Research Article

Cooperation and cheating in microbial exoenzyme production – Theoretical analysis for biotechnological applications

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The engineering of microorganisms to produce a variety of extracellular enzymes (exoenzymes), for example for producing renewable fuels and in biodegradation of xenobiotics, has recently attracted increasing interest. Productivity is often reduced by "cheater" mutants, which are deficient in exoenzyme production and benefit from the product provided by the "cooperating" cells. We present a game-theoretical model to analyze population structure and exoenzyme productivity in terms of biotechnologically relevant parameters. For any given population density, three distinct regimes are predicted: when the metabolic effort for exoenzyme production and secretion is low, all cells cooperate; at intermediate metabolic costs, cooperators and cheaters coexist; while at high costs, all cells use the cheating strategy. These regimes correspond to the harmony game, snowdrift game, and Prisoner's Dilemma, respectively. Thus, our results indicate that microbial strains engineered for exoenzyme production will not, under appropriate conditions, be outcompeted by cheater mutants. We also analyze the dependence of the population structure on cell density. At low costs, the fraction of cooperating cells increases with decreasing cell density and reaches unity at a critical threshold. Our model provides an estimate of the cell density maximizing exoenzyme production.

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1 Introduction

Many biotechnologically relevant processes involve the action of extracellular enzymes (exoenzymes). An example of high commercial interest nowadays is biofuel production. It often involves the exoenzyme, cellulase, for saccharification [1, 2]. Extracellular bacterial cellulases, xylanases and amylases are important in digestive processes in many habitats such as the rumen [3]. *Saccharomyces cerevisiae* secretes, among others, acid phosphatase [4] and invertase [5]. Further examples are fungal lignolytic enzymes such as lignin peroxidase, laccase, and manganese peroxidase [6]. Exoenzymes play an important role in biodegradation of xenobiotics and, thus, in bioremediation of polluted areas. For example, lignolytic enzymes are also active in the breakdown of toxic polycyclic aromatic hydrocarbons [6].

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Abbreviation: ESS, evolutionarily stable strategy

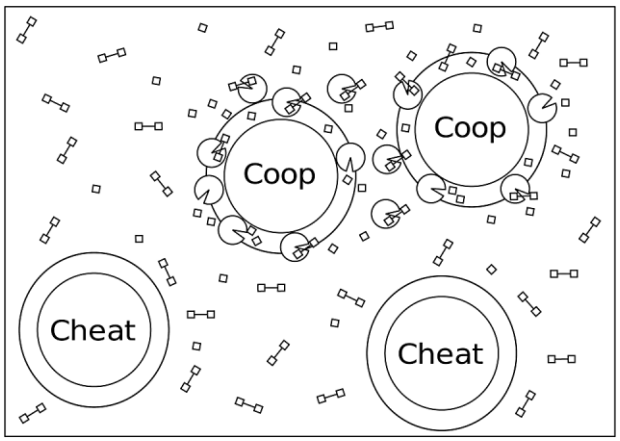

Figure 1. Interplay between microbial cells upon secretion of exoenzymes. Cheater cells (denoted by 'Cheat') do not produce exoenzymes (incised circles) and benefit from the growth substrate (monomers) released by the enzyme secreted by cooperating cells ('Coop'). The initial substrate is indicated by dimers (*e.g.*, sucrose), even though it may consist of polymers such as cellulose. Besides this "desirable" substrate, other substrates may be present. A possibly present periplasmic space is represented by the double envelope. Note that the concentration of the growth substrate is higher near cooperating cells in this spatially heterogeneous system.

The total production of extracellular enzymes by a population of microorganisms is often diminished by the existence of a subpopulation of non-producing cells [3, 7]. These cells do not invest the metabolic costs of producing and secreting these enzymes, but benefit from the substrates released by the action of the exoenzymes generated by other cells (Fig. 1). An illustrative example is provided by yeast invertase, encoded by the *SUC* genes. Some strains of *S. cerevisiae* carry a non-functional *SUC2* as the only *SUC* gene [8]. Moreover, the species *S. italicus* does not harbor any *SUC* gene [9], but benefits from the extracellular glucose generated by the invertase secreted by other *Saccharomyces* species. Greig and Travisano [10] studied wild-type cells of *S. cerevisiae* and cells in which the gene *SUC2* had been deleted. They observed coexistence between both cell types (although the subpopulations were generated artificially in that case). Another example is provided by *Candida albicans*. The activity of extracellular enzymes such as proteinase and phospholipase differs significantly among three different genotypic strains [11, 12] and among karyotypes of that fungus [13].

