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PROBABILISTIC SAFETY AND OPTIMAL CONTROL FOR SURVIVAL ANALYSIS OF *BACILLUS SUBTILIS*[1]

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Abstract: This work investigates the problem of determining switching conditions for the production of the lantibiotic subtilin by *Bacillus subtilis*. These conditions are synthesized as the outcome of an optimal control problem based on a survival analysis interpretation. The problem is built upon the general modeling framework of stochastic hybrid systems, by translating the survival analysis into a probabilistic optimal safety verification procedure.

Keywords: Stress response network; Survival analysis; Stochastic hybrid systems; Safety analysis; Optimal control.

1. INTRODUCTION

The investigation of the stress response network of *Bacillus subtilis* ATCC 6633 offers a detailed explanation of how the bacterium reacts to competitive environmental conditions, among the many options, by producing the antibiotic subtilin in order to directly suppress other cells while getting immunized (T. Msadek 1999). The mechanisms of this generation are fairly well understood and described by a genetic and protein pathway that involves some non-deterministic interplay between its quantities. In particular, the presence of switching modes exhibits the activation/deactivation of certain genes and the increase/decrease in production of the corresponding proteins.

The concept of *optimum* is common and shared between engineering and biological systems. Before its employment, though, it is necessary to answer some fundamental questions: optimality with respect to what? And at what level? The biology literature offers numerous examples where optimality appears to regulate a certain behavior, or to explain the properties of a particular entity. (R. Rosen 1967) presents probably the first attempt, rather qualitative in nature but nevertheless very stimulating, to systematically frame the concept of optimality in biology. More recently, (D. Segre *et al.* 2002) employs a similar take in the context of metabolic networks, while (J. Weibull 1995, D. Wolf *et al.* 2005) look at dynamical game theory as a means to think about optimality in the context of evolution. On the other hand, many notable instances from the same domain caution that the abuse of this notion may yield to incorrect conclusions. However, according to the general tenets of evolution, it is indisputable to claim that *a biological structure is "optimal" (at least locally and temporarily) because it has survived evolution under the pressure of natural selection.* In our study,

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this translates into postulating that the functioning of the pathway follows certain criteria and levels of optimality. In this context optimality is intended as a measure of personal fitness or, in the particular instance, of own survival. In particular, one would expect that the activation/deactivation switches in the network happen "optimally" in the above sense.

In this work, we look at a recently developed dynamical model for the genetic network describing the biosynthesis of the lantibiotic subtilin (J. Hu *et al.* 2004) and propose a few improvements and modifications to the model to bring it in line with newer evidence reported in the literature (H. Tjalsma *et al.* 2004, T. Stein 2005). We obtain a system that presents partially decoupled high-level dynamics (those dealing with the population size and the nutrient level) and low-level ones (those describing the mechanism of production of subtilin by a single cell). The high-level model is non-linear and deterministic, while the low-level one is hybrid and stochastic. The model, while somewhat simplistic and limited in scope, has to be intended as a framework for the methodological procedure presented in this work.

The system in its entirety can be interpreted as a stochastic hybrid system (SHS) (J. Hu *et al.* 2004, A. Abate *et al.* 2006). This allows one to study the survival of the single *B. subtilis* cell as a probabilistic, decentralized safety specification problem. It is "probabilistic" because of the certainly stochastic dynamics. It is naturally "decentralized" because each entity, while optimizing for its own fitness (which depends on global information), does not communicate with the competitors, nor has knowledge of their actions. Furthermore, the solution of the problem may not be globally optimal.

Using recently developed techniques for probabilistic verification in a stochastic hybrid systems setting (A. Abate *et al.* 2006), we reinterpret the above probabilistic safety problem as a (stochastic) optimal control one, where the controls are functions of the state-space that encode the switches in the network. Finally, the solution of this stochastic and decentralized optimal control problem yields the location and the structure of the switching behaviors under study. This methodological approach may shed light on the strategies employed by the organisms to improve their chances of survival when it is possible to codify such a condition into a cost function. In general, we argue that a genetic network function can be viewed as an optimization problem where the objective is to maximize the probability of survival of the individual. Moreover, this approach suggests that it is according to this survival interpretation, rather than other ad hoc characterizations, that the thresholds in the system should be specified and determined.

