Molecular Tweeting: Unveiling the Social Network Behind Heterogeneous Bacteria Populations

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ABSTRACT

It is well established that bacteria engage in social behavior and form networked communities via molecular signaling. However, most studies published to date focus on the intracellular molecular networks rather than the intercellular networks formed across strains and species. Therefore, in this paper, we define for the first time a bacteria intercellular network and describe its dynamics and contribution to biofilm formation. We apply our methods to a heterogeneous bacteria population consisting of strains that are often identified from clinical isolates, namely, the wild-type (cooperator), and its signal-blind and signal-negative (cheater) mutants. We analyze the network dynamics and biofilm metrics, showing that our method can effectively reveal the underlying intercellular communication process and community organization within the biofilm. We claim that the application of social and network sciences to understanding bacteria population dynamics can aid in developing better drugs to control the many pathogenic bacteria that use social interactions to cause infections.

Categories and Subject Descriptors
J.3 [Life and Medical Sciences]: Biology and genetics; J.4 [Social and Behavioral Sciences]: Sociology; B.4.3 [Interconnections (Subsystems)]: Topology

General Terms
Theory

Keywords
Biofilm, Dynamic network evolution, Quorum sensing

1. INTRODUCTION

Despite their size, bacteria hold an overwhelmingly significant influence over earth’s biota [41], playing both beneficial [6] and pernicious [12, 7] roles in human health. Until relatively recently, the prevailing notion of bacterial behavior was one in which individual cells coexisted within a population but acted independently with respect to their neighbors. However, over the past two decades, it has become widely acknowledged that bacteria, in fact, communicate and organize to perform a broad range of collective behaviors through a form of molecular signaling called quorum sensing (QS) [40, 21]. Consequently, a substantial share of research has been devoted to elucidating the mode, mechanisms, and effects of intercellular organization [36]. Empirical evidence suggests that the intracellular production and secretion of signaling molecules, known as autoinducers, are controlled by a positive feedback genetic pathway dependent on the ambient environmental concentration of autoinducer surrounding a cell [38, 27]. This genetic machinery facilitates the increase of intracellular concentration of autoinducer within local cells until a molecular consensus, or quorum, is met. Thus the QS system equips individual bacteria with the ability to detect and measure local cell density by proxy, enabling group level coordination for carrying out population sensitive collective behaviors including, but not limited to, the production of a variety of virulence factors, digestive enzymes, and extracellular polymeric substances (EPS) used in biofilm formation [31, 35, 37]. Moreover, these population level behaviors are vital to the fitness, community coherence, and drug resistance of a bacterial species at newly colonized infection sites, highlighting the critical role QS plays in pathogenesis.

Although the intracellular biochemical pathways which give rise to QS have been well studied from both molecular and systems biological perspectives [32, 30, 9, 10], a higher level description of intercellular communication—one capable of capturing population level patterns—has yet to be explored. To this end, we adopt a network centric description of intercellular signaling in which we draw from canonical network models as a basis of abstraction for communication among agents [29]. Figure 1 depicts several phases throughout the development of a bacterial network as described by our methods.

Biofilms rarely exist as pure, homogenous colonies, rather, hundreds or even thousands of distinct bacterial species tend to coexist in cooperation or conflict with one another at a single site. Within microbiomes, distinct types of bacteria can establish molecular communication networks, compete for resources, and collaborate towards common goals in similar ways as in macroscale societies. The interactions among different types of bacteria within the community—each hav-
conversion of public goods to private goods. We argue that the commons is avoided in the experimental cases via the following explanation of how the tragedy of the commons arises. Furthermore, we observe that the emergence of important exoproducts such as EPS and virulence factors can be largely predicted from these network dynamics. We show that molecular social network dynamics track closely to biofilm dynamics, and that the production of these virulence factors—have a critical influence on the subsequent population dynamics of a developing community, as well as biofilm formation. For example, three types of pathogenic bacterial strains often found in clinical studies—wild-type (WT) cooperators, signal-blind (SB), and signal-negative (SN) mutant cheaters—belong to the same species but differ in their QS behavior, leading to dramatically different population evolution outcomes. The communication network dynamics among the three strains can be easily understood using a terminology adopted from social networks. The WT cooperators compose molecular tweets such as “generate public goods like EPS or virulence”, and broadcast them to others through molecular diffusion. Upon receiving these messages, nearby WT cooperators retweet the message immediately, while starting to produce goods following the instructions in the message. However, the SB cheater strain tweets cooperative messages but ignores the messages it receives, saving resources for its own growth rather than producing public goods. The SN cheater strain, on the other hand, does not tweet or retweet messages; instead, they keep the message secret and produce the goods silently. In these cases, it is clear that variations in communication behavior can allow certain types of bacteria to exploit the behaviors of others. More precisely, bacterial cheaters do not cooperate to produce public goods, but gain the benefit from those who do, would gain a growth advantage and be able to take over the population—an instantiation of the “tragedy of the commons” [15]. However, if this were the case, what would be the evolutionary drive for cooperative behaviors [14, 13]?

