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An age-dependent model to analyse the evolutionary stability of bacterial quorum sensing

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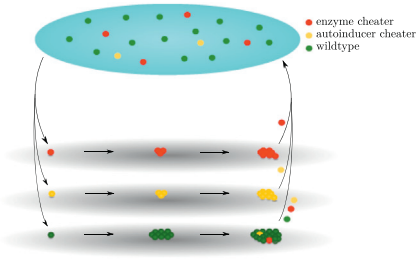
HIGHLIGHTS

- We model quorum sensing in bacteria switching between plankton and biofilm.
- We assess the evolutionary stability against different types of cheaters.
- The long-term outcome depends on nonlinear parameter combinations.
- Growth in colonies can stabilize cooperation in plankton.
- Intermediary colony death rates promote cooperators.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacterial communication is enabled through the collective release and sensing of signalling molecules in a process called quorum sensing. Cooperative processes can easily be destabilized by the appearance of cheaters, who contribute little or nothing at all to the production of common goods. This especially applies for planktonic cultures. In this study, we analyse the dynamics of bacterial quorum sensing and its evolutionary stability under two levels of cooperation, namely signal and enzyme production. The model accounts for mutation rates and switches between planktonic and biofilm state of growth. We present a mathematical approach to model these dynamics using age-dependent colony models. We explore the conditions under which cooperation is stable and find that spatial structuring can lead to long-term scenarios such as coexistence or bistability, depending on the non-linear combination of different parameters like death rates and production costs.

1. Introduction

Cooperation between bacterial cells seems to be the rule rather than the exception, which has led to the development of a field of research called sociomicrobiology (Parsek and Greenberg, 2005). Forms of cooperation often include the release of public goods, i.e., extracellular molecules that benefit all neighbouring cells (such as antibiotica, sidephores or certain virulence factors). Some of these molecules play a crucial role for bacterial nutrition (e.g. exoglycosidase, exoprotease). Production and release of public goods is often regulated by bacterial cell–cell communication (usually termed quorum sensing, QS) using released signals (autoinducers) (Fuqua et al., 1994). Once a certain environmental concentration of autoinducers is reached, which is usually associated with a certain cell density or number of cells, the population starts a coordinated release of public goods. The evolutionary purpose of such a control has been described as guaranteeing a reasonable cost/benefit ratio or efficiency (Hense et al., 2007; Hense and Schuster, 2015; Darch et al., 2012).

Understanding the evolutionary stability of bacterial cooperation is challenging (Keller and Surette, 2006; West et al., 2007a; Ghoul et al., 2014; Leggett et al., 2014; Harrington and Sanchez, 2014). “Cheater” mutants (also called “defectors” or “free riders”),

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which do not contribute to the cooperation, e.g. which do not release public goods, are assumed to save costs, although they do benefit from the public goods provided by cooperators. This theoretically predicted fitness advantage of cheaters has been confirmed with and without QS regulation in vitro and in vivo (Diggle et al., 2007; Sandoz et al., 2007; Köhler et al., 2009; Rumbaugh et al., 2009; Popat et al., 2012; Pollitt et al., 2014). In terms of game theory, such a behaviour is usually described as prisoners dilemma, where the non-cooperative behaviour is the dominant strategy (Archetti et al., 2011). This raises the question, why bacterial cooperation nevertheless exists, i.e., why in the long term cheaters do not outcompete honest cooperators in nature.

With respect to evolutionary stability, QS represents a specific situation as it involves two levels of cooperation: (a) cooperation at the signalling level, as autoinducers themselves are public goods, (b) cooperation on the level of QS-controlled target genes. Both are prone to cheater mutants.

Several mechanisms explaining evolutionary stability of cooperation and QS have been described (for a recent overview see Ross-Gillespie and Kümmerli, 2014). The concepts of kin selection and multi-level selection provide additional approaches from evolutionary theory (Lehmann et al., 2007). In short, these concepts require assortment by a privileged allocation of the benefits of public goods to cooperative producers (Damore and Gore, 2012).

Spatial structuring of populations is a fundamental principle allowing for assortment in bacteria. Such separation could serve to stabilize cooperation in combination with population bottlenecks (Brockhurst, 2007). Spatial structuring can be caused by environmental heterogeneities, but also by self-organization via bacterial interactions (Frey and Reichenbach, 2011). In biofilms, for example, cells and cheaters tend to grow in clusters (Nadell et al., 2010). Both theoretical and experimental studies (Cremer et al., 2012; Chuang et al., 2009; Melke et al., 2010; Rumbaugh et al., 2012) showed that under certain conditions, cyclic separations of the whole population into small subpopulations and subsequent re-mixing events can protect cooperative behaviour from being completely outcompeted.

Studies analysing the influence of fragmentation/re-assortment processes usually do not discuss specifically how these processes may be realized in nature. Most bacteria live as free-floating single cells (plankton) or in aggregates, most frequently attached to surfaces (colonies or biofilms). Fragmentation in colonies usually works as follows: Aggregates normally start with cells that attach to a surface and divide while staying together, if the conditions fit. From a growing colony, eventually cells leave, disperse and found new colonies. Initiating usually from single cells, such a lifestyle presents an extreme form of fragmentation, providing in this respect optimal conditions for the maintenance of cooperation.

In contrast, the realization of fragmentation in plankton is more challenging as cyclic spatial structuring will probably only exceptionally occur (e.g. in cases of growth to flocs). Nevertheless, although a number of genes are differentially expressed under planktonic and attached conditions, QS has been described for both, meaning QS is not switched off in plankton. Values of quorum sensing parameters have even been reported to be almost identical both under planktonic and attached conditions (Meyer et al., 2012; Fekete et al., 2010; Buddrus-Schiemann et al., 2014).

QS signalling within microcolonies seems to be isolated to a certain degree towards signals in the surrounding fluid, which strengthens the degree of separation (Meyer et al., 2012). Although the amount of production can be assumed to vary quantitatively depending on the environmental conditions, QS-controlled public goods as nutritional exoenzymes and siderophores are released in both life styles (Evans et al., 1994). Accordingly, a number of QS-regulated genes are expressed both under planktonic and biofilm conditions (Waite et al., 2006).

There have been different theoretical (modelling) approaches to investigate evolutionary stability of cooperation, using a broad spectrum of analytical tools. For an illustrative review on the evolution of cooperation see West et al. (2007b). Czárán and Hoekstra (2009) modelled cooperation through cellular automata, investigating the spatial aspects of cooperation. Since bacteria procreate through cell division, cells in the vicinity tend to be closely related. In this way, the results could also be explained by Hamilton's Rule, which has been used in Chuang et al. (2010).

Cremer et al. (2012) presented an individual-based model of cooperation in microbial populations, following the experimental results of Chuang et al. (2009).

Garcia et al. (2014) addressed the evolutionary dynamics of attachment and group cohesion. Frank (2010) presented an ODE model which suggests that it is the combination of mutation and demographic processes (such as local density, colony survival and dispersal) which determines the relative fitness of cooperators versus cheaters. In his model, cheaters are just the endpoint of a continuum of secretion rates capability.

As mentioned, most bacteria switch between two states: attached to surfaces, which actually represents the main life style of bacteria, and plankton, which allows to disperse to new niches. A theoretical analysis about evolutionary stability of (QS regulated) cooperation regarding explicitly the biphasic life style of these bacteria is missing yet. In this paper, we thus investigate stability of QS controlled cooperation under such conditions, including mutation rates which are ignored in most similar models. Our aim is the identification of critical factors for cooperation and performing an analysis of the conditions for domination of wildtype or cheater mutants, or coexistence of both. We hypothesize that cooperative behaviours like the production of exoenzymes or siderophores, which are expressed both in plankton and in colonies/biofilms, can be evolutionarily stabilized for both conditions through inter-subpopulation selection in the colony state.