Secretion of exoenzymes and partial or complete failure to do so can be regarded as different strategies of cells in an evolutionary "game" on fitness (growth rates). The strategies correspond to genetic or epigenetic states, and switches between them can occur, for example, by mutations or gene silencing, respectively. Evolutionary game theory [14-18] can be used to determine equilibrium states of the population (*i.e.*, mixtures of strategies) when the fitness of an organism depends not only on its own strategy but also on the strategies of others. That theory has been used for analyzing many properties of living organisms such as sex ratios [14], the evolution of cooperation [16, 18–20], biofilms [21], and the selection of biochemical pathways [22, 23]. Moreover, it can be used for classification in data analysis [24]. Game theory is also a well-suited tool for analyzing and optimizing biotechnological setups in which the balance between competition and cooperation determines productivity. Here we present a mathematical framework for modeling exoenzyme production based on evolutionary game theory. We analyze the difference in fitness of cooperating and cheating cells in dependence on total cell density and on the costs of enzyme production and secretion.

In game theory, a number of typical games can be distinguished. Probably the most famous is the Prisoner's Dilemma [25]. In the traditional Prisoner's Dilemma, defection (cheating) is the only stable strategy. Thus, much work has been done on how cooperation can evolve in a Prisoner's Dilemma setting, for example, by stochastic or spatial effects or iteration of the game [15, 16, 26, 27]. Another game is the snowdrift game, also known as game of chicken or hawk-dove game [14, 15, 18, 20]. The name snowdrift originates from a cover story in which two car drivers got stuck in a blizzard, and shoveling snow by one of them is sufficient for both to move on. There, cooperation occurs naturally because that game leads to two Nash equilibria, in which one player cooperates and the other one defects. Nash equilibria are situations where none of the players would benefit from switching strategy unilaterally [15, 28]. In the snowdrift game, the payoff for the cooperating player would be reduced when switching to defection because this player would then – in the cover story – remain stuck in the snowdrift. An example from everyday life is the situation where two people want to pass a narrow door. In the two Nash equilibria, one person goes first while the other waits a moment. In a population, the two equilibria in the snowdrift game imply that defectors and cooperators can coexist. Another relevant game is the harmony game [23, 26], also called mutually beneficial game, in which the only stable solution is where all players cooperate.

An advantage of using game theory rather than traditional dynamic simulation or other techniques is that the complicated process of finding the stable solution is not considered. For example, it usually takes some communication and, thus, some time, until the players agree upon the Nash equilibrium,

as we know from the situation of passing a door. Only the final situation is determined. For the present calculations, we use the concept of "evolutionarily stable strategy" (ESS) [14, 15]. It is a generalization of the Nash equilibrium to n-player situations with large n. An ESS is a strategy which, if chosen by a population of agents (*e.g.*, microbial cells), cannot be outcompeted by any alternative strategy that is initially rare. The ESSs can be attained by different processes, notably epigenetic processes or mutations, implying that cells switch between strategies and, thus, between subpopulations, or by frequency-dependent selection among subpopulations, implying varying growth rates. For our calculations, the exact nature of these processes is not relevant. In the Prisoner's Dilemma, the only ESS is that all players defect (which is a pure ESS), while in the snowdrift game, the only ESS is that a certain subpopulation of cells cooperate and the remaining fraction of cells defect (a mixed ESS) [15]. In the harmony game, the only ESS is that all cells use the cooperative strategy.

In the experiment by Greig and Travisano [10], cheaters showed a higher or lower fitness (growth rate) than cooperators when total cell density was high or low, respectively (starting with equal fractions). The conclusion drawn by Greig and Travisano [10] that this would correspond to a Prisoner's Dilemma has been questioned [17, 21], mentioning that it could rather be a snowdrift game (see also [27, 29]). As mentioned above, stable cooperation arises naturally in the snowdrift game. Gore *et al.* [30] showed by experiments (again with baker's yeast) that a stable coexistence between invertase-secreting and non-secreting yeast cells can be established even in well-mixed cultures. They concluded that generally a snowdrift game applies and supported this by a phenomenological mathematical model, which implies Prisoner's Dilemma, snowdrift, or harmony games depending on parameter values.