In this work we investigate the population dynamics of simple heterogeneous biofilms consisting of WT, SB, and SN strains. We show that molecular social network dynamics track closely to biofilm dynamics, and that the production of important exoproducts such as EPS and virulence factors can be largely predicted from these network dynamics. Furthermore, we offer an explanation of how the tragedy of the commons is avoided in the experimental cases via the conversion of public goods to private goods. We argue that abstracting the complex interactions of cellular communication into a representation readily treated with methods from network science will not only further our understanding of complex microbial societies, but also aid the discovery of effective alternative therapeutics in the fight against multiresistant bacteria [5, 33, 16, 3].

In the following subsections, we first describe the LuxI/R QS system used by most gram-negative bacteria, including how signal molecules are generated, how they bind to receptors, and how the binding product-receptor complex regulates the public goods production. Then, we derive a single substrate growth model used in our biofilm simulation environment with a constant nutrition concentration at the top bulk layer (Figure 2). Last but not least, we define a QS-based bacteria network by identifying the signal spreaders, receivers, and responders in bacterial communities.

2. BIOFILM MODEL

As computers become more and more powerful, a “bottom-up” agent-based approach to study the evolution of pathogen populations is the next milestone towards understanding their social behaviors and for treating the infectious diseases they cause [24], especially for chronic infections. Therefore, we adopt a “bottom-up” approach and develop a mixed cell- and ordinary differential equation based model for the cell-to-cell/cell-EPS physical interactions and molecular dynamics of a QS system, respectively. In the cell-based model, bacteria are considered as spheres, and physical interaction among various agents is explicitly modeled. 2.1 Cellular Dynamics Model

2.1.1 Quorum Sensing

Using the LuxI/R system as a basis for the QS model is relevant for describing species such as the opportunistic pathogen Pseudomonas aeruginosa [32, 8]. The LuxI/R system is mediated by autoinducer signal molecules, such as acylated homoserine lactone (AHL), produced by the synthase luxI homologs. The signal molecule AHL binds
to LuxR receptors and activates the transcription regulator (LuxR homologs). This LuxR – AHL complex leads to the transcription of a plurality of genes that are directly involved in bacterial metabolism and collective behavior.

The molecular network of the LuxI/R QS system has two positive feedback loops. More precisely, as shown in Figure 3, the LuxR – AHL complex upregulates the expression of both LuxR and LuxI genes, which generate more AHL molecules and LuxR receptors. Based on the ODE-models proposed in [25, 42], we have the following equations to describe the time-varying dynamics of the LuxI/R QS system:

\[
\frac{d[A]}{dt} = c_A + \frac{k_A[A]^C}{K_A + [C]} - k_0[A] - k_1[R][A] + k_2[RA] \quad (1)
\]

\[
\frac{d[R]}{dt} = c_R + \frac{k_R[R]^C}{K_R + [C]} - k_3[R] - k_4[R][A] + k_5[RA] \quad (2)
\]

\[
\frac{d[R][A]}{dt} = k_1[R][A] - k_2[RA] - 2k_4[RA]^2 + 2k_5[C] \quad (3)
\]

\[
\frac{d[C]}{dt} = k_4[RA]^2 - k_5[C] \quad (4)
\]

where \([X]\) denotes the concentration of a particular molecular species \(X\), \(t\) represents time. In our formulation, \(A\) is AHL, \(R\) is LuxR homolog, \(RA\) is the LuxR – AHL complex, \(C\) is the dimerized complex, \(c_A\) and \(c_R\) account for the basal level transcription of \(A\) and \(R\), respectively. The values and references of these parameters (see Table 1) are adapted from a general LuxI/R system [25] and reflect those used to characterize \(P.\ aeruginosa\).

