In Silico Evaluation of the Impacts of Quorum Sensing Inhibition (QSI) on Strain Competition and Development of QSI resistance Supplementary Information

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Supplementary Figures

**Applications**

**Tools**

**Models**

1. Biofilm dynamics (M1-M4)
2. Effects of QSI (M5-M10)
3. Spread of QSI resistance

Figure 1. Fig. 1R: Our simulation framework consists of three layers. The modeling layer contains the intracellular and intercellular model; this layer provides the basis to build up the tool and application layers. One of our main contributions is to develop a cell-cell interaction model that characterizes the biofilm dynamics which are discussed in the main manuscript (the Cell-Cell Interaction Network under Methods section). Next, we run these models to capture the biofilm dynamics at ideal growth conditions (M1-M4 in the main manuscript) Finally, we model the spread of QSI resistance under different classes of inhibitors (M5-M10 in the main manuscript).
Figure 2. Bacterial growth of $QS^+$ cooperators and $QS^-$ cheaters where cooperators produce non-beneficial goods, and suffer strict metabolic losses. In the simulations, spherical particles are used to represent cells; $QS^+$ cells are represented as red particles, $QS^-$ cells are blue.

Figure 3. Bacterial growth on a secondary limiting nutrient, accessible only when metabolized by QS-mediated private enzymes within a cell. $QS^+$ cells grow exponentially while the growth of $QS^-$ cells is impaired. $QS^+$ cells are represented as red particles, $QS^-$ cells are blue.

Figure 4. Bacterial growth with QS-mediated quasi-public goods secretion. $QS^+$ cells gain a competitive advantage by jettisoning themselves and their lineages into higher nutrient rich regions while suffocating $QS^-$ cells below. In these simulations, yellow spherical globules represent quasi-public goods which provides structural support for the biofilm.
Figure 5. Bacterial growth with QS-mediated production of diffusible public goods and colonization pattern where there is physical segregation of two genotypes. The three-dimensional heat maps depict the extracellular concentration of diffusible public good and demonstrates that, while the exoproduct is freely available to cheaters, the diffusivity and spatial configuration of cooperators constrains their growth. This suggests that genotypic locality and segregation are critical in determining the exploitability of a resource.
Figure 6. Dynamics of strain growth with biofilms composed of $QS^+$, $QS^-$, and $QSI$-resistant strains, when QS regulates the production of non-beneficial goods. Strains are represented by different colors (red: $QS^+$; blue: $QS^-$; green: $QSI$-resistant).

Figure set A: Projections of biofilm growth up to 10 days. Figure set Bi-Biii: Graphs displaying the relative ratio of each strain, before and after QSI addition under various types of QSI (i, ii, iii correspond to scenarios SE5, SE6, and SE7, respectively).

Figure set Biv-viii: Graphs comparing multiple QS and bacteria population measurements. Dotted lines corresponds to addition of QSI. Note that resistance to QSIs targeting signal accumulation is not considered, SE6 does not have $QSI$-resistant strain in the mix.
Figure 7. Dynamics of strain growth with biofilms composed of $QS^+$, $QS^-$, and QSI-resistant strains, when QS regulates the production of private goods (colored red, blue, and green respectively). Figure set A: Projections of biofilm growth up to 10 days. Figure set Bi-Biii: Graphs displaying the relative ratio of each strain, before and after QSI addition under various types of QSI (i, ii, iii correspond to scenarios SE8, SE9, and SE10, respectively). Figure set Biv-viii: Graphs comparing the consequences of different modes of QSI on multiple QS and bacteria population measurements. Note that resistance to QSIs targeting signal accumulation is not considered, SE19 does not have $QSI$-resistant strain in the mix.
Figure 8. Dynamics of strain growth with biofilms composed of $QS^+$, $QS^-$, and QSI-resistant strains, when QS regulates the production of diffusive public goods (red, blue, and green, respectively). Figure set A: Projections of biofilm growth up to 10 days. Figure set Bi-Biii: Graphs displaying the relative ratio of each strain, before and after QSI addition under various types of QSI (i, ii, iii correspond to scenarios SE11, SE12, and SE13, respectively). Figure set Biv-viii: Graphs comparing multiple QS and bacteria population measurements. Note that resistance to QSIs targeting signal accumulation is not considered, SE12 does not have QSI-resistant strain in the mix.
Figure 9. *Pseudomonas aeruginosa* uses *LasI/R* quorum-sensing networks. Specifically, *LasI* is an AHL synthase, *LasR* is the receptor which can bind the AHL molecules, and the *LasR – AHL* complex regulates a set of genes (red), including EPS and various virulence factors.