Identifying the correct game appropriate for describing a particular biological situation is important because, as explained before, different games result in different stable population structures. For biotechnological applications, it is of interest to manipulate the system such that this population structure induces a maximal level of exoenzyme production. Here we present a mathematical model based on the Monod kinetics, which is widely used for quantifying growth in microbiology. Moreover, our model takes into account the dependence on total cell density, a parameter that is crucial for optimizing the efficiency of exoenzyme production. This enables us to describe mathematically the experimentally observed dependence of the relative fitness of cheaters on total cell density in Greig and Travisano's experiment [10].

The presented approach has a wide range of potential applications. This includes the economically important production of renewable biofuels such as ethanol from non-food sources. For this purpose, various microorganisms can be engineered to secrete exoenzymes for degrading celluloses and hemicelluloses [1].

2 Methods

In accordance with the experiment by Greig and Travisano [10] and earlier modeling studies [3, 7], here we consider two types of cells only: cooperating cells secreting a given quantity of exoenzyme and complete defectors not secreting any exoenzyme at all. Throughout, the term "enzyme" refers to the extracellular enzyme under study. The product of that enzyme is often a growth substrate for the cell, for example, glucose in the case of invertase and cellobiase (a special cellulase cleaving cellobiose). Therefore, we make a distinction between "initial substrate" (of the enzyme) and "growth substrate". The latter may then be taken up into the cell and act as a substrate for further biotransformations.

We consider exponentially growing populations. The basic growth rate is denoted by μ_0. This is the growth rate when the desirable growth substrate under study is absent, while alternative substrates may be present, and it includes the death rate of cells if this is non-negligible. The concentration of the desirable growth substrate at the surface of a given cell is denoted by S. To quantify the dependence of the growth rate on S, we use the Monod equation [31]:

$$\mu'(S) = \frac{\mu'_{\max} \cdot S}{K + S} \tag{1}$$

where the prime refers to the surplus in growth rate above μ_0 due to the desirable external growth substrate. Note that μ_0 can be described by Monod kinetics in terms of alternative substrates, but this dependence is irrelevant for the model because we assume that alternative substrates (if any) have constant concentrations. K and $\mu'_{\max}$ denote the half-saturation constant and maximum increase in growth rate at saturating growth substrate levels, respectively. Note that this function is monotonically increasing and concave.

Since most of the enzyme acts in the vicinity of the cell (*e.g.*, in the periplasmic space if any, Fig. 1), part of the growth substrate generated by it diffuses straight into the enzyme-secreting cell. This can

be regarded as a "privileged share" of the growth substrate available to cooperating cells, even in the case of well-mixed systems. Moreover, in the case of spatially heterogeneous systems, cooperating cells have access to an additional bonus due to spatial gradients, which imply higher growth substrate levels around them (Fig. 1). This is another important "privileged share" factor in compensating for the costs of cooperation. The overall effect of the privileged shares for a cooperating cell is an increase in concentration of growth substrate, here denoted by δ. This quantity depends, in a monotonically increasing way, on the level of the initial substrate and is zero if none is available. For simplicity's sake, in our model, we assume that the initial substrate and, thus, δ are constant. The variable x stands for the frequency (fraction) of cooperating cells and ρ for total cell density. Both S and ρ relate to volume in three-dimensional media (*e.g.*, batch cultures) and to area in two-dimensional media (*e.g.*, agar plates). The total cost of enzyme production and secretion is counted by a reduction, c, in growth rate. The growth rates of cooperating cells, μ_C, and of defector cells, μ_D, can then be expressed as

$$\mu_C = \mu_0 - c + \mu'(q\rho x + \delta) \tag{2}$$

$$\mu_D = \mu_0 + \mu'(q\rho x) \tag{3}$$

where q is a proportionality constant relating the cell density of cooperators to the growth substrate level. Like δ, q is considered constant throughout.