In a generic modelling approach, we will analyse whether and under which conditions this hypothesis holds. For that purpose we use differential equations, as in Frank (2010). The model includes a switch between habitation in separated colonies and in plankton, growth and death, QS-controlled release of a nutritional exoenzyme, and mutations toward both signal and exoenzyme cheaters. In a first step, we will analyse the model with respect to which parameter sets promote the long term dominance of honest cells, cheater cells of both types or the co-existence of both. We first build up our model in Section 2 and analyse it mathematically in Section 3. As a second step, we investigate the behaviour of the model through numerical simulations, using experimentally derived parameters when known. In particular, the influence of key parameters (such as cooperation costs, number of colonies and colony death rate) on the stability of the system are tested. The results are shown in Section 4.

2. The basic age-dependent model

As we want to analyse the effect of repeated mixing and separating, our model will be composed by two parts, namely population dynamics and lifestyle switch: *plankton*, where the bacteria are well mixed and from which they can separate to continue growing in *colonies*, the second lifestyle. Every bacterium in the plankton has an equally distributed chance to do so. Entire colonies can die out due to external influences, e.g. grasers, while the plankton cannot die out at once. Additionally, we assume that there are only a limited number of colony places that are fit for settlements, due to space restrictions. We consider the important processes in plankton and colonies as similar enough to assign

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them the same model, for simplicity's sake, since dropping this assumption would not change the general outcome of our analysis.

For both lifestyles we assume a logistic growth, which we realize through a density-dependent mortality rate, with parameter μ. The bacteria propagate with a set rate r, which is enhanced under production of QS-regulated exoenzyme. Compared to the standard formulation for the logistic growth, this corresponds to a carrying capacity $K=r/\mu$. Therefore the availability of exoenzyme enhances both the growth rate as well as the environmental capacity. Table A1 gives an overview over all occurring variables and parameters.

2.1. Population dynamics

We consider two levels of cooperation, namely QS signal and enzyme production. Without double mutations, this translates into three sub-populations: one cheater that does not produce autoinducer (we call it AI-cheater, and denote it by y); another cheater that does not produce enzyme (we call it enzyme cheater and denote it by z) and a fully cooperative wildtype (which we denote by x). If we take a signal-blind cheater instead of a cheater that does not produce functional enzyme, our analysis still remains valid. Therefore we will concentrate on the cheater types y and z.

We assume that wildtype bacteria turn into cheaters during replication with a constant mutation rate m_y or m_z, respectively, with no reverse or double mutations, due to the very low probabilities of these happening. Because of the metabolic costs for signal and enzyme production, the cheaters will have a growth advantage over the wildtype, which is reflected in different basic growth rates: $r_x<r_y<r_z$. In order to keep the effects of mutation better visible, we formulate the mutations as separate terms; the rates m_y, m_z are to be interpreted relative to the replication rates. This interpretation is in line with that of, for example, zur Wiesch et al. (2010), connecting the generation of mutants to the population size. The model for the three subpopulations then reads:

$$x' = (r_x-\mu(x+y+z))x-(m_y+m_z)x, \tag{1a}$$

$$y' = (r_y-\mu(x+y+z))y+m_y x, \tag{1b}$$

$$z' = (r_z-\mu(x+y+z))z+m_z x. \tag{1c}$$

We further assume that the regulated enzyme provides nutrients, which will speed up growth with a rate $r_n\cdot n$, the main driver of bacterial growth. These nutrients are present in a non-digestible form $\overline{n}$, which regenerate with a rate $\overline{n}_0$, and enzymes e are needed to turn them into nutrients n. The resulting equations for the two forms of nutrients are:

$$n' = c_1 e\overline{n}-c_2 n(x+y+z)-\gamma_n n, \tag{2a}$$

$$\overline{n}' = \overline{n}_0-c_1 e\overline{n}-\gamma_n\overline{n}, \tag{2b}$$

where c_1 is a measure of the enzyme "efficiency" and c_2 the nutrient uptake rate of the bacteria. Additionally, both nutrients have a decay rate of γ_n. Since this process is much faster than bacterial growth, we can consider the nutrient enzyme dynamic to be in a steady state. It follows from (2):

$$n=\frac{c_1 e}{c_1 e+\gamma_n}\cdot\frac{\overline{n}_0}{c_2(x+y+z)+\gamma_n}. \tag{3}$$

We add equations for the QS signal (s) and enzyme (e) concentrations. While there is a baseline production (α) for signal, enzyme is only produced in induced cells with rate β_e. Every single cell decides whether or not to produce enzyme according to the signal concentration. But as we are only interested in the overall enzyme production and cells can be induced at slightly different signal levels, the overall induction is a sigmoid function of signal concentration. At the same time, signal production is induced (β_s). To model this behaviour, we use a Hill-function with Hill coefficient h and τ as the threshold value for induction. This way to describe autoinducer dynamics has become quite standard, see e.g. Dockery and Keener (2001).

In combination with decay rates γ_s,γ_e we obtain our basic model:

$$x' = (r_x+r_n n-\mu(x+y+z))x-(m_y+m_z)x, \tag{4a}$$

$$y' = (r_y+r_n n-\mu(x+y+z))y+m_y x, \tag{4b}$$

$$z' = (r_z+r_n n-\mu(x+y+z))z+m_z x, \tag{4c}$$

$$s' = (x+z)\alpha+\beta_s(x+z)\cdot\frac{s^h}{\tau^h+s^h}-\gamma_s s, \tag{4d}$$

$$e' = \beta_e(x+y)\cdot\frac{s^h}{\tau^h+s^h}-\gamma_e e. \tag{4e}$$

These equations (together with Eq. (2)) describe the population dynamics of two types of cooperation. If we study the long-time behaviour of model (4) without additions, the cheaters will inescapably drive the wildtype to extinction, due to the mutation rates. To take into account the impact of the different bacterial lifestyles, namely living in plankton and/or in colonies, we include an age-dependent model.

2.2. Lifestyle switch

2.2.1. Age-dependent model for the colonies

We will use an age-dependent framework to track the amount of time passed after a colony is colonized. The bacteria living in colonies will be represented as follows. We assume that there is an arbitrary but fixed number L of suitable places for colonies of which $l(t)$ are empty at time t, see Fig. 1. These are colonized at a rate ξ when a bacterium encounters them. Since there are three kinds of bacterial populations, there will be three different types of colonies whose frequency we denominate by u, v and w for colonies colonized by a wildtype, an AI-cheater or an enzyme cheater, respectively. Finally, these colonies will die out again with an age-dependent colony mortality rate $\mu_K(a)$, where the age of a colony is defined as the amount of time passed since it was first colonized. We can therefore put together an age-dependent model of colony frequencies:

$$(\partial_t+\partial_a)u(t,a) = -\mu_K(a)\cdot u(t,a),\quad u(t,0)=\xi x(t)\cdot l(t), \tag{5a}$$

$$(\partial_t+\partial_a)v(t,a) = -\mu_K(a)\cdot v(t,a),\quad v(t,0)=\xi y(t)\cdot l(t), \tag{5b}$$

$$(\partial_t+\partial_a)w(t,a) = -\mu_K(a)\cdot w(t,a),\quad w(t,0)=\xi z(t)\cdot l(t). \tag{5c}$$