Figure 10. The evolution dynamics of different initial composition ratios of three genotypes. More precisely, the ratio of *QS*<sup>+</sup>, *QS*<sup>−</sup> and QSI-resistance cells is as follows: (i) 1:1:0.1, (ii) 1:1:0.3, (iii) 1:1:0.5, and (iv) 1:1:1.
Supplementary Note 1: Preliminary Success of QSIIs

Previous work on using QSI inhibitors can be characterized to target at least one of these three categories: (1) signal generation;\(^1\) (2) extracellular signal accumulation;\(^2\) (3) signal reception.\(^3\)

For targeting on the signal generation, extensive research has evaluated the mechanisms involved in AHL production. LasI synthase proteins utilize components of the amino acid and fatty acid biosynthesis pathways to produce AHLs. Recent in vitro studies have also shown that alterations in the biosynthesis of the LasI synthase proteins can decrease the production of active AHL molecules.\(^1\) This research data demonstrate that LasI is very sensitive to environmental conditions and that controlling the LasI substrates may have a significant effect on functional AHL production. Therefore, targeting the expression of LasI substrates can prevent the production of AHL, and thus QS activation.

For targeting on the signal accumulation, another extensively studied QSI strategy is the degradation and modification of the QS signaling molecules. Most enzymes identified thus far target AHL molecules. Lactonases degrade AHLs as they leave the bacteria, thus inhibiting their activation of LasR and host cells. Indeed, when these lactonases were expressed in P. aeruginosa, once can observe a significant decrease in AHL production and virulence factors expression.\(^2\)

For targeting on the signal generation, a recent study used a reporter assay to identify a group of compounds to inhibit the activation of LasR and elastase production.\(^3\) The antagonists are similar in structure to natural AHLs produced by P. aeruginosa and would compete for binding to LasR proteins; thus, inhibit the activation of LasR.

Supplementary Note 2: Simulations with QSI Effects and QSI Resistance, where QS Leads to Production of Non-beneficial Goods

We consider QS\(^+\) and QSI-resistant cells produce non-beneficial goods in response to QS activation. Also, we consider three QSI strategies, namely, a) targeting signal generation (SE5), b) targeting signal accumulation, and c) targeting signal reception (SE7).

When QS regulates the production of non-beneficial goods, the QS-regulated expression, QS\(^+\) genotype ratio, and QSI-resistant cell frequencies do not increase; in fact, the QSI-resistant can not dominate the population in all three scenarios due to the production of non-beneficial goods has a metabolic costs on QS\(^+\) and QSI-resistant cells, and this is not balanced by any fitness benefit. Overall, the use of QSI in the non-beneficial goods cases can effectively inhibit the QS-regulated expressions without inducing any bad outcome. That is to say, if the virulence factors are regulated by QS, and they confer no growth benefits to the producers, then QSI therapy should work very well.