To give some intuition, the first term of Eq.(1) describes the basal level transcription, the second term captures the positive feedback loop regulated by the dimerized complex \(C\), the third and forth terms describe the AHL concentration changes caused by the binding and unbinding reactions of AHL and LuxR receptor, respectively. Eq.(2) and Eq.(4) describe the binding reaction of AHL and LuxR receptor, as well as dimerization process of the binding product \([RA]\).

Therefore, to model the SB mutant strain, based on the WT cooperator model parameters in Table 1, we set \(c_R = 0\) and \(k_R = 0\) to make sure LuxR molecules are never produced. Similarly, we set \(c_A = 0\) and \(k_A = 0\) to make sure SN mutants do not generate any molecular message.

### 2.1.2 Cell Growth Model

For simplicity, we consider a single nutrient controlled kinetics; Monod introduced this concept to describe the micro-

\[
\mu = \frac{S}{S + K_s}
\]

The kinetic parameters, maximum specific growth rate \((\mu_{max})\) and substrate affinity \((K_s)\), are assumed to be constant, but dependent on strain, medium, and growth conditions, such as temperature and pH. When cells are metabolically active, but not growing or dividing, they may still take up substrate. This effect is not considered in the original model in Eq.(5) [28]. To address this, a maintenance rate \((m)\) is generally used to describe the reduction, hence Eq.(5) is modified to:

\[
\mu = \mu_{max} \cdot \frac{S}{S + K_s} - m
\]

To balance the limiting substrate \(S\), we introduce a utility parameter \(U\) to model the consumption of substrate nutrition due to cell growth:

\[
\frac{d[S]}{dt} = -UX \cdot \mu = -UX \frac{d[X]}{dt} \quad (7)
\]

Therefore, higher cell densities can lead to a decreased growth rate \(\mu\) in a nutrition-limited environment.

Also, we need to take into account the cost of generating QS-related molecules; therefore, we modify the consumption equation to be:

\[
\frac{d[S]}{dt} = -UX \frac{d[X]}{dt} - U_{AHL} \frac{d[A]}{dt} - U_{LuxR} \frac{d[R]}{dt} \quad (8)
\]

where utility parameters \(U_{AHL}\) and \(U_{LuxR}\) model the consumption of substrate nutrition due to the production of AHL and LuxR receptors, respectively.

### 2.1.3 EPS Production

We model the production of EPS as a function of the intracellular dimerized LuxR – AHL complex \(C\) while incurring a cost on the carbon substrate \(S\) in Eq.(7):

\[
\frac{d[EPS]}{dt} = k_{EPS} \frac{[C]}{[C] + K_C}\n\]

\[
\frac{d[S]}{dt} = -UX \frac{d[X]}{dt} - U_{AHL} \frac{d[A]}{dt} - U_{LuxR} \frac{d[R]}{dt} - U_{EPS} \frac{d[EPS]}{dt}\n\]

where \(k_{EPS}\) is the maximum EPS production rate and \(U_{EPS}\) is the utility of EPS production. Using this model, we expect a maximum rate of EPS production once cells reach a quorum through communication (i.e. the level of AHL in the extracellular environment exceeds a given threshold).

### 2.2 Intercellular Network Model

The QS-based bacteria network is significantly different from traditional networks in several aspects:

- The signal molecules do not have specific destinations encoded in the information (e.g. the address in the IP packet); they simply diffuse in the environment. Therefore, information cannot be precisely “routed” to target recipients in a network.

- The diffusion of molecules is spatially limited and is significantly slower than the kinetic dynamics of bacteria. In other words, each bacterium has a limited influence range in space; for efficient communication, bacteria need to stay close to each other.
Bacterial networks based on QS use signaling molecule concentrations to represent information that is generated collectively by a large number of bacteria; each bacterium contributes minimally to the overall extracellular AHL concentration.