As mentioned before, these colonies have the same basic dynamics as the plankton, which means they follow Eqs. (4) and grow from one cell to their capacity with increasing a. This implies that we are not able to find an explicit expression for these dynamics (there is no explicit expression for $f(a)$). But given that

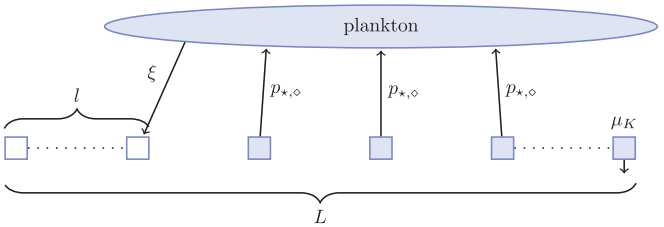


Fig. 1. Interactions between plankton and colonies. Empty colony places are colonized at a rate ξ, from colonies differing amounts $p_{*,\diamond}$ of bacteria will go into the plankton before they die at a rate μ_K.

we are not interested any further in the colonies themselves, the amount of bacteria of type $\diamond$ in one such colony will just be given by $\tilde{f}_{*,\diamond}(a)$, which is dependent on the type of bacteria that started the colony $*$ and the age of the colony a. For example $\tilde{f}_{x,y}(a)$ would denote the amount of AI-cheater bacteria in a wildtype colony of age a. From those, some will migrate into the plankton and we will call this amount $f_{*,\diamond}(a)$. The total amount of bacteria that migrate will be given by

$$p_{x,\diamond}(t) = \int_0^\infty f_{x,\diamond}(a) \cdot u(t,a)\,\mathrm{d}a \tag{6a}$$

$$p_{y,y}(t) = \int_0^\infty f_{y,y}(a) \cdot v(t,a)\,\mathrm{d}a \tag{6b}$$

$$p_{z,z}(t) = \int_0^\infty f_{z,z}(a) \cdot w(t,a)\,\mathrm{d}a \tag{6c}$$

that is, we integrate over all ages.

2.2.2. Plankton dynamics

To obtain the plankton dynamics, we add the migration terms to the respective equations:

$$x' = (r_x + r_n n - \mu(x+y+z))x - (m_y+m_z)x + p_{x,x}, \tag{7a}$$

$$y' = (r_y + r_n n - \mu(x+y+z))y + m_y x + p_{x,y} + p_{y,y}, \tag{7b}$$

$$z' = (r_z + r_n n - \mu(x+y+z))z + m_z x + p_{x,z} + p_{z,z}, \tag{7c}$$

$$s' = (x+z)\alpha + \beta_s(x+z)\cdot\frac{s^h}{\tau^h+s^h} - \gamma_s s, \tag{7d}$$

$$e' = \beta_e(x+y)\cdot\frac{s^h}{\tau^h+s^h} - \gamma_e e. \tag{7e}$$

3. Analysis

Having built our model, we now proceed to analyse it. To give us an indication whether or not the wildtype will be able to survive in the long term. In the following section, we will look at the behaviour of the plankton, not the colonies, because they are dependent on the plankton.

3.1. Stationary states

We determine the stationary solutions of Eqs. (5). Setting $\partial_t u(a) = 0$ leads to terms of the form

$$u(a) = \xi x l \exp\left(-\int_0^a \mu_K(\tau)\,\mathrm{d}\tau\right). \tag{8}$$

We define

$$\theta := \xi \cdot \int_0^\infty \exp\left(-\int_0^{\bar{a}} \mu_K(\tau)\,\mathrm{d}\tau\right)\mathrm{d}\bar{a},$$

$$\varphi(a) := \frac{\exp(-\int_0^a \mu_K(\tau)\,\mathrm{d}\tau)}{\int_0^\infty \exp(-\int_0^{\bar{a}} \mu_K(\tau)\,\mathrm{d}\tau)\mathrm{d}\bar{a}}. \tag{9}$$

Since $\int_0^\infty \varphi(a)\,\mathrm{d}a = 1$ holds, we can write down the stationary solutions of the wildtype colony dynamics as

$$u(a) = xl\theta\varphi(a), \tag{10}$$

with $v(a)$ and $w(a)$ defined similarly. After a short calculation we obtain

$$l = \frac{L}{1+\theta(x+y+z)}. \tag{11}$$

If we define

$$\hat{p}_{\star,\diamond} = \int_0^\infty f_{\star,\diamond}(a)\cdot\varphi(a)\,\mathrm{d}a, \tag{12}$$

the colony input rates in the stationary case are

$$p_{x,\diamond} = \frac{xL\theta}{1+(x+y+z)\theta}\cdot\hat{p}_{x,\diamond}, \tag{13a}$$

$$p_{y,y} = \frac{yL\theta}{1+(x+y+z)\theta}\cdot\hat{p}_{y,y}, \tag{13b}$$

$$p_{z,z} = \frac{zL\theta}{1+(x+y+z)\theta}\cdot\hat{p}_{z,z}. \tag{13c}$$

Plugging these results into (7), we can show that there must exist the following stationary states:

- the empty state;
- two states with one kind of cheater each;
- a state with wildtype bacteria only (if we disregard mutation rates for a moment).

As the model is too complex to check stability of these steady states through the Jacobian matrix, we will instead do a spectral analysis.

3.2. Analysis of the eigenvalues

In this analysis, we ask if a stationary point of one type of bacteria can be invaded by another bacterial type. To this end, we determine the eigenvalues of (7a)–(7c) in the different stationary states, following the ideas as introduced in Webb (1985); Müller and Kuttler (2015). If these are positive, the corresponding bacterial type will be able to invade the stationary state. To calculate these eigenvalues we first use separation of variables on (5), which leads us to

$$u(t,a) = \xi l x \cdot \exp\left(-\int_0^a \lambda+\mu(\tau)\,\mathrm{d}\tau\right), \tag{14a}$$

$$v(t,a) = \xi l y \cdot \exp\left(-\int_0^a \lambda+\mu(\tau)\,\mathrm{d}\tau\right), \tag{14b}$$

$$w(t,a) = \xi l z \cdot \exp\left(-\int_0^a \lambda+\mu(\tau)\,\mathrm{d}\tau\right). \tag{14c}$$

We plug these results into the ansatz $\lambda\cdot v = f(v)$, with f being the functional dependency of the right hand side of Eqs. (7a)–(7c).