3 Results

3.1 Initial relative fitness

Using Eqs. (2) and (3), the relative fitness of defectors can be calculated as

$$\frac{\mu_D}{\mu_C} = \frac{\mu_0 + \mu'(q\rho x)}{\mu_0 - c + \mu'(q\rho x + \delta)} \tag{4}$$

Together with Eq. (1), this equation gives the dependence of the relative fitness on cell density. In Fig. 2 (note the logarithmic scale), the nonlinear dependence is plotted for $x = 0.5$, in accordance with the initial conditions in the experiment of Greig and Travisano [10]. They empirically fitted the data to a straight line. The curve plotted in Fig. 2 is in agreement with the asymptotic behavior for very low and very high cell densities. In the extreme cases, the curve tends to asymptotic values, which can be explained as follows. Defectors in isolation grow at a constant rate. At extreme crowding, the enzyme concentration is in excess and the uptake of the growth substrate is saturated, so that the growth rate is constant again. The nonlinear curve generated by the model shows that the advantage of defectors and cooperators at high and low total densities, respectively, can be readily computed and, thus, explained on theoretical grounds. A quantitative comparison with the data of Greig and Travisano [10] will be published elsewhere.

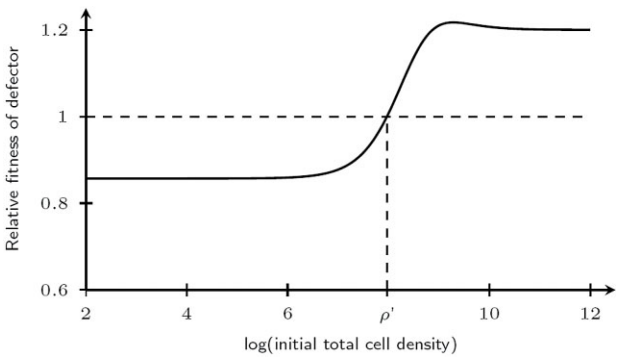

Figure 2. Relative fitness of defectors as a function of total cell density according to Eqs. (1) and (4). ρ', density at which the fitness of defectors and cooperators is equal and both have frequency 0.5 in the population. Parameter values: $\mu'_{max} = 0.4$/h, $\mu_0 = 0.2$/h, $c = 0.1$/h, $K = 2$ mM, $\delta = 1$ mM, $q = 8 \times 10^{-9}$ mM. This leads to $\rho' = 10^{7.97}$.

3.2 Equilibrium frequencies

Next, we calculate the frequency x^* that is finally attained in the ESS. At equilibrium (*i.e.*, in an ESS), the frequency-dependent growth rates of cooperators and defectors are equal. Thus, Eqs. (2) and (3) yield

$$\mu_0 - c + \mu'(S^* + \delta) = \mu_0 + \mu'(S^*) \tag{5}$$

with

$$S^* = q\,\rho\,x^* \tag{6}$$

$$\mu'(S^*+\delta) - c = \mu'(S^*) \tag{7}$$

where the asterisk refers to the situation in the ESS. Provided that δ is small, $\mu(S^* + \delta)$ can be expanded into a Taylor series up to the first-order term. Using Eq. (7), this gives

$$\mu'(S^* + \delta) = \mu'(S^*) + \left.\frac{d\mu'(S)}{dS}\right|_{S^*} \delta \tag{8}$$

$$\left.\frac{d\mu'(S)}{dS}\right|_{S^*} = \mu'_{\max}\frac{K}{(K + q\rho x^*)^2}. \tag{9}$$

Together with Eq. (7), we derive that the right-hand side of Eq. (9) must equal c/δ. This leads to the equilibrium frequency of cooperators:

$$x^* = \frac{\sqrt{\mu'_{\max} K\delta / c} - K}{q\rho} \quad (10)$$

Interestingly, this equilibrium frequency does not depend on the basic growth rate, μ_0, because the latter is the same for cooperators and cheaters. However, if μ_0 is very large in comparison to μ', the computation of the equilibrium frequency of cooperators becomes less robust because the difference in growth rates between cooperators and cheaters does not exceed "noise".

If Eq. (10) gives values of x^* lower than zero or larger than one, Eq. (5) cannot be fulfilled. If the growth rate of cooperators (left-hand side of Eq. 5) is larger than that of the defectors (right-hand side), x tends to one, while in the opposite case it tends to zero. Thus, the equilibrium frequencies x^* in these two cases equal one and zero, respectively. Accordingly, we can distinguish the following three cases (see Fig. 3).