Based on the above observations, we propose a QS-based network definition that considers both intracellular and extracellular factors. More specifically, a directed link from bacterium A to bacterium B is established under three conditions (Figure 4):

- Bacteria A and B must be within a diffusion-limited signal influence range $T_D$.
- The autoinducer signal concentration inside bacterium A is larger than that of bacterium B. Therefore, there is a descending AHL gradient from A to B.
- The concentration of the signal receptor (e.g., LuxR) of bacterium B is above an activation threshold $T_R$.

The first condition represents the fact that molecular diffusion is relatively slow and distance limited, thus the AHL produced and secreted by a bacterium has direct impact only within a range $T_D$ depending on AHL production rate, properties of the signal molecules and their diffusion medium. The second condition specifies the link direction, while the third ensures that bacterium B is able to receive QS signals.

To account for different AHL productivity of spreader bacteria, we classify them into four categories based on intracellular AHL production rates $P_A$. More precisely, the first category includes spreaders with $P_A < \frac{1}{4}P_{A^{\text{max}}}$, where $P_{A^{\text{max}}} = c_A + k_A$ is the maximum production rate, and cells have an influence range $T_D = 5\mu m$. Similarly, $T_D = 10\mu m$ for the second category: $\frac{1}{4}P_{A^{\text{max}}} < P_A < \frac{1}{2}P_{A^{\text{max}}}$; $T_D = 20\mu m$ for the third category: $\frac{1}{2}P_{A^{\text{max}}} < P_A < \frac{3}{4}P_{A^{\text{max}}}$. Finally, $T_D = 40\mu m$ for the super spreader category: $\frac{3}{4}P_{A^{\text{max}}} < P_A$.

3. RESULTS

Using an open-source simulator [39], we set up a 3D environment of size $(300\mu m, 300\mu m, 500\mu m)$ in which 1000 non-overlapping (generic) bacterial agents are randomly attached to the surface at the bottom. To support the survival and growth of bacteria cells, we assume a constant nutrition concentration $S = 100\mu M$ in the bulk layer such that the cells can get more nutrition as they grow upwards from the attachment surface towards the bulk layer (Figure 2).

A cell is considered to be a receiver if its intracellular LuxR concentration is above a threshold $T_R = 0.2\mu M$. A cell divides into two daughter cells when its radius is above $T_{\text{div}} = 2\mu m$, and dies when the value goes below $T_{\text{div}} = 0.5\mu m$. Dead cells are not removed, but exist as cell debris.

To show the social dynamics in the formation of heterogeneous biofilms, we consider four scenarios, namely, (S1) pure WT; (S2) half WT and half SB mutant; (S3) half WT and half SN mutant; (S4) 1/3 WT, 1/3 SB mutant, and 1/3 SN mutant. In the following subsections, we discuss these four cases from different perspectives.

3.1 Biofilm Evolution

To give some intuition, let us first see the 24-hour biofilm evolution dynamics for all scenarios (videos are available at http://goo.gl/hyrMol). As it can be seen from Figure 5, we take the snapshots of the biofilm development at time $t = 0.2\text{hrs}$, $t = 1.5\text{hrs}$, $t = 10\text{hrs}$, and $t = 24\text{hrs}$, which capture the most significant changes of the system as a result of the social evolution [26]. In our simulations, we use particles to represent both cells and EPS; SB cells are represented as red particles, SB mutants are blue, and SN mutants are yellow. Small green particles represent EPS which provides structural support to the biofilm. Both WT and SN bacteria generate EPS particles while SB mutants do not. Note that the boundary is periodic, which is why some cells appear to be floating without support (they are actually connected to the other side of the world.)

As shown in Figure 5, in all scenarios WT cooperators start tweeting and retweeting the message of producing EPS around $t = 1.5\text{hrs}$. In scenario S1, where the WT cooperators is the only strain present, all bacteria participate in tweeting and retweeting the message of producing EPS, and the EPS generation process quickly spans the spatial domain. In the other three scenarios, EPS generation only takes place in locations with high density of WT cooperators.