2. A MODEL FOR ANTIBIOTIC SYNTHESIS

Resorting to a schematization proposed in (J. Hu *et al.* 2004), based on a wealth of recent research (S. Banerjee and Hansen 1988, K. Entian and de Vos 1996, P. Kiesau *et al.* 1997, T. Msadek 1999, T. Stein *et al.* 2002, T. Stein 2005), it is possible to abstract the biosynthesis network into a five-dimensional model (see figure 1, taken from (J. Hu *et al.* 2004)). The model, which for the sake of simplicity disregards some of the components in the otherwise complex subtilin biosynthesis pathway, as well as some behavior only tangentially of interest at this level, encompasses two "global" variables (population and nutrient level) and three "local" ones (the concentration of the sigma factor SigH and of the two proteins SpaRK and SpaS). In order to prune away details that may be uninteresting at this level, the presence of the peptide SpaS is equated to represent the actual antibiotic subtilin. Furthermore, for the sake of simplicity (and, as it shall be seen in the following, of computation), given the symmetric and repetitive structure of the dynamics of SpaRK and SpaS in their dependence on, respectively, SigH and SpaRK, in the current case study we shall disregard the evolution of SpaRK and just hypothesize a direct influence of SigH on SpaS, without much change in the final behaviors (cfr. also trajectory plots in (J. Hu *et al.* 2004)).

Ideally, a model that keeps track of each of the dynamics of a variable set of species in a certain environment may yield extremely precise results. However, this approach is discarded because of its sheer impracticality and of the difficulty in its dynamical analysis. Hence, it is preferred to introduce a model that decouples high-level from low-level dynamics (see figure 2). The higher level, which encompasses the first two global variables, is deterministic and based on average dynamics. The lower one, involving the last three local coordinates, describes cellular processes and is made up of stochastic and switching dynamics. The reader may notice that the model is endowed with a decentralized structure (see figure 2).

We shall denote with [SigH] and [SpaS] the concentration of the respective species, and with $\overline{[SpaS]}$ the corresponding averaged value throughout the whole environment. In the following, a modification of the model in (J. Hu *et al.* 2004) is described.

Let us start from the lower-level relations. The level of the sigma factor follows a controlled switching behavior:

$$\frac{d[SigH]}{dt} = \begin{cases} -\lambda_1[SigH] & \text{if production is OFF} \\ -\lambda_1[SigH] + k_3 & \text{if production is ON.} \end{cases}$$

In (J. Hu *et al.* 2004) the conditions determining the ON/OFF status depended on a fixed, arbitrary threshold on the food level. In (P. Kouretas *et al.*

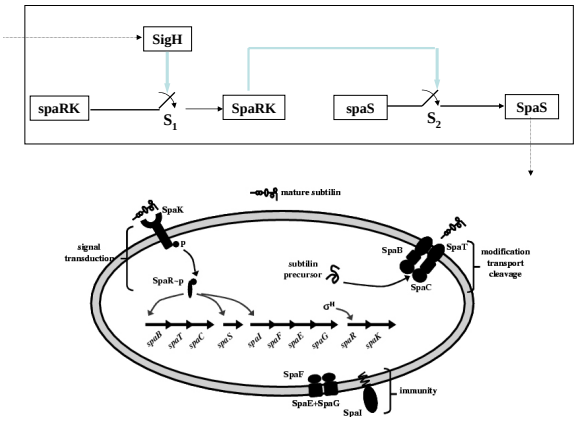

Fig. 1. The subtilin biosynthesis pathway.