3.2.1. Empty state

If we only add a few bacteria to the empty plankton state, the density dependent death rate as well as the signal and enzyme production can be neglected. Thus, there will be no nutrient enhanced growth and, as all available colony places are empty, $l = L$ holds. With these simplifications the equation reads

$$\lambda\begin{pmatrix}\hat{x}\\ \hat{y}\\ \hat{z}\end{pmatrix} = \begin{pmatrix} r_x - (m_y+m_z) + \hat{p}_{x,x,\lambda} & 0 & 0\\ m_y + \hat{p}_{x,y,\lambda} & r_y + \hat{p}_{y,y,\lambda} & 0\\ m_z + \hat{p}_{x,z,\lambda} & 0 & r_z + \hat{p}_{z,z,\lambda}\end{pmatrix}\begin{pmatrix}\hat{x}\\ \hat{y}\\ \hat{z}\end{pmatrix}, \tag{15}$$

where

$$\hat{p}_{\star,\diamond,\lambda} = \xi L\int_0^\infty f_{\star,\diamond}\cdot\exp\left(-\int_0^a \lambda+\mu(\tau)\,\mathrm{d}\tau\right)\mathrm{d}a,$$

$$\text{with } \star,\diamond \in \{x,y,z\}. \tag{16}$$

The wildtype will therefore be able to invade the empty patch, if

$$\lambda = r_x - m_y - m_z + \hat{p}_{x,x,\lambda} \tag{17}$$

has a positive solution λ. Since the right hand side of this equation is monotone decreasing while the left hand side is monotone increasing, it has a positive solution if and only if the right hand side is positive for $\lambda=0$. After a short calculation, which works similarly for the cheaters, we arrive at the following conditions:

$$r_x - m_y - m_z + L\theta\hat{p}_{x,x} > 0 \;\Rightarrow\; \text{wildtype able to invade,} \tag{18}$$

$$r_b + L\theta\hat{p}_{b,b} > 0 \;\Rightarrow\; \text{cheater able to invade,} \quad b \in \{y,z\}. \tag{19}$$

This tells us when a single bacterial type is able to live on its own, without other types nearby.

3.2.2. Solely cheaters present

As before, no matter what type of cheater we have, there will be no nutrient enhanced growth as either signal or enzyme is produced. But in this situation the density depended death rate amounts to μb_0, where b_0 is the number of mutants in this steady state. Additionally, l is reduced to $\frac{L}{1+\theta b_0}$. Consequently, the eigenvalue equation changes to

$$\lambda\begin{pmatrix}\hat{x}\\ \hat{y}\\ \hat{z}\end{pmatrix} = \begin{pmatrix} r_x - \mu b_0 - (m_y+m_z) + \hat{p}_{x,x,\lambda} & 0 & 0 \\ m_y + \hat{p}_{x,y,\lambda} & r_y - \mu b_0 + \hat{p}_{y,y,\lambda} & 0 \\ m_z + \hat{p}_{x,z,\lambda} & 0 & r_z - \mu b_0 + \hat{p}_{z,z,\lambda}\end{pmatrix}\begin{pmatrix}\hat{x}\\ \hat{y}\\ \hat{z}\end{pmatrix}, \tag{20}$$

where

$$\hat{p}_{\star,\diamond,\lambda} = \frac{\xi L}{1+\theta b_0}\int_0^\infty f_{\star,\diamond} \cdot \exp\left(-\int_0^a \lambda + \mu(\tau)\,\mathrm{d}\tau\right)\mathrm{d}a, \quad \text{with } \star,\diamond \in \{x,y,z\}. \tag{21}$$

As before, we want to find out under which conditions there will be positive solutions for λ. Analogously as in the empty patch

$$r_x - \mu b_0 - (m_y+m_z) + \frac{L\theta}{1+\theta b_0}\hat{p}_{x,x} > 0 \;\Rightarrow\; \text{Wildtype able to invade.} \tag{22}$$

3.2.3. Solely wildtype present

With a wildtype-only-state we have to incorporate the nutrient enhanced growth rate. As we assume a steady state, the amount of nutrient would also have stabilized at an amount n_0. The equation for the eigenvalues thus is

$$\lambda\begin{pmatrix}\hat{x}\\ \hat{y}\\ \hat{z}\end{pmatrix} = \begin{pmatrix} r_x + n_0 r_n - \mu x_0 - (m_y+m_z) + \hat{p}_{x,x,\lambda} & 0 & 0 \\ m_y + \hat{p}_{x,y,\lambda} & r_y + n_0 r_n - \mu x_0 + \hat{p}_{y,y,\lambda} & 0 \\ m_z + \hat{p}_{x,z,\lambda} & 0 & r_z + n_0 r_n - \mu x_0 + \hat{p}_{z,z,\lambda}\end{pmatrix}\begin{pmatrix}\hat{x}\\ \hat{y}\\ \hat{z}\end{pmatrix}, \tag{23}$$

which leads to the invasion condition being

$$r_b + r_n n_0 - \mu x_0 + \frac{L\theta}{1+\theta x_0}\hat{p}_{b,b} > 0 \;\Rightarrow\; \text{Cheater able to invade.} \tag{24}$$

3.2.4. Combinations

We can now combine the invasion conditions to look at several scenarios of how cheaters and wildtype interact with each other and can thus explore the long term effects the parameter constellations have. To simplify notation, we will denote the wildtype by W, a mutant by M and $\varnothing$ denotes the empty patch. To indicate invasiveness we will use the arrow $\to$, to indicate that an invasion cannot happen we will use the negated arrow $/\!\to$. Broadly speaking, there are three different possible outcomes: either mutant and wildtype coexist, one type dominates the other or the population dies out.

Coexistence

True coexistence: If every bacterial type is able to invade any of the stationary points, this will result in a stable coexistence point. We call that "true coexistence", because here the cheaters form a more or less independent sub-population instead of constantly rising anew from the wildtype through mutation. For that to happen, the following inequalities must hold:

$$W \to \varnothing \qquad r_x - (m_y+m_z) + L\theta\hat{p}_{x,x} > 0,$$

$$M \to \varnothing \qquad r_y + L\theta\hat{p}_{y,y} > 0,$$

$$W \to M \qquad r_x - \mu b_0 - (m_y+m_z) + \frac{L\theta}{1+\theta b_0}\hat{p}_{x,x} > 0,$$

$$M \to W \qquad r_y + r_n n_0 - \mu x_0 + \frac{L\theta}{1+\theta x_0}\hat{p}_{y,y} > 0.$$

Suppose we start out with a patch of only wildtype bacteria. Due to the rates, a few cheater bacteria will arise and increase in frequency because $M \to W$ holds. As a result of $W \to M$, however, the mutants will not drive the wildtype to extinction but to a mixed state. The same happens when starting with a mixed population. And while at first a cheater-only population will remain that way, adding just a few wildtype bacteria will bring the population to the coexistence point again. So in this scenario the only stable point is the coexistence point, all others are unstable.

Mutant does not spread: Here, the wildtype is able to invade the mutant while the mutant cannot invade the wildtype. Whether or not the mutant is able to live on its own ($\overset{\to}{\underset{\to}{\not\to}}$ means invasibility does not matter), in a mixed population there will always be primarily wildtype bacteria with a small sub-population of mutants, thanks to the mutation. The conditions here are

$$W \to \varnothing \qquad r_x - (m_y+m_z) + L\theta\hat{p}_{x,x} > 0,$$

$$M \not\rightleftarrows \varnothing \qquad r_y + L\theta\hat{p}_{y,y} \neq 0,$$

$$W \to M \qquad r_x - \mu b_0 - (m_y+m_z) + \frac{L\theta}{1+\theta b_0}\hat{p}_{x,x} > 0,$$

$$M \not\to W \qquad r_y + r_n n_0 - \mu x_0 + \frac{L\theta}{1+\theta x_0}\hat{p}_{y,y} < 0.$$

3.2.5. One bacterial type only

Mutant outcompetes wildtype: If the mutant is able to invade the wildtype-only state but the wildtype is unable to compete, the mutant will outcompete the wildtype no matter the starting condition. The conditions for this to happen are

$$W \to \varnothing \qquad r_x - (m_y+m_z) + L\theta\hat{p}_{x,x} > 0,$$

$$M \to \varnothing \qquad r_y + L\theta\hat{p}_{y,y} > 0,$$

$$W \nrightarrow M \qquad r_x - \mu b_0 - (m_y + m_z) + \frac{L\theta}{1+\theta b_0}\hat{p}_{x,x} < 0,$$

$$M \to W \qquad r_y + r_n n_0 - \mu x_0 + \frac{L\theta}{1+\theta x_0}\hat{p}_{y,y} > 0.$$