Like macro-scale evolution, the evolution of microbes given certain environmental conditions converges to only a few cases. At time $t = 10\text{hrs}$, S1 develops a thick biofilm uniformly supported in space by EPS particles. Surprisingly, in S2, the SB mutant cheaters do not replicate as much as the WT cooperators. On the contrary, they suffocate at the bottom, losing the ability to compete for nutrition coming from the top bulk layer. Similar dynamics can be observed in S4. On the other hand, the SN strain, which does produce EPS particles to support its growth towards upper layer, survives the competition in both S3 and S4. However, due to the fact that the SN strain does not tweet or retweet any chemical message, the overall AHL concentration within the spatial domain is lower than the S1 and S2 cases, resulting in lower EPS and virulence production rates.

These simulation results can explain how pathogenic bacteria are able to avoid the “tragedy of the commons”. More precisely, due to the nature of immobile EPS, it cannot provide structural support to cheaters that are too far away, therefore, the cheaters cannot efficiently exploit these exoproducts. From this perspective, a locality principle seems to govern when “public goods” ought to be more suitably considered “private goods”. Therefore, it is this locality
Figure 5: Biofilm evolution in four different cases, (S1) pure WT; (S2) half WT and half SB mutant; (S3) half WT and half SN mutant; (S4) 1/3 WT, 1/3 SB mutant, and 1/3 SN mutant. In our simulations, we use particles to represent both cells and EPS; WT cells are represented as red particles, SB mutants are blue, and SN mutants are yellow. Small green particles represent EPS which provide structural support to the biofilm; both WT and SN bacteria generate EPS particles while SB mutants do not. Note that the boundary is periodic, which is why some cells appear to be floating without support (they are actually connected to the other side of the world.)
Figure 6: Bacteria network evolution for S4 case. (a) $t = 0$ hrs. (b) $t = 12$ hrs. (c) $t = 24$ hrs. Communities emerge in regions with the highest WT cooperator densities, which means that $AHL$ accumulation in the initial stage is carried out by the WT cooperators. This is because SN mutants do not generate $AHL$ and SB mutants do not have the $AHL$ positive feedback loop involving the $LuxR$ receptor (The video is available at http://goo.gl/hyrMol).

and population structure which prevents the “tragedy of the commons” in bacterial society. Note that EPS as the sole public good is an extreme case; many other types of public goods are diffusible in the extracellular space and therefore can be exploited to a larger extent by the cheaters. However, the locality/population structure still plays a major role here in constraining the cheaters.

3.2 Biofilm Metrics

To get a deeper understanding of the biofilm formation process, we characterize some network metrics as well as classic biofilm measurements. Figure 8.a shows that the maximum biofilm thickness for the WT cooperators is about $40\mu m$ more than all other cases. This is confirmed by Figure 5 where the S1 biofilm at $t = 24hrs$ has several large mushroom-like structures separated by water-filled channels, which are often observed in wet-lab experiments [34].

On the other hand, biofilm roughness—the standard deviation of the thickness distribution divided by mean thickness—shows some interesting behaviors (Figure 8.b). At the beginning, the roughness coefficients in all cases are high as a result of the random spatial attachment of the initial 1000 cells. Then the S1 (green) and S3 (blue) mixed cases dip down to almost a homogenous flat film due to the generation of EPS which quickly fills the free extracellular space. As the biofilm develops, both the S1 (green) and S4 (red) cases end up with a high roughness for different reasons. In S1, the emergence of mushroom- or pillar-like structure induced the rise of roughness while the spatial imbalance caused by the genotype heterogeneity is the roughness source in S4.

To be clinically relevant, we are focused on virulence production, as it has direct consequence on patient survival rate. In this work, we assume virulence is positively related by QS system as supported by experimental evidence [19], and virulence is measured indirectly through the concentration of $LuxR - AHL$ complex. As shown in Figure 8.c, the cases producing the highest levels of virulence are S1 (green) and S2 (pink). This outcome is clear in Figure 5, where the WT becomes the dominant strain as the most virulent genotype. However, to get a deeper understanding of the evolution dynamics, we need to change our view to a network perspective; this is discussed in the following sections.

3.3 Zooming in the Biofilm Formation

To see more clearly the network communication process and its relationship to biofilm formation, we study in detail scenario S4, in which we have 1/3 WT, 1/3 SB mutant, and 1/3 SN mutant in the initial population. As can be seen from Figure 6, individual network communities emerge in the regions that have the highest WT cooperator density. The small communities consisting of tens or hundreds of cells (Figure 7) then expand to have a few thousand cells within them. However, the spatial population structures and genotype segregation clearly prevent them from merging into one large network component. From this perspective, the bacterial communication networks are distributed networks without a clear center.