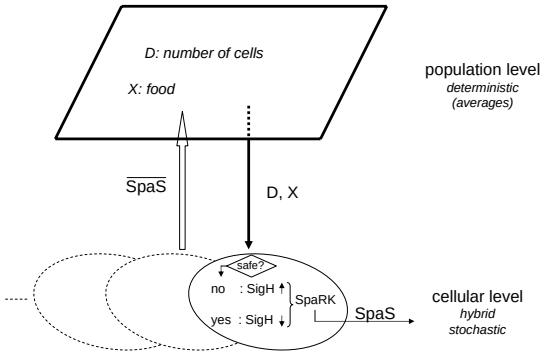

Fig. 2. The decentralized structure of the model under study.

2006), an identification scheme is employed to find out parameters of the system, and could in principle be applied to compute this threshold. The underlying hypothesis is again that the threshold is a constant level on any of the model coordinates. Notice further that, since all the models built so far are time-invariant, no threshold dependence on the growth phase of the species is taken into account (T. Stein 2005). Both approaches rule out the possibility of having a more complex dependence on the state space, which is indeed what this work aims at finding. Assuming fictitiously that the production can be controlled, it is instructive to write out the above dynamical relations as

$$\frac{d[SigH]}{dt} = -\lambda_1[SigH] + k_3 u. \qquad (1)$$

The essential assumption is that the control $u = f(D, X, [SigH], [SpaS]) : \mathcal{D} \to \{0, 1\}$, where $\mathcal{D} = [0, D_M] \times [0, X_M] \times \mathbb{R}^2_+ \subset \mathbb{R}^4$ is a general binary function of the variables of the model.
The concentration of the protein SpaS depends on one of the two possible states of a switch S_1 as:

$$\frac{d[SpaS]}{dt} = \begin{cases} -\lambda_3[SpaS] & \text{if } S_1 \text{is OFF} \\ -\lambda_3[SpaS] + k_5 & \text{if } S_1 \text{is ON.} \end{cases} \qquad (2)$$

$S_1 = \{OFF, ON\}$ is assumed to be a Markov Chain, whose transition probability matrix is:

$$P_1 = \begin{bmatrix} 1 - b_0 & b_0 \\ b_1 & 1 - b_1 \end{bmatrix}, \qquad (3)$$

where b_0, b_1 depend directly on [SigH] according to (J. Hu *et al.* 2004) $b_0([SigH]) = \frac{e^{-\Delta G_{rk}/RT}[SigH]}{1+e^{-\Delta G_{rk}/RT}[SigH]}$, $b_1([SigH]) = 1 - b_0([SigH])$. The quantity ΔG_{rk} represents the Gibbs free energy of the molecular configuration, R is a gas constant and T the environment temperature in Kelvin. Intuitively, SigH promotes the production of SpaS by increasing the likelihood of S_1 to be in the ON state. The above choice for the transition probabilities makes S_1 reversible, which helps in its analysis in that the steady state corresponds to the vector $[\pi_{OFF}, \pi_{ON}]^T = [b_1, b_0]^T$.
The dynamics of the population is modeled by a logistic equation as follows:

$$\frac{dD}{dt} = rD\left(1 - \frac{D}{D_\infty}\right). \qquad (4)$$

The quantity D_∞ is the carrying capacity, which turns out to be the stable equilibrium point for the dynamical system. We a priori define it to be $D_\infty = \frac{X}{X_M} D_M$, where D_M and X_M represent the maxima for the population and the food levels in the environment. As intuitive, the steady state dynamically depends on the relative quantity of food in the environment.
The food dynamics are taken to be:

$$\frac{dX}{dt} = -k_1 DX + k_2\overline{[SpaS]}. \qquad (5)$$

The above dynamical relation says that the food gets consumed at a rate proportional to its present level and the population density, while the average production of subtilin decreases this rate because of its indirect negative influence on the population level. This average quantity is introduced to be the following:

$$\overline{[SpaS]} = \frac{D}{D_M}\left(1 - \frac{X}{X_M}\right)\frac{k_5}{\lambda_3}\bar{b}_0 h(X), \qquad (6)$$

where $\bar{b}_0 = b_0(\overline{[SigH]})$ and $\overline{[SigH]} = \frac{k_3}{\lambda_1}$, and $h(X)$ is the Heaviside step function over the food level, i.e. it is equal to 1 if $X > 0$, and to 0 if $X = 0$. The above relation stresses a dependence on the "competition" in the environment (first two terms), and on the steady-state dynamics for [SpaS], which in turn depends on the steady state of SigH (last terms, taken from eqns. (1-2)).
From the dynamical relations in (4-5) and eqn. (6), the steady state of the high level variables is going to be either $[D_{eq}, X_{eq}]^T = [0, \beta]^T, \beta \in [0, X_M]$, or $[D_{eq}, X_{eq}]^T = [\alpha D_M, \alpha X_M]^T$, where $\alpha \leq \frac{k_2 k_3 k_5}{k_2 k_3 k_5 + k_1 \lambda_1 \lambda_3 D_M X_M} \leq 1$. While the first equilibrium is unstable, the second is stable for any combination of the model parameters.

The above set of dependencies and dynamical relations can be formally reframed as a SHS (A. Abate *et al.* 2006). In order to exploit some results from this literature, from now on we shall work in discrete time, assuming that the above dynamical relations have been properly approximated. Notice that the probabilistic terms in the model are concentrated on the switching structure of S_1. The hybrid state space is made up of a discrete component θ (the state of S_1), and a continuous one (the vector $\boldsymbol{x} \in \mathcal{D}$)—it will be denoted $\mathcal{S} = \{OFF, ON\} \times \mathcal{D}$. The dynamics of SigH depends on a binary function that depends, according to eqn. (1), on a general feedback contribution of the (continuous component of the) state space. The objective will then be that of synthesizing this function according to certain optimality criteria that will be made explicit in the following. The shape of the outcome will then dictate whether the dynamics of SigH will present a threshold (as suggested in the literature) or, more generally, what vector field will describe it. A control profile along a certain interval of time, i.e. a sequence of mappings $\mu = (\mu_0, \mu_1, \ldots)$ of the form in (1), shall be named a *strategy*, or a *policy*. The binary control space, denoted $\mathcal{U} = \{0, 1\}$, is in this case discrete and finite.

A solution of the above SHS model is a stochastic process with two components $\boldsymbol{s}(k) = (\theta, \boldsymbol{x})(k), k \geq k_0$ which, given an initial condition at time k_0 (possibly sampled from an initial probability distribution), continuously evolves in either of the two discrete modes until a jumping condition is verified (which reduces to sampling, along the evolution of the trajectory, from the inhomogeneous probability distribution of the MC S_1). Once a transition is triggered, the discrete state changes mode, while the continuous state remains unchanged (in the hybrid systems parlance, it is said the "reset" is the identity), and the evolution continues from the unaltered conditions in the new mode. By construction, the solution of the model is a controlled stochastic process. Furthermore, given the structure of the policy and its sole dependence on the current state at each time, once a strategy is selected the process is simply Markovian. For more details, especially on the probabilistic structures and properties embedded in the model, please refer to (A. Abate *et al.* 2006). Along with the general SHS interpretation, the model can be also though as being a piecewise-deterministic Markov process, as in (M.H.A. Davis 1993, P. Kouretas *et al.* 2006).

3. SURVIVAL ANALYSIS AS PROBABILISTIC SAFETY VERIFICATION

The literature on antibiotic synthesis as a stress response for *B. subtilis* suggests that the production activation or de-activation follows some sort of "switching" profile (T. Stein *et al.* 2002, T. Stein 2005). In other words, it is believed that there exist certain thresholds that are function either of the species concentration, or of the food or the population level, that are characteristic of these switches. A wealth of research has been spent, assuming a special structure for these functions, on automatically identifying these thresholds from the data (P. Kouretas *et al.* 2006).