Bistability: In this scenario, none of the bacterial types can invade the others but everyone can invade the empty patch. This means that whichever type is present first in greater quantities will assert itself. The one-type-only stationary states will therefore be stable while the coexistence point and the point of origin are unstable:

$$W \to \varnothing \qquad r_x - (m_y + m_z) + L\theta\hat{p}_{x,x} > 0,$$

$$M \to \varnothing \qquad r_y + L\theta\hat{p}_{y,y} > 0,$$

$$W \nrightarrow M \qquad r_x - \mu b_0 - (m_y + m_z) + \frac{L\theta}{1+\theta b_0}\hat{p}_{x,x} < 0,$$

$$M \nrightarrow W \qquad r_y + r_n n_0 - \mu x_0 + \frac{L\theta}{1+\theta x_0}\hat{p}_{y,y} < 0.$$

3.2.6. Extinction

Evolutionary suicide: This scenario is very similar to "mutant outcompetes wildtype" with one marked difference: the mutant is unable to invade the empty patch and therefore unable to live by itself. After driving the wildtype to extinction, the remaining mutant-only population will then die out. As it is impossible to have a population consisting solely of wildtype bacteria because of the mutation rate, the bacterial population will become extinct.

$$W \to \varnothing \qquad r_x - (m_y + m_z) + L\theta\hat{p}_{x,x} > 0,$$

$$M \nrightarrow \varnothing \qquad r_y + L\theta\hat{p}_{y,y} < 0,$$

$$W \nrightarrow M \qquad r_x - \mu b_0 - (m_y + m_z) + \frac{L\theta}{1+\theta b_0}\hat{p}_{x,x} < 0,$$

$$M \to W \qquad r_y + r_n n_0 - \mu x_0 + \frac{L\theta}{1+\theta x_0}\hat{p}_{y,y} > 0.$$

4. Numerical simulations

In this section, we present how the model behaves under different parameter sets. To this end we implement the differential equations in Matlab (Mathworks). The ordinary differential equations were solved numerically with a Runge–Kutta-solver. In order to solve the partial differential equations (5), we need to reformulate them. Following the methods mentioned by Webb (1985), we can write $u(t,a)$ as

$$u(t,a) = \begin{cases} 0 & a > t \\ x(t-a)l(t-a)\xi \exp\left(-\int_0^a \mu(s)\,\mathrm{d}s\right) & a \le t \end{cases}. \tag{25}$$

$v(t,a)$ and $w(t,a)$ can be calculated in the same manner. Lastly, $f_{*,\diamond}(a)$ is computed through spline interpolation of the solution curves of our basic model, which without colony input, describes the colony dynamics as well. Examples for both the colony and plankton dynamic can be found in Fig. 2. Note that the seemingly very small values for the cheaters in colonies are due to the continuous model with non-vanishing mutation rates, also acting for very low wildtype cell numbers. They can be interpreted better for a large number of colonies, as multiplied by the colony number they express the expected number of this cell type in the whole system. The standard set of parameters used in our simulations can be found in A.2. We assume that although cheaters are able to survive on their own, the benefit derived from secreting the enzyme ($r_n \cdot n$) is the main driver of bacterial growth. For the simulation, we used a fixed volume for the planktonic phase of 1^{-8} L. Because the long-term development is foreseeable after a short time span, we stopped our calculations at $t=400$.

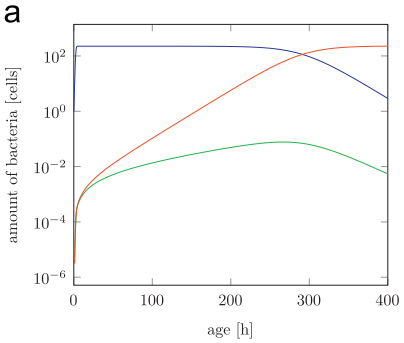
4.1. Colony number

We start by exploring how changing of the number of available colony places, L, influences the behaviour of the solution. We find that cooperation collapses in simulations as long as $L \le L_0$ (Fig. 3). It is not possible to calculate a closed expression for this critical value L_0, but one can see that $L_0 = 80$ in our calculations. The more L approximates to L_0, the more time it takes until cooperators are outcompeted, which is reflected by an increasing fraction of cooperators for $t >= 400$ in Fig. 3. For $L > L_0$, cooperators dominate, although a small number of cheaters may still be present. Cooperation is thus stabilized more if the proportion of bacteria in colonies is high compared to the number of bacteria in plankton.

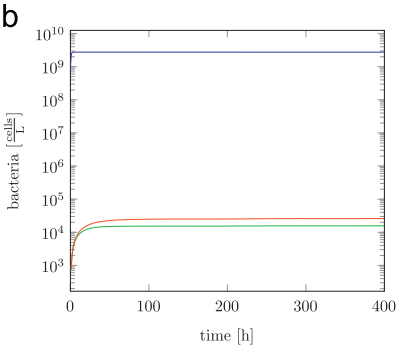
4.2. Enzyme production cost

By raising the production cost for the enzyme, we reduce the reproduction rates for the wildtype and the signal cheater, r_x and r_y. As suspected, this destabilizes cooperation, although this effect is markedly decreased if the benefit of cooperation, represented by r_n, is high. See Fig. 4 for illustration.

We did the same calculations with higher signal production cost. The figure is omitted here, because the long-term behaviour remains the same. If the wildtype dies out, the dominating type will always be the mutant with the highest growth rate which



Growth curve of a single colony started by a wildtype.



Growth curve of the associated plankton.

Fig. 2. Time course of plankton and colony dynamics with standard parameter values. The blue line represents the amount of wildtype bacteria, the green line denotes the amount of signal cheating mutants and the red line indicates the amount of enzyme cheater. One can see that regular death events are needed to preserve the abundance of wildtype bacteria. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of this paper.)

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means the lowest metabolic costs. If we assume that enzyme is more costly than signal, then the dominating type will always be the enzyme cheater, thus rendering changes in the signal cost irrelevant for the long term dynamic.

4.3. Colony death rate

The effect changes of the colony death rate μ_K have on the wildtype-mutant-dynamic is not so straightforward. If the colony death rate is very high, colonies will not grow to large numbers meaning that the colony influence on plankton is low. This, in turn, leads to a cooperation decline. On the other hand, if the colony death rate is very low, the amount of wildtype bacteria in the colonies will decline through mutation and subsequent growth of mutants. An intermediate death rate is most favourable for cooperation stability. The corresponding simulation can be found in Fig. 5.

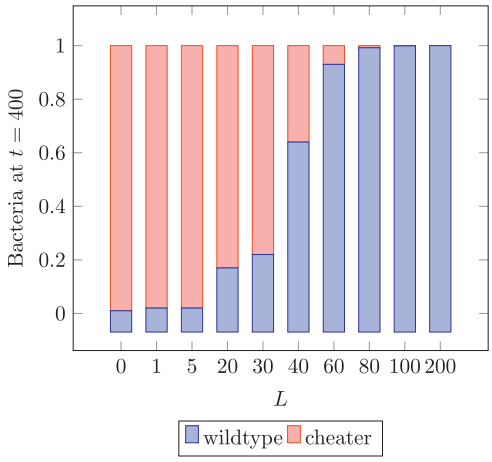

Fig. 3. Influence of colony number L on the survival of wildtype against mutant. Ratio between wildtype and cheater after t=400. In scenarios with $L \leq 80$, the wildtype will die out in the long term, whereas in scenarios with $L > 80$ the wildtype dominates cheaters in long term equilibrium. Parameter values can be found in Section A.2.