Figure 7: A small region at $t = 10hrs$ for S4. It is clear that WT cooperators form densely-knit individual communities. The purple lines represent the network links.

In Figure 7, we focus on a small region of the biofilm to get a better sense of how bacterial networks form in our simulations. The snapshot is taken at $t = 10hrs$, when most of the network links exist among the WT cooperators and some links are formed from WT cooperators to SN mutants. Accordingly, EPS particles are produced inside and around the network communities. As the $AHL$ accumulation/diffusion process continues, most of the SN cells join the communities and generate EPS to support their growth upwards.
Figure 8: Biofilm evolution dynamics. (a) Maximum biofilm thickness. WT cooperators form mushroom-like structures that are much thicker than others. (b) Biofilm roughness measures the heterogeneity of a biofilm. Spatial irregularities and genotype segregation result in a rough surface. (c) Virulence production. In this paper, virulence production is assumed to be positively regulated by the LuxR–AHL complex, thus the amount of virulence being produced is proportional to the complex concentration. (d)(e) Number of network communities and the diameter evolution in time. Bacteria form small to medium sized communities during the transition period, the number of communities stabilizes and decreases monotonically thereafter as community expansion ensues. However, the average clustering coefficient of around 0.5 indicates that the cells have tight connections with each other inside individual communities; this is also clearly shown in Figure 7. The high clustering coefficients not merge into a few strongly connected giant components as one would have expected. In fact, due to genotype segregation, AHL diffusion constraints, and various 3D spatial structures formed across the biofilm, bacterial cells always stay in small to medium size communities, usually consisting of a few thousand interacting bacteria and having a diameter of 20-40 links (Figure 8.e). Therefore, trying to find and attack the “central control” of a bacteria population in order to cure infections may not be a wise strategy.

As shown in Figure 8.f, the average clustering coefficient spikes to its maximum as communities initially form locally due to proximity and initial local AHL accumulation and decreases monotonically thereafter as community expansion ensues. However, the average clustering coefficient of around 0.5 indicates that the cells have tight connections with each other inside individual communities; this is also clearly shown in Figure 7. The high clustering coefficients

3.4 Network Metrics

The first metric we calculate is the number of communities in the bacterial society. As shown in Figure 8.d, the communities emerge around \( t = 1.5 \text{hrs} \), followed by the transition period during which cells replicate very fast in a relatively nutrient rich environment, then communities form, merge, and recombine with each other until \( t = 10 \text{hrs} \). After the transition period, the number of communities stabilizes and the difference among the four cases becomes clear. Specifically, the S1 and S4 cases have the most independent network communities; we find a strong correlation between the community number and the biofilm roughness coefficient (Figure 8.b). Indeed, more independent communities result in a higher roughness coefficient of the biofilm.

We also note that the number of communities does not decrease as the biofilm matures, even in scenario S1 where WT is the only strain. This implies that distributed groups do
observed in the mixed strain cases are induced by more structured bacterial population due to genotype segregation.

In Figure 8.g, the early “explosion” of links can be noticed immediately following QS activation ($t = 2\text{hrs}$), after which the link count increases monotonically, approaching a saturation level constrained by the limited nutrition availability. As mentioned before, the number of links is directly correlated with information propagation efficiency in the network. An efficient network can lead to a much higher virulence production as observed in Figure 8.c. Figure 8.h, on the other hand, shows the strain composition in the biofilm which can better explain the evolution in Figure 5. For example, in the S2 case the WT cooperators clearly become the dominant species and take up to nearly 90% of the entire population at $t = 24\text{hrs}$; this confirms the evolution advantage of cooperators over the SB cheaters. On the contrary, the WT cooperators do not have a clear growth advantage compared to the SN cheaters; this is because although SN cheaters do not tweet or retweet any message, they do receive and follow the instructions in the messages sent by others and generate EPS particles to help them get nutrition. Note that the cooperators have a growth advantage at early stages ($t < 10\text{hrs}$) because the AHL concentration is much higher in the cooperator clusters, resulting in a higher EPS production rate; this advantage becomes negligible as AHL continuously diffuses across the space, making the concentration landscape more homogeneous.