1. *Low-benefit case* (compared to costs): The condition relevant in this case follows from Eq. (10) by assuming $x^* \le 0$. This leads to

$$c \ge \mu'_{\max}\, \delta/K = c_2. \quad (11)$$

Pure defection is the only ESS in this case: $x^* = 0$; the cells are trapped in a Prisoner's Dilemma (black lower plane in Fig. 3). The population can then survive only if additional factors intervene, such as the presence of alternative growth substrates or intracellular enzyme forming growth substrate. Otherwise, the basic growth rate μ_0 would be negative, implying that the population, which then consists of cheaters only, would become extinct. This fatal situation may no longer be observed in nature just because the populations became extinct [32]. However, in biotechnological setups, it can indeed occur, but should, of course, be avoided.

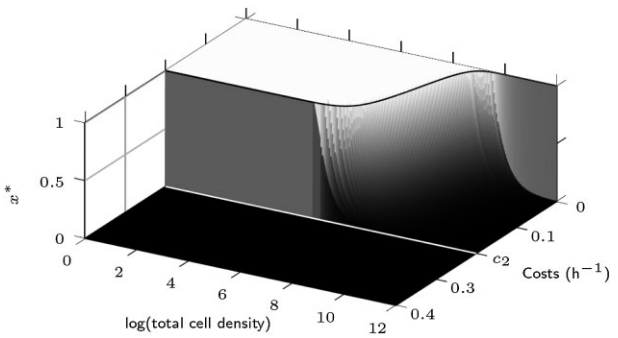

Figure 3. 3-D plot of the equilibrium frequency of cooperators, x^*, *vs.* the total cell density, ρ , and the cost of cooperation, c. The white plateau corresponds to the case of pure cooperation (harmony game, high-benefit case). For $c \ge c_2$ (black plane), the Prisoner's Dilemma always results, irrespective of cell density (low-benefit case, *cf.* Eq. 11). The area between these planes corresponds to coexistence (snowdrift game, intermediate-benefit case). For $c < c_2$ and large population densities, the frequency of cooperators tends to zero (Eq. 10), so that practically pure defection occurs. Accordingly, the black color at the bottom of the surface representing the snowdrift game is the same as for the Prisoner's Dilemma game. Parameter values are the same as in Fig. 2 except the varying costs.

2. *High-benefit case*: From Eq. (10), it follows that the frequency x^* equals one if population density is lower than the critical density threshold

$$\rho'' = \frac{\sqrt{\mu'_{\max} K\delta / c} - K}{q} \quad (12)$$

At these low densities there is effectively no interaction between cells and therefore the cooperator cells take over the population by virtue of their higher growth rate. In terms of the costs, the condition relevant in this case reads

$$c \le \frac{\mu'_{\max} K\delta}{(q\rho + K)^2} = c_1. \quad (13)$$

Below the critical density ρ'' given by Eq. (12), the cheater frequency tends to zero. In the high-benefit case, the only ESS is pure cooperation: $x^* = 1$ (white plateau in Fig. 3). Thus, a harmony game applies. The curve of relation (13) in the equality case can be seen by the curved black line on the upper end of the "cliff" in Fig. 3.

3. *Intermediate-benefit case*: The relevant constraint reads

$$c_1 = \frac{\mu'_{\max} K\delta}{(q\rho + K)^2} < c < \frac{\mu'_{\max}\delta}{K} = c_2. \quad (14)$$

In this case, x^* lies between 0 and 1. The dependence of x^* on the costs of enzyme secretion and cell density as given by Eq. (10) is depicted in Fig. 3. From Eq. (10), it follows that for large population densities, the frequency of cooperators tends to zero, so that practically pure defection occurs. Moreover, note that for low total densities, ρ, the lower bound in relation (14) tends to the upper bound. Then, the intermediate-benefit case practically disappears as can be seen in Fig. 3. The critical density, ρ', at which the cooperating and cheater cells have equal fitness and equal frequency (Fig. 2) can be determined by setting x^* equal to 1/2 in Eq. (10):

$$\rho' = 2\frac{\sqrt{\mu'_{\max} K\delta / c} - K}{q} = 2\rho'' \quad (15)$$

This means that this critical density is twice as high as the critical threshold at which the harmony game turns into a snowdrift game. It is worth noting that both Eqs. (12) and (15) have

been derived on the basis of a Taylor expansion. In Fig. 2, the more precise value of ρ' without using that approximation is shown.