5. Discussion

The main results of our study are the following:

- Switch between growth in colonies and in biofilms can promote evolutionary stability of QS-regulated cooperation in plankton.
- The specific combination of different parameters as described in Chapter 3.2.4 determines the outcome, whereby both low costs

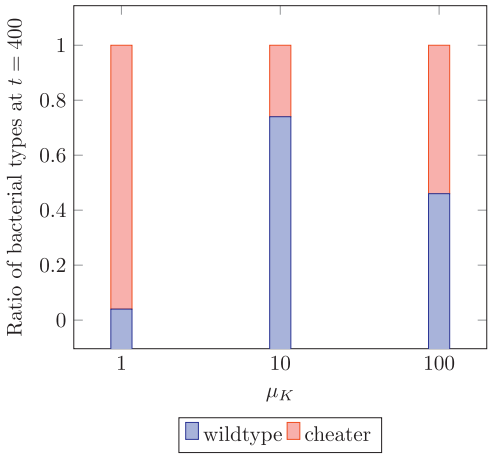

Fig. 5. Influence of colony death rate on the survival of wildtype against mutant. Parameter values can be found in the A.2.

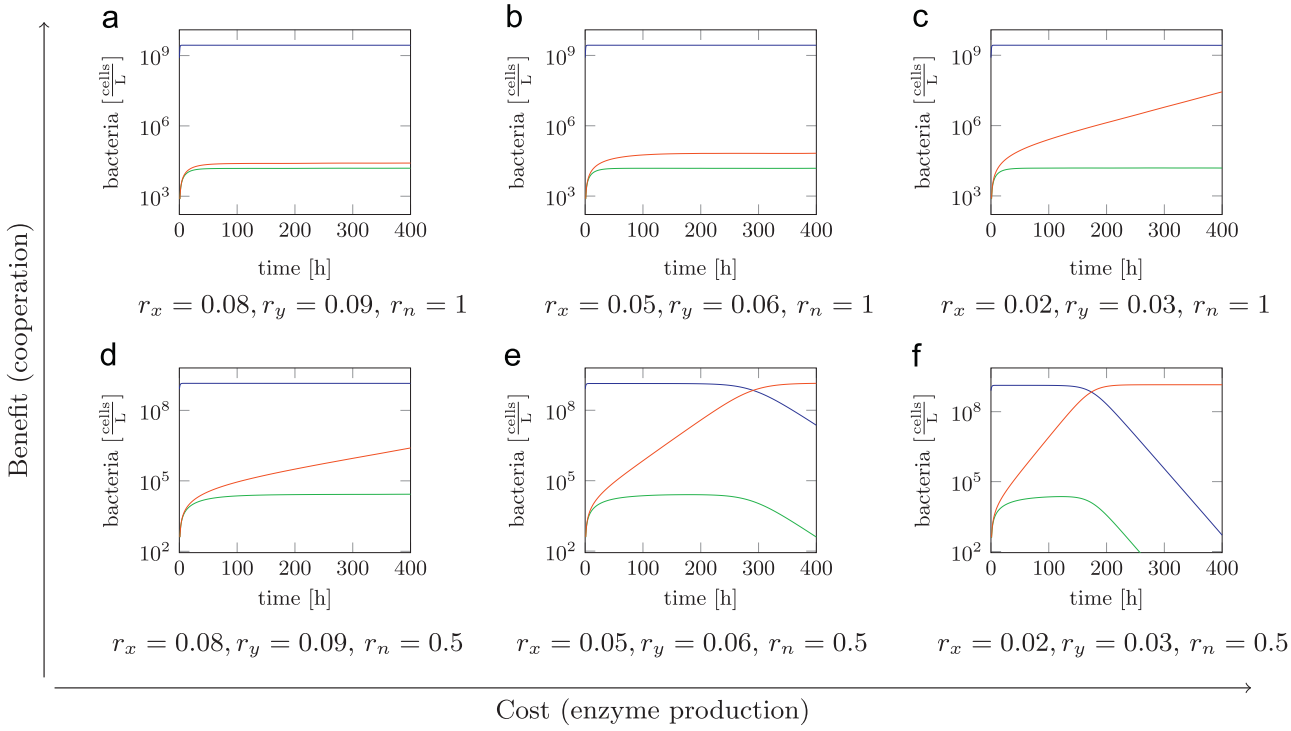

Fig. 4. Comparison of resulting dynamics with higher enzyme production costs for two different cooperation benefits. The blue line represents the amount of wildtype bacteria, the green line denotes the amount of AI-cheaters and the red line indicates the amount of enzyme cheater. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of this paper.)

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and high benefit of cooperative traits promote cooperators. The same holds for high carrying capacity for pure wildtype colonies and low carrying capacity for cheater colonies.

- Depending on the parameter values, four different types of long term equilibria could be achieved: cheater dominates, wildtype dominates, co-existence of both, bistability.
- Values of one parameter unfavourable for cooperation can to some degree be compensated by more favourable values in other parameters. Exemplarily, high costs of cooperation can be compensated by high number of colony patches in combination with high benefit of cooperation in a way as described in Chapter 3.2.4. Some parameters, such as high number of patches for colonies, and high switching rate from plankton to colonies tend to promote true coexistence. These factors can be described as promoting the influence of colonies in the system under investigation. In nature, this would indicate a high ratio between the size of the surface area suitable for colony growth and the volume of the plankton.
- There exists an optimum with respect to colony death rate, as too high or too low rates promote cheaters.

Our study shows that a switch between plankton and biofilm state can promote evolutionary stability of QS-controlled cooperation. Most bacterial species regularly undertake such switches. We thus suggest that the results contribute to the explanation of the evolutionary puzzling fact that in well-mixed planktonic cultures most, if not all, QS systems are expressed and control a specific set of highly costly target genes. A prerequisite for this stabilization is that QS controls genes under both planktonic and biofilm modes of growth.

Low costs of cooperation and high carrying capacity for the wildtype in combination with high death rate in plankton tend to inhibit spread of mutants, whereas high mutation rates, high carrying capacity for mutant and low benefit promote mutants. The basic growth rate of wildtype and cheaters and the growth rate promotion by QS-regulated exoenzyme hereby reflect costs and benefit of cooperation.

Factors promoting the relevance of colonies in relation to plankton may enable true coexistence, for example when cell death rate and carrying capacities of both populations in plankton are not too large. In contrast, large death and carrying capacities rates for wildtype and mutants in plankton, in combination with low values of colony patch number, growth rates and benefit of cooperation, may under certain cases result in bistability, i.e. in an assertion of the cell type which is present first.

High benefits and low costs promote stability of cooperation, as described for other scenarios (Hummert et al., 2010; Ruppin et al., 2010; Chuang et al., 2010; Xavier et al., 2011; Schuster et al., 2010). The amount of available substrate for the exoenzyme, i.e., nutrient concentration, determines the benefit of exoenzyme production. If, as in our set-up, costs for target genes are higher than costs of signal production, signal-blind- or target gene mutants will dominate over mutants of signal production. This will depend on the frequencies of mutants found, as it has been shown in in situ experiments such as clinical samples of pathogens (Strateva and Mitov, 2011; Cullen and McClean, 2015; Pollitt et al., 2014). For reasons of compactness, we only analysed a target gene mutant, omitting a signal blind mutant. As the signal induces its own production, and because in reality most signals control more than one costly target gene, both types of cheaters will gain quantitatively different outcomes. However, qualitatively our results will hold.