Supplementary Note 3: Simulations with QSI Effects and QSI Resistance, where QS Leads to Production of Private Goods

As discussed earlier, private goods are typically secondary metabolites that are only accessible by producer cells, including QSI-resistant cells and QS\(^+\) cells (if their signal reception is not inhibited), therefore providing exclusive fitness benefits to the producer cells. In the following three scenarios, we model the 10-day biofilm evolution consisting of QS\(^−\) cells, QS\(^+\) cells, and QSI-resistant cells where QS\(^+\) and QSI-resistant cells produce private goods in response to QS activation. Similarly, we consider three QSI strategies: a) targeting signal generation (SE8), b) targeting signal accumulation (SE9), and c) targeting signal reception (SE10).

Compared to SE5, SE6, SE7, coupling between QS-regulated products and bacterial fitness leads to dramatically different evolution results, yet each QSI strategy present distinct risks of the development of resistance. Specifically, targeting signal accumulation results in weak resistance selection while targeting signal reception results in strong selection of QSI-resistance cells. Nevertheless, targeting signal molecule accumulation in the extracellular space continue to show potential as the best approach.

Supplementary Note 4: Simulations with QSI Effects and QSI Resistance, where QS Leads to Production of Diffusive Public Goods

As previously shown, the QS-mediated production of diffusive public goods shared by all cells imposes a significant negative impact on the fitness of QS\(^+\) cells. However, under QSI treatment, an important, but often overlooked detail is whether or not QS\(^+\) cells remain actively producing public goods. If not, QS\(^+\) cells incur no metabolic cost and are able to achieve growth rates comparable to QS\(^−\) cells. In the following three scenarios, we consider the 10-day biofilm growth consisting of QS\(^−\) cells, QS\(^+\) cells, and QSI-resistant cells where QS\(^+\) and QSI-resistant cells produce diffusive public goods in response to QS activation. Similarly, we consider three QSI strategies: a) targeting signal generation (SE11), b) targeting signal accumulation (SE12), and c) targeting signal reception (SE13).
In general, QSI therapy works well when the micro-environment at infection sites encouraging $QS^+$ and QSI-resistant cells to generate public goods, and the public goods can be effectively shared by $QS^-$ cheaters. More precisely, QSI treatment not only shuts down the QS-regulated expression immediately, but also prevents the increase of resistance and $QS^+$ genotype ratio (see Supplementary Fig. 8).

**Supplementary Note 5: Robustness of Simulation Parameters**

We examined the impact of considering different ratios of each strain at the beginning of simulations. More precisely, we use scenario M5 as an example to illustrate that the initial ratio among genotypes does not change the long-term evolution dynamics. Here, we consider four different composition ratios of $QS^+$, $QS^-$ and QSI-resistance cells. As shown in Supplementary Fig. 10, when the initial compositions of QSI-resistance cells increases, QSI-resistance cells enter the exponential growth phase earlier; this is due to the fact that QSI-resistance cells begin to massively produce QS-controlled public goods earlier. However, the long-term evolution dynamics is robust and not influenced by this selection of initial composition ratios of the three genotypes of cells (e.g. the QSI-resistance cells have growth advantage compared to other cell types).

We also conduct a robustness analysis of the model parameters to show the range of model parameters that can still capture the desired behavior. Since we calibrate the model parameters to fit the relative changes of the LasR-AHL concentration, the model parameters depend on the relative values. For example, the production rates $k_A$ and $k_R$ in eq (2) and (3) (in the main manuscript) are calibrated to be equal (i.e., $k_A = k_R$) and the binding and unbinding rates are calibrated to be of the same magnitude order. Therefore, we vary the values of these parameters to verify the robustness of the model.

As shown in Supplementary Table 3, the model parameters can accommodate 100X changes with respect to the nominal values we used in the simulations.
Table 1. Comparison of our proposed model with current bacteria simulators and models.

| Simulator | Environment | Intracellular Modeling | Physicochemical Interaction | Inter cellular Interaction |
|-----------|-------------|------------------------|-----------------------------|-----------------------------|
| Our Model | Complex 3D  | Multiscale stochastic   | Yes                         | Yes                         |
| NFSim[13] | Flat 3D     | Stochastic simulation   | No                          | No                          |
| AgentCell[13] | 3D       | Stochastic simulation   | No                          | No                          |
| RapidCell[14] | 2D       | Stochastic ODE          | No                          | No                          |
| Bsim[15]  | Complex 3D  | Rate-based              | Yes                         | No                          |