As described in the preceding section, in this work we take a rather different perspective. The presence of the thresholds will not be a-priori postulated, but possibly obtained with regards to a certain survival property. It is intuitive to think that a species activates or halts the "production pipeline" for the antibiotic with the main objective of maximizing its own survival likelihood. Now, reinterpreting survival within the dynamic model introduced in section 2, we can introduce certain safety regions within the state space that are associated with a survival status. Next, because a solution of the SHS is dependent on a Markovian control, we aim at synthesizing these thresholds from their dependence on certain *safety levels*.

In general terms, in a stochastic setting a *safety analysis* problem consists in evaluating the probability that the state of the system remains outside a certain set deemed to be *unsafe* during a given time horizon, starting from some set of initial conditions.

According to this interpretation and associating a safety set to a survival condition, we will say that the single *B-subtilis* bacterium is safe if $[SpaS] > \overline{[SpaS]}$, meaning that the subtilin production level of the single species under study is higher than the average subtilin present in the surrounding environment. This condition encodes in a higher likelihood for the species to kill other bacteria, rather than being killed by their antibiotic. Equivalently, exploiting the expression in equation (6), we define the *safe region* $\mathcal{A}$ to be the set of points

$$\mathcal{A} = \left\{ \boldsymbol{s} \in \mathcal{S} : [SpaS] > \frac{D}{D_M} \left(1 - \frac{X}{X_M}\right) \frac{k_5}{\lambda_3} \bar{b}_0 h(X) \right\}.$$

The objective of the study then becomes that of modeling antibiotic production via safety analysis in a SHS framework. In the following section 4, we formally set up the problem and recall results in the literature to work it out.

4. SAFETY VERIFICATION FOR SHS

Let us recall the SHS model introduced in section 2. The variables into play are the population level D, the nutrient level X, the concentrations of the sigma factor SigH and of the peptide SpaS. Let us consider an arbitrary finite time horizon N.

For a given initial state $\boldsymbol{s}_0 \in \mathcal{S}$ and a Markov

policy μ (sequence of N binary control functions $u \in \mathcal{U}$), a safety analysis problem consists in determining the probability that the execution associated with μ and initialization $\boldsymbol{s}_0$ will stay within the set $\mathcal{A}$ during the time horizon $[0, N]$:

$$p^{\mu}_{\boldsymbol{s}_0}(\mathcal{A}) := P^{\mu}_{\boldsymbol{s}_0}(\mathbf{s}(k) \in \mathcal{A} \text{ for all } k \in [0, N]). \quad (7)$$

The set of initial conditions that guarantees a safety level ϵ, when the control policy μ is assigned, $S^{\mu}(\epsilon) = \{s \in \mathcal{S} : p^{\mu}_s(\mathcal{A}) \geq \epsilon\}$, is then referred to as *probabilistic safe set* with safety level ϵ. Analogously, $S^*(\epsilon)$, obtained by maximizing over the allowed controls, is called *maximal probabilistic safe set*. Finally, a policy μ^* is *maximally safe* if $p^{\mu^*}_s(\mathcal{A}) = \max_{\mu} p^{\mu}_s(\mathcal{A})$, $\forall s \in \mathcal{A}$.

Let $\mathbf{1}_C : \mathcal{S} \to \{0, 1\}$ denote the indicator function of a set $C \in \mathcal{S}$. Observe that

$$\prod_{k=0}^{N} \mathbf{1}_{\mathcal{A}}(s_k) = \begin{cases} 1, & \text{if } s_k \in \mathcal{A} \text{ for all } k \in [0, N] \\ 0, & \text{otherwise,} \end{cases}$$

where $s_k \in \mathcal{S}$, $k \in [0, N]$. Then, interpreting this set of $N + 1$ points s_k as a realization of $\boldsymbol{s}(\cdot)$,

$$p^{\mu}_{\boldsymbol{s}_0}(\mathcal{A}) = P^{\mu}_{\boldsymbol{s}_0}\left(\prod_{k=0}^{N} \mathbf{1}_{\mathcal{A}}(\mathbf{s}(k)) = 1\right) = E^{\mu}_{\boldsymbol{s}_0}\left[\prod_{k=0}^{N} \mathbf{1}_{\mathcal{A}}(\mathbf{s}(k))\right].$$