Generally, plankton tends to destabilize cooperation, whereas colony growth tends to stabilizes it. Therefore, all parameters affecting the interrelationship between both have significant influence. The relevance of “colony death rates” implies that external disturbing factors such as grazers or death of hosts affect stability of cooperation. Similar effects are caused by other events that interfere with the life span of e.g. colonies, such as self-induced disorganization of whole colonies (Cárcamo-Oyarce et al., 2015).

In our model, higher numbers of available colony patches promote cooperation. Ultimately, the relation between available space for plankton growth, which was kept constant in our model, and potential for colony growth is critical. In accordance with our modelling results, spatial structuring in separated microcolonies connected in a limited way by free floating cells has been reported to stabilize QS-cooperation in *Bacillus thuringiensis* during infections of larvae of the diamondback moth and in *Plutella xylostella* (Zhou et al., 2014). The same has been shown for siderophore production by *Pseudomonas fluorescens* in soil (Luján et al., 2015).

Most game theory approaches employed to study cooperation assume a linear relation between cost and benefit (such as prisoner's dilemma or snow drift) (Damore and Gore, 2012; Archetti et al., 2011; Nowak et al., 2010). In many cases, this relation can better be described by a non-linear term, often including saturation effects (Hense and Schuster, 2015; Chuang et al., 2010; MacLean et al., 2010). Exemplary, in case of an increasing amount of exoenzymes released by an increasing number of cells, the benefit (amount of transformed substrate or the increases of growth rate) obviously saturates. In other cases, benefit may be described as sigmoid or stepwise function of costs (Archetti et al., 2011; Popat et al., 2015).

Our model contains non-linearity of benefits as nutrients are limited. Consequently, during growth of plankton and microcolonies, the benefits/cost ratio declines affecting the outcome with respect to evolutionary stability of cooperation. Interestingly, non-linearity has been identified as a factor which can under certain conditions promote cooperation independent on assortment, allowing for co-existence of cooperators and cheaters (Frey and Reichenbach, 2011; Archetti et al., 2011; Perc et al., 2013; Zhang et al., 2013). As a prerequisite, benefit has to be a concave function of costs and there needs to be an intersection between the curves describing cooperator, respectively cheater fitness. In our model, such an intersection does not exist, so non-linearity tends to weaken cooperation at high densities of cooperators as the benefits saturate while the costs remain.

Depending on parameter values, four different outcomes in the long term are predicted in our study. (A) Only cheaters survive, (B) Cheaters are repressed, i.e. only a low amount of cheaters, arisen from recent mutations, exist, (C) true coexistence and (D) bistability. (A) can be easily explained by a dominance of the fast growing cheaters. Here, effect of colony growth is insufficient to rescue cooperation. In (C) we have an equilibrium between within- and between group selection. Outcome (B) seems surprising at first sight, however very low costs ($r_x \approx r_y$) have been reported to promote such a game of harmony-scenario (1194), together with a high benefit ($x_0, \hat{p}_{x,x} \geqslant b_0, \hat{p}_{y,y}$). Interesting is the bistability in (D). Here, no strain can invade the other strain. Such a behaviour is promoted when the competition for resources is high, but benefit and cost of cooperation are low.

Co-existence is enabled by negative frequency dependencies of fitness in both strains (cheaters and wildtype). In contrast, bistability reflects a scenario with positive frequency dependency of fitness for all strains (Damore and Gore, 2012). For both scenarios, examples have been described for non-spatially structured environments. However, these examples required specific properties such as green beard genes (promotion of bistability) or privileged share, i.e. if a fixed amount of benefit is directly redirected to the producer of the public good (Gore et al., 2009). Although our generic model does not include such privileged assortment, the assumption of complete separation has a similar effect.

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There is increasing evidence that cooperation found in nature may be more often connected with co-existence of cooperators and non-cooperators, rather than with populations in which all cells contribute to cooperation. This may seem comprehensible due to the fact that mutations, which always occur, may more frequently switch from cooperator to cheater than vice versa. However, our study indicated under which conditions true co-existence in equilibrium may be the result of counteracting driving forces, in our case between within-group selection (benefiting cheaters) and between group selection (benefiting cooperators).

Beyond pure cheaterism, co-existence of cooperators and non-cooperators may have other implications. Under certain conditions, it might be advantageous for the fitness of populations if only a fraction of the population contributes to cooperation (Elhanati et al., 2011; Perc et al., 2013; Diard et al., 2013; MacLean et al., 2010). This especially holds if non-cooperators, possibly by chance, express other properties beneficial for the population as shown in *Pseudomonas fluorescens*, where "cooperators" optimize access to nutrients by building biofilms, whereas "cheaters" have better dispersal traits, allowing cells to spread and occupy new locations (Rainey and Kerr, 2010; Rainey and Rainey, 2003). Note that the notions "cooperators" and "cheaters" increasingly loose sense in such examples.

Even for QS, a strategy which due to the existence of positive feedback loops in most species (e.g. signal induced signal production) was assumed to enable an all-or-none behaviour, co-existence of QS active and defective strains may not always be explained by cheaterism, but at least sometimes reflects division of work in isogenic populations (Anetzberger et al., 2009; Pradhan and Chatterjee, 2014). Thus, co-existence observed in nature has to be interpreted with care. The question whether it represents rather cheaterism or division of work is not always straightforward and requires thorough ecological and evolutionary investigations, but the distinction might be relevant from various perspectives, including development of adequate treatment strategies, e.g. for antibiotic substitution (Schuster et al., 2013; Brown et al., 2009). Mechanistic models as the one presented here can be valuable tools.

Several other factors promoting stability of QS controlled cooperation have been reported, namely heterogeneity of cooperation between cells (Perc et al., 2013; Pérez-Velázquez et al., 2015), stochastic fluctuations (Houchmandzadeh, 2015), pleiotropy (Dandekar et al., 2012; Foster et al., 2004; Wang et al., 2015; Strassmann et al., 2011), punishment of social cheats (Friman et al., 2013), costly over-expression of certain QS regulated genes in QS defective mutants (Oslizlo et al., 2014; Wilder et al., 2011), negative feedback loop on the public good production (Gore et al., 2009) and preferred adhesion of cells with identical cooperative behaviour (Rainey and Rainey, 2003; Strassmann et al., 2011). Interestingly, QS itself is a strategy to limit development of cheaters, as it limits costly and thus exploitable production of public goods (Czárán and Hoekstra, 2009; Perc et al., 2013; Travisano and Velicer, 2004; Popat et al., 2015).