Table 2. Table of model parameters

| Parameter                                      | Symbol | Value         | Source |
|------------------------------------------------|--------|---------------|--------|
| AHL maximum production rate                   | $k_A$  | $2 \times 10^{-4} /s$ | [2 \times 10^{-4}, 2 \times 10^{-3}] $ |
| LasR maximum production rate                  | $k_B$  | $2 \times 10^{-4} /s$ | [2 \times 10^{-4}, 2 \times 10^{-3}] $ |
| AHL constitutive production rate               | $c_A$  | $1 \times 10^{-4} /s$ | [1 \times 10^{-4}, 1 \times 10^{-3}] $ |
| LasR constitutive production rate              | $c_B$  | $1 \times 10^{-4} /s$ | [1 \times 10^{-4}, 1 \times 10^{-3}] $ |
| LasR - AHL half saturation concentration      | $K_C$  | $1 \mu M$     | this study |
| Small molecule decay rate                     | $k_{d}$ | $1 \times 10^{-3} /s$ | [1 \times 10^{-3}, 1 \times 10^{-2}] $ |
| Binding rate of LasR and AHL                  | $k_1$  | $2 \times 10^{-3} /s$ | [2 \times 10^{-3}, 2 \times 10^{-2}] $ |
| Unbinding rate of LasR - AHL complex          | $k_2$  | $1 \times 10^{-3} /s$ | [1 \times 10^{-3}, 1 \times 10^{-2}] $ |
| Formation rate of LasR - AHL complex          | $k_3$  | $1 \times 10^{-3} /s$ | [1 \times 10^{-3}, 1 \times 10^{-2}] $ |
| Diffusion coefficient of extracellular molecules | $D_{ex}$ | $1 \times 10^{-6} cm^2/s$ | [1 \times 10^{-6}, 1 \times 10^{-5}] $ |

Table 3. Robustness of model parameters

| Parameter                                      | Symbol | Value         | Source |
|------------------------------------------------|--------|---------------|--------|
| AHL maximum production rate                   | $k_A$  | $2 \times 10^{-4} /s$ | [2 \times 10^{-4}, 2 \times 10^{-3}] $ |
| LasR maximum production rate                  | $k_B$  | $2 \times 10^{-4} /s$ | [2 \times 10^{-4}, 2 \times 10^{-3}] $ |
| AHL constitutive production rate               | $c_A$  | $1 \times 10^{-4} /s$ | [1 \times 10^{-4}, 1 \times 10^{-3}] $ |
| LasR constitutive production rate              | $c_B$  | $1 \times 10^{-4} /s$ | [1 \times 10^{-4}, 1 \times 10^{-3}] $ |
| LasR - AHL half saturation concentration      | $K_C$  | $1 \mu M$     | this study |
| Small molecule decay rate                     | $k_{d}$ | $1 \times 10^{-3} /s$ | [1 \times 10^{-3}, 1 \times 10^{-2}] $ |
| Binding rate of LasR and AHL                  | $k_1$  | $2 \times 10^{-3} /s$ | [2 \times 10^{-3}, 2 \times 10^{-2}] $ |
| Unbinding rate of LasR - AHL complex          | $k_2$  | $1 \times 10^{-3} /s$ | [1 \times 10^{-3}, 1 \times 10^{-2}] $ |
| Formation rate of LasR - AHL complex          | $k_3$  | $1 \times 10^{-3} /s$ | [1 \times 10^{-3}, 1 \times 10^{-2}] $ |

Supplementary Video 1: Biofilm Formation in Homogenous Populations of Bacteria
Supplementary Video 2: Biofilm Formation in Heterogeneous Populations of Bacteria
Supplementary Table
Supplementary References

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