinducers becomes autocatalytic and experiences a massive increase. If we assume that the siderophore production rate is proportional to autoinducers intracellular concentration, then for the portion of the diverted resources, ξ we can write

$$\xi = \begin{cases} \xi_L + N\kappa_L & if\ N < N_{cr} \\ \xi_H + N\kappa_H & if\ N > N_{cr} \end{cases} \quad (9)$$

Here we assumed that the bacteria population is growing (moving from left to right in Fig 2). Sub-indexes L and H indicate low (below critical) and high (above critical) bacterial concentration regions. In this approximation, we can solve Eq.4 onto two intervals: $N \in [0, N_{cr}]$ and $N \in [N_{cr}, K)$, separately (here K is the carrying capacity). Then, we can stitch them together at the point $N=N_{cr}$. If we consider the first interval, then Eq.4 can be rewritten as

$$dC = -(\xi_L + N\kappa_L)dS \quad (10)$$

If we express dS through dN using Eq.2 and substitute it into Eq.10, we will get

$$dC = \frac{\xi_L + N\kappa_L}{\gamma} dN \quad (11)$$

This equation can be solved

$$C = C_0 + \xi_L(N - N_0) + \frac{\kappa_L}{2\gamma}(N^2 - N_0^2) \quad (12a)$$

For *[0, N_{cr}]* interval, C_0 most likely is equal to zero (no initial siderophore).

Similarly, for the second interval, we will have

$$C = C_1 + \xi_H(N - N_{cr}) + \frac{\kappa_H}{2\gamma}(N^2 - N_{cr}^2) \quad (12b)$$

We can stitch solutions at the point $N=N_{cr}$. In particular, we can find that $C_1 = C_0 + \xi_L(N_{cr} - N_0) + \kappa_L(N_{cr}^2 - N_0^2)/2\gamma$

Most likely, siderophore production above critical value is higher than the below critical one. Bacteria colony has grown to a significant size, and now bacteria may focus on improving their fitness.

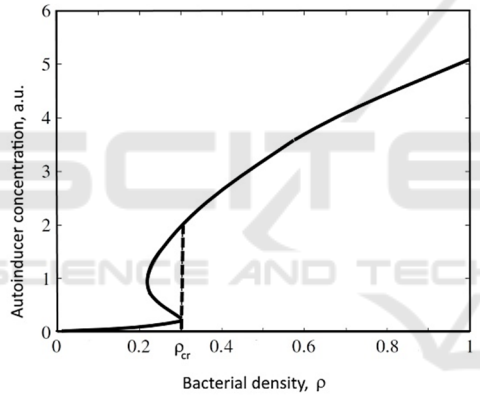

Figure 2: Quorum sensing mechanism: autoinducer concentration as a function of bacterial density (adopted from (Dockery, 2001) with modifications).

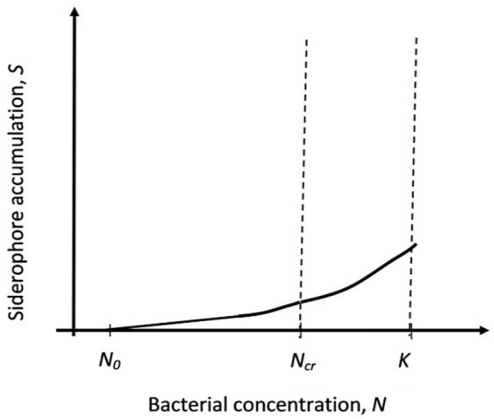

Figure 3: Siderophore accumulation as a function of bacterial concentration.

The siderophore accumulation as a function of bacterial concentration for the Quorum Sensing model is depicted in Figure 3.

3.4 Nutrient-dependent and QS Models Interplay

If nutrient-dependent and QS mechanisms are responsible for siderophore regulation, we have more potential siderophore accumulation scenarios. In particular, we can anticipate several major scenarios:

High resource availability: In this case, $N_{th}>N_{cr}$ and the dynamic of the siderophore accumulation is entirely driven by the QS mechanism

Low resource availability: In this case, $N_0<N_{th}<N_{cr}$, and we can observe all phases: I, II, and III.

Ultra-low resource availability: In this case, $N_0>N_{th}$, fast siderophore production starts immediately, and we can observe phases II and III only.

4 DISCUSSION

We have investigated bacterial growth and siderophores production using the same analytical framework based on Monod's approach. It allows us to express siderophore accumulation as an explicit function of bacterial concentration in two realistic cases: when siderophore production is a) resource-mediated and b) quorum sensing-mediated. Nutrient-dependent and quorum sensing models predict completely different behaviors at high bacterial concentrations. The nutrient-dependent model predicts saturation or slow growth, while the QS model predicts rapid siderophore accumulation.

A few words about model parameters: N_{cr} has an "absolute" value. It is dependent on bacterial strain only. N_{th} has a "relative" value. In addition to the bacterial strain, it also depends on inoculums concentration and the initial nutrient concentration. Some parameters can be measured (like *a, r, K*) or set (like N_0, C_0) experimentally.

It should be noted that all equations were derived under the assumption of homogeneous conditions. In reality, it is not obviously the case. Differences in microenvironment for each bacteria will result in different starting points for each phase for each bacterium. It will result in smoothening curves near N_{th}, N_{cr}, and *K/2*. The higher the homogeneity (e.g., in a continually shaking environment), the closer the curve shapes will be to the model prediction.

We assumed that the temperature remains constant. Obviously, various parameters may have different temperature dependencies, which can further complicate the model.

The initial validation of our theoretical predictions was performed on *Pseudomonas aeruginosa* elsewhere (Caschera, 2021). *P. aeruginosa* are a clinically relevant bacterial species and produce pyoverdine, a fluorescent siderophore. It can be of particular importance for remote quantification of bacterial presence using fluorescence bioimaging (Saiko, 2020). In Caschera et al. (Caschera, 2021), the model parameters (N_0 and C_0) were set experimentally. The experimental data show clear sigmoid dependence of bacterial fluorescence on bacterial concentration. It persisted through variations in temperature and inoculum starting condition. While the results are very preliminary, they indicate that *P. aeruginosa* fluorescence is primarily nutrient-driven.

5 CONCLUSIONS

We have built two simple siderophore production models (quorum sensing and resource dependency) and linked them with Monod's growth model. As a result, siderophore accumulation is explicitly expressed through bacterial concentration, which allows direct experimental verification.

The nutrient-dependent model predicts a sigmoid curve: three siderophore accumulation phases with bacteria concentration growth: slow accumulation for $[N_0, N_{th}]$, fast linear accumulation for $[N_{th}, K/2]$, and slow or no accumulation for $[K/2, K)$.

The quorum-sensing model predicts two regimes of siderophore accumulation: relatively slow accumulation for $[N_0, N_{cr}]$ and much faster non-linear accumulation for $[N_{cr}, K)$.

These models' interplay introduces more complex behavior (e.g., start and stop of siderophore production with bacterial population growth).

Such as models predict entirely different behavior, experimental data may help differentiate between them.

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