3.3 Maximum productivity is attained at "the edge of harmony"

In view of technological applications, it is of great interest to maximize the effectivity of exoenzyme production. The optimization problem is non-trivial because an increase in total cell density also facilitates the occurrence of cheater cells. The question arises whether the goal is to maximize specific productivity or volumetric productivity. Note that the equilibrium density of cooperating cells is the (mathematical) product of total equilibrium density and cooperator frequency (Fig. 4). (Equilibrium is defined here with respect to frequencies rather than total growth). The volumetric enzyme productivity can be assumed to be (nearly) proportional to the equilibrium density of cooperating cells. The curve in Fig. 4 consists of two parts: at low densities, proportionality holds because $x^* = 1$, so that $\rho x^* = \rho$. At high densities, the function is a constant, as can be seen by multiplying Eq. (10) by ρ. It can be seen in Fig. 4 that the mathematical solution to the optimization problem of maximizing exoenzyme production is not unique. It is sufficient to have any equilibrium density above the threshold value ρ''. This ambiguity (degeneracy) of the solution can be resolved by also maximizing specific productivity. This is achieved by decreasing cell density. Therefore, the best value is reached at the critical value, ρ'', *i.e.*, at the upper end of the "cliff" in Fig. 3. The same solution would be obtained by first maximizing the specific productivity and then resolving the resulting ambiguity by maximizing volumetric productivity.

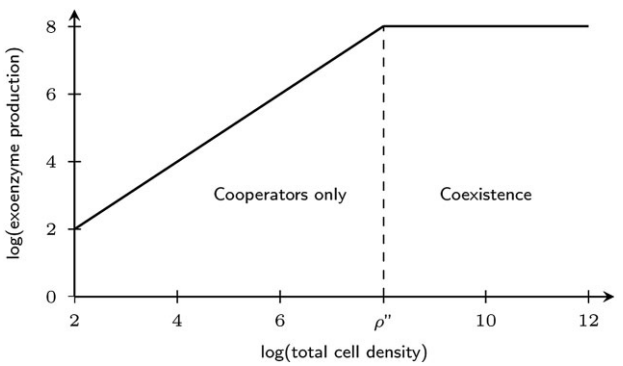

Figure 4. Plot of the exoenzyme production (assumed to be proportional to equilibrium density of cooperators, x^*) *vs.* the total equilibrium cell density, ρ. ρ'', threshold between snowdrift and harmony games (Eq. 12). For further explanations, see text. Parameter values are as in Fig. 2. This leads to $\rho'' = 10^{8.02}$.

4 Discussion

Here we have presented a game-theoretical model of cooperation and competition among microbial cells upon secretion of exoenzymes, and have discussed this from a biotechnological perspective. As shown by our theoretical analysis, secretion of exoenzymes is not always a Prisoner's Dilemma. Under a wide range of physiological conditions, it rather is a snowdrift game and can even turn into a harmony game. For the special case of yeast invertase, this has been shown both experimentally and theoretically by Gore *et al.* [30]. Notably, a snowdrift game occurs when cell density is neither very low nor very high. From our model, it can be deduced that all cells eventually cooperate (harmony game) when the process is run at constant low densities, as has indeed been observed [33]. Another testable prediction is that, when cell density increases upon growth, a transition from pure cooperation (harmony game) to coexistence (snowdrift game) should occur. In summary, our results indicate that microbial strains engineered for exoenzyme production will not, under appropriate conditions, be outcompeted by non-producing mutants. Thus, our presentation demonstrates that game theory is useful in designing biotechnological setups that are robust against "takeover" by cheater mutants.

In both the snowdrift and harmony games, cooperation is a direct consequence of the payoff structure of the game. Thus, more complex means to escape the Prisoner's Dilemma such as formation of aggregates are not required, in contrast to, for example, the competition between microorganisms that use respiration for ATP production and those using fermentation or respirofermentation [22, 34].