Figures 8.i and g show that virulence production correlates not only with biofilm thickness/roughness - physical properties - but also with the percentage of networked cells in a bacteria population and the number of network links - network metrics. The highest virulence is produced in the WT (pure) strain case, the second highest being in the WT and SB mixed case. Similar to other correlations, our network metrics always identify the trend in advance, showing its predictive power for key biofilm factors such as thickness and virulence, based on the underlying communication process. Given such observations, it is desirable for some of our in silico characterization techniques to be implemented in vitro, analyzing clinical samples in the lab. Possible approaches include adding fluorescence flags to the luxR receptors or synthesizing “probe” bacteria with genes of fluorescence proteins fused to the downstream of its QS system to measure the AHL concentration level [11, 1]; such approaches can easily measure overall network activities in the biofilm and help physicians understand the underlying dynamics.

### 3.5 Degree Distribution

The degree distributions in the four scenarios are indicative not only of the impact that mutant phenotypes have on connectivity from a network perspective, but also of the biological implications of reaching a quorum.

Figure 9 shows the degree distributions of the different mutant frequency cases in the incipient network formation stage (red) and advanced development (blue), under the same environment conditions. Early stages of network formation are uniform across all cases due to bacteria initializing connections only with the closest neighbors, thus we...
see consistent normal fits in node degree distributions. The advanced development stage is characterized by significantly higher cell density, thus elevated quantities of AHL in the extracellular medium. In the wild type case (Figure 9.a), the degree distribution maintains a normal shape throughout the network development stages, mirroring the population homogeneity. In contrast, mutants are characterized by a deficiency in forming links, either on the incoming (SB) or outgoing (SN) direction; this limitation explains the lower average degree and skew towards lower degrees, which is well fitted by a gamma distribution in all three mutant cases. Also, we notice that although there are some high degree nodes and the networks show small world network properties, the physical diffusion limit constrains the formation of super hubs in the networks. This is also confirmed by the clustering coefficient and the network diameter as shown in Figure 8. Thus, unlike scale-free or small world networks, diffusion-based QS networks consist of many small size communities with a few thousand cells each, a network structure resilient against a hub-targeted attack strategy [29, 2].

It is noteworthy that the node degree distribution skew exhibited in each of the mutant cases is reflected directly in the amount of virulence they produce. Generally speaking, the imbalance in the network degree distribution is caused by inefficiencies in the information propagation process. Therefore, it is natural to come up with strategies to cut the connections in the molecular social network. Indeed, researchers recently developed compounds that can inhibit the LuxR receptors or hydrolyze the AHL signal molecules in the extracellular space [5, 33, 16, 3]. Initial experiments have shown that these compounds can quickly and significantly reduce the pathogen virulence and make pathogens more susceptible to conventional antibiotics as well as the immune system due to the lack of EPS protection [23, 20, 17]. In the future, we plan to evaluate the efficiency of these compounds in inhibiting the QS network through our network analysis.

4. CONCLUSION

Much of the population dynamics of heterogeneous bacterial colonies can be explained by intercellular communication. Yet, a high level description of such communication has not previously been explored. In this paper, we have provided four in silico scenarios in which we analyze biofilm population dynamics using a network-centric approach. We have shown that the network metrics correlate well with many traditional biofilm metrics and can thus be used as measures of population level behavior. In addition, we have emphasized the importance of spatial locality for microbial populations in overcoming the “tragedy of the commons.” In future, we plan to explore the evolutionary dynamics involving diffusive public goods and the effects of QS inhibition.

With the rise of bacterial resistance to standard drugs, as well as a plethora of unanswered questions regarding emergent properties in population evolution, we aim to inspire researchers to adopt a network-centric perspective in future investigations.

5. ACKNOWLEDGMENTS

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APPENDIX

A. SUPPLEMENTARY VIDEOS

The biofilm evolution videos for the four scenarios discussed in the paper can be accessed at http://goo.gl/hyrMol.

B. PARAMETERS

The parameters used in our QS and growth model are listed here. The QS parameters are adapted from a general LuxIR system [25]. Other parameters used in our model, such as nutrition concentration $S$ and influence range $T_D$ are variables, and are described in the main body of the paper.

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Table 1: Model parameters

C. REFERENCES