Our approach can be interpreted both in terms of multilevel and kin selection (West et al., 2006). Colony patches may be regarded as main entities of between-group selection connected by plankton, whereas within-group selection occurs in the patches as well as in plankton. However, new colonization of patches also represents a realization of kin selection, as all cells within a patch descend from a single founder cell. This extreme bottleneck set-up allows for cyclic complete separation of cooperators and cheaters, supporting an almost complete suppression of cheaters under suitable conditions. However, such an extreme set-up is not a *sine qua non* condition. In reality, many bacteria grow rather in large biofilms, composed of a number of independently founded microcolonies. However, spatial assortment can maintain, or may even develop in completely mixed, growing biofilms under certain conditions, as long as mechanisms of mixing, e.g. mobility of single cells in the biofilms, do not dominate (Kerr et al., 2002; Rumbaugh et al., 2012; Nadell et al., 2010). Even more, the assumption that plankton is ideally well mixed probably does not necessarily always hold in reality. Transient assortments of cooperative cells, respectively public goods, may exist due to limited diffusion rates or limited connection between planktonic subpopulations in highly structured environments such as micro cave systems in porous soil or within hosts. It remains to be investigated to which degree such weaker forms of separation can support cooperation. Note that too low diffusion rates may turn QS useless and, in extreme cases, eventually may change a public good into a private good (Czárán and Hoekstra, 2009). Furthermore, plankton and colonies are not fully separated, as shown by influence of auto-inducers produced in biofilms on plankton in overlying fluid (Nigaud et al., 2010).

Our model has some simplifications which we assume not to interfere with the qualitative outcome. We chose the QS-related parameters of signal productions rates and threshold to be constant and identical for plankton and colonies, in accordance with the results of a series of studies of *P. putida IsoF* QS system (Meyer et al., 2012; Buddrus-Schiemann et al., 2014; Fekete et al., 2010). The model does not consider gradients of signals, which occur in colonies, biofilms or between biofilms and plankton (Hense et al., 2012).

Similarly, it is clear that neither the fitness benefit provided by public goods nor the costs of their production are necessarily constant, as assumed in the model, but can vary spatio-temporally depending on the environmental conditions. For example, fitness costs for public good production may be low when resources for their production are available in high amounts, i.e. when these resources do not limit growth (Brockhurst et al., 2008). The so-called metabolic prudence concept, which has gained some experimentally support, states that cells tend to induce the production of public goods under low/no cost conditions (Xavier et al., 2011; Mellbye and Schuster, 2014). Analogously, the fitness benefit of producing exoenzymes depends, for instance, on the availability of their substrate.

Information about environmental factors, e.g. substrate availability, is often integrated into QS systems (Juhas et al., 2005; Schaefer et al., 2008; Darch et al., 2012; Hense and Schuster, 2015). Generally, it has been suggested that optimal QS regulation with respect to benefit and costs depends on the properties of the public good, e.g. on the way how its benefit is realized (Cornforth et al., 2012; Heilmann, 2015). Hense et al. (2012) suggested that this complex regulation in QS can be understood as a hybrid push-pull control to optimize the cost/benefit interplay, where "push" refers to the potential strength of regulated public good activity, and "pull" to the cells' demand of the public good. The spatio-temporal dynamics of costs and benefit therefore have an important impact on the evolutionary stability of QS and could represent an interesting extension of our work.

Our model focusses on the stability of cooperation versus cheater mutants and is not dedicated to explain the evolutionary development of cooperation, which might often occur in small steps. The latter task requires different methods, e.g. tools of adaptive dynamics.

Understanding under which conditions cheaters arise or existence of cooperators and non-cooperators in equilibrium emerges is of high interest, e.g. in developing treatment strategies. Our study sheds light on the question how switches between plankton and attached mode of growth can contribute to this.

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Appendix A. Variables and parameters

A.1. Table of used variables and parameters

See Table A1 .

A.2. Standard parameter values

See Tables A.2 and A.3.

Table A1
Table of all occurring variables and parameters.

Name	Unit	Stands for
α	mol/L cells h	Basic production rate of signal molecule
β_e	mol/cells h	Induced production rate of enzyme
β_s	mol/L cells h	Induced production rate of signal molecule
γ_e	1/h	Enzyme degradation rate
γ_n	1/h	Nutrient degradation rate
γ_s	1/h	Signal molecule degradation rate
θ	1/cells	Measure for the lifespan and recolonization frequency of colonies
μ	1/cells h	Bacterial death rate in plankton
μ_K	1/cells h	Death rate for the colonies
μ_{colony}	1/cells h	Bacterial death rate in colonies
ξ	1/cells h	Recolonization rate of empty colony patches
τ	mol/L	Threshold value for induction
φ	1/h	Normed measure for the survival chances of colonies
b_0	cells	Number of bacteria from an arbitrary cheater type in a stationary state
c_1	1/mol h	Effectiveness of enzyme
c_2	1/cells h	Nutrient uptake of bacteria
$e(t)$	mol	Existing amount of enzyme at time t
$f_{\star,\diamond}(a)$	cells	Number of bacteria of type $\diamond$ that migrate into plankton from colonies of age a that were started by type $\star$
h		Hill coefficient
$l(t)$		Number of empty colony places at time t
L		Total number of colony places available
m_y	1/h	Mutation rate from wildtype bacteria to AI-cheaters
m_z	h	Mutation rate from wildtype bacteria to enzyme cheaters
$n(t)$	mol	Amount of digestible nutrient at time t
n_0	mol	Amount of digestible nutrient in a stationary state
$\overline{n}_0$	mol/h	Nutrient regeneration rate
$p_{\star,\diamond}(t)$	cells	Number of bacteria of type $\diamond$ that migrate into plankton from all colonies started by type $\star$ at time t
$\hat{p}_{\star,\diamond}$	cells	Number of bacteria of type $\diamond$ that migrate into plankton from a single colony started by type $\star$ during its life-span in the stationary state
r_b	1/h	Basic growth rate for an arbitrary cheater
r_n	1/mol h	Nutrient dependent growth rate
r_x	1/h	Basic growth rate for wildtype bacteria
r_y	1/h	Basic growth rate for AI-cheaters
r_z	1/h	Basic growth rate for enzyme cheaters
$s(t)$	mol/L	Concentration of signal molecule at time t
$u(t,a)$	1/h	Number of colonies of age a at time t that were started by a wildtype
$v(t,a)$	1/h	Number of colonies of age a at time t that were started by a AI-cheater
$w(t,a)$	h	Number of colonies of age a at time t that were started by a enzyme cheater
$x(t)$	cells	Number of wildtype bacteria at time t
x_0	cells	Number of wildtype bacteria in a stationary state
$y(t)$	cells	Number of AI-cheaters at time t
$z(t)$	cells	Number of enzyme cheaters at time t

Table A2
Standard parameter values for the numeric simulations if not explicitly stated otherwise.

Name	Value		Source
α	1.5×10^{-11}	mol/L h	
β_e	1.2	mol/cells h	Vetter et al. (1998)
β_s	1.5×10^{-10}	mol/L h	
γ_e	0.021	1/h	
γ_n	2.3	1/h	
γ_s	0.0055	1/h	
μ	0.0208	1/cells h	Heymann (2010)
μ_K	1	1/h	
μ_{colony}	0.01	1/cells h	
ξ	0.5		
τ	7×10^{-8}	mol/ L	
c_1	2.4×10^{-15}	1/mol h	Böckle et al. (1995)
c_2	1×10^{-19}	1/cells h	Simon (1985)
h	2		
L	100		
m_y	3.5×10^{-7}	1/h	
m_z	3.5×10^{-7}	1/h	
n0	5	mol/h	
r_n	0.5	1/mol h	
r_x	0.08	1/h	Heymann (2010)
r_y	0.09	1/h	Heymann (2010)
r_z	0.12	1/h	Heymann (2010)

Table A3
Differing parameters used in simulations for Fig. 5.

Name	Value		Source
γ_e	0.0021	1/h	
c_1	3.6×10^4	1/mol h	Böckle et al. (1995)
c_2	10×10^{-19}	1/cells h	Simon (1985)
$\overline{n}_0$	1×10^{-18}	mol/h	
r_n	0.01	1/mol h	

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