For cooperation to occur it is crucial that the benefit (payoff) from the public goods produced by a given cooperator exceeds the costs of its production. This is fulfilled if each cell obtains a sufficient privileged share of the growth substrate. A privileged share, which has been taken into account here by a higher growth substrate concentration, may arise when part of the substrate is retained by cooperating cells, for example, when the exoenzyme is bound to the cell wall or to a periplasmic space and/or if the medium is not well mixed. The local growth substrate around cooperating cells will then be higher than around defecting cells [35]. In the important case of well-mixed systems, the privileged share only consists of the part directly retained by cooperating cells. Thus, the same model can be applied, with a smaller privileged share, δ. This leads to a shift of the boundaries between the three cases (games). In particular, the area of the

harmony game is smaller than in spatially heterogeneous setups because δ is then smaller, while the area of the Prisoner's Dilemma is larger. Gore *et al*. [30] studied this case both experimentally and theoretically. They modeled this by an efficiency of capture, ε, and by raising the payoff for defectors and cheaters to an exponent α being a phenomenological (not necessarily integer) parameter. Here, in contrast, we use the Monod equation (Eq. 1), an established kinetics in microbiology.

From the above reasoning about privileged shares (both in well-mixed and heterogeneous systems), it follows that "cooperators" operate in their selfish interest (rather than for the well-being of the population, let alone by altruism). It is worth noting that there are alternative possibilities of advantages for cooperators [35]. For example, some secreting bacteria physically attach to a water-insoluble substrate and can, thus, escape from predation by protozoa [3, 36].

Upon variation of density, at most two games can occur for a given cost value: at low costs, a harmony game or a snowdrift game can be observed, while at high costs, it is always a Prisoner's Dilemma independent of cell density (Fig. 3). Accordingly, in biotechnological applications, first the costs of exoenzyme production and secretion should be determined or at least estimated. Only if they are low enough to allow for cooperation (snowdrift or harmony games), is a further investment worth making. Thus, enzyme production should not be upregulated to the extent that costs will be too high and defecting mutants are likely to outcompete the engineered strain. Moreover, it is useful to engineer the enzyme so that it can remain attached to the cell; thus cooperators get an advantage.

Our model is based on a number of simplifying assumptions. The first simplification is the restriction to exponentially growing populations. The case of logistic growth eventually leading to stationary phases is left to future extensions of the model. The simplifications allow a largely analytical treatment – the nonlinear dependencies of the initial relative fitness on total cell density and of the equilibrium frequencies on both cell density and the costs can be calculated in closed form. Our results are complementary to, and consistent with, the numerical simulations based on a grid model by Allison [7]. Those simulations predicted that cooperators would outcompete defectors in the case of high benefit, while intermediate benefit leads to coexistence. The assumptions of our model are, in spite of the simplification, justified on biological grounds.

The physiological advantage of extracellular hydrolysis is obvious for macromolecular substrates such as cellulose because they cannot enter the cell. It is less obvious for smaller substrates such as sucrose. Osmotic effects [37], foraging by chemotaxis and the lower costs of uptake of glucose and fructose, which is performed by facilitated diffusion in contrast to the active sucrose uptake [33] were suggested in the literature. Moreover, intracellular space for proteins is severely limited due to macromolecular crowding [38, 39]. Therefore, we hypothesize that secretion of extracellular enzymes provides an advantage by relieving intracellular packing.

Of course, maximizing the volumetric productivity of exoenzyme secretion is of interest for biotechnological applications. Our approach allows one to delineate specific production conditions under which the synthesis of a desired exoenzyme would be maximized by adjusting total cell density on the basis of estimates of parameters such as costs of exoenzyme production/secretion. Our model shows, for the case of sufficiently low costs, a degenerate maximum above the density threshold between snowdrift and harmony games. Thus, somewhat counter-intuitively, the case of coexistence between cooperators and cheaters allows a higher exoenzyme production than most situations of pure cooperation. Since, for economic reasons, it is worth using a low density and, thus, in addition maximizing specific productivity, the best value is reached at the "edge of harmony". This shows again that it is very helpful to analyze the types of "games played" by microorganisms upon extracellular conversion of diverse substrates.

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