The quantity $p^{\mu}_{\boldsymbol{s}_0}(\mathcal{A})$ can be computed through a backward iterative procedure. For the sake of notation, let us introduce a stochastic kernel $T_s : \mathcal{B}(\mathcal{S}) \times \mathcal{S} \times \mathcal{U} \to [0, 1]$ on $\mathcal{S}$ given $\mathcal{S} \times \mathcal{U}$, which assigns to each $\boldsymbol{s} = (\theta, \boldsymbol{x}) \in \mathcal{S}$, $u \in \mathcal{U}$, a probability measure on the Borel space $\mathcal{B}(\mathcal{S})$ according to the dynamics of the SHS. This mapping is probabilistic because of the presence of the MC S_1. For each $k \in [0, N]$, define the map $V^{\mu}_k : \mathcal{S} \to [0, 1]$ as follows, $\forall s \in \mathcal{S}$:

$$V^{\mu}_k(s) := \mathbf{1}_{\mathcal{A}}(s) \int_{\mathcal{S}^{N-k}} \prod_{l=k+1}^{N} \mathbf{1}_{\mathcal{A}}(s_l) \cdot \quad (8)$$

$$\cdot \prod_{h=k+1}^{N-1} T_s(ds_{h+1}|s_h, \mu_h(s_h)) T_s(ds_{k+1}|s, \mu_k(s)).$$

Then, $V^{\mu}_k(s) = E^{\mu}\left[\prod_{l=k}^{N} \mathbf{1}_{\mathcal{A}}(\mathbf{s}(l)) | \, \mathbf{s}(k) = s\right]$ denotes the probability of remaining inside $\mathcal{A}$ during the (residual) time horizon $[k, N]$ starting from a specific $s \in \mathcal{S}$ at time k, under policy μ. It follows that $p^{\mu}_s(\mathcal{A}) = V^{\mu}_0(s)$. It can be shown through some algebraic manipulation that the terms $V^{\mu}_k(s)$ can be inductively expressed in terms of $V^{\mu}_{k+1}(s)$, with initialization $V^{\mu}_N(s) = \mathbf{1}_{\mathcal{A}}(s)$ (A. Abate *et al.* 2006).

As argued above, it is natural to set up an optimal control problem. In the following theorem (A. Abate *et al.* 2006), we describe an algorithm to compute $\max_{\mu} p^{\mu}_s(\mathcal{A})$ and ensure the existence of a maximally safe policy μ^*.

Theorem 1. Define the maps $V^*_k : \mathcal{S} \to [0, 1]$, $k = 0, 1, \ldots, N$, by the backward recursion:

$$V^*_k(s) = \max_{u \in \mathcal{U}} \mathbf{1}_{\mathcal{A}}(s) \int_{\mathcal{S}} V^*_{k+1}(s_{k+1}) T_s(ds_{k+1}|s, u),$$

initialized with $V^*_N(s) = \mathbf{1}_{\mathcal{A}}(s)$, and with $s \in \mathcal{S}$. Then, $V^*_0(s) = \max_{\mu} p^{\mu}_s(\mathcal{A})$ for all $s \in \mathcal{S}$. Moreover, there exists a maximally safe policy $\mu^* = (\mu^*_0, \ldots, \mu^*_{N-1})$, with $\mu^*_k : \mathcal{S} \to \mathcal{U}$, $k \in [0, N-1]$, given $\forall s \in \mathcal{S}$, by

$$\mu^*_k(s) = \arg\max_{u \in \mathcal{U}} \mathbf{1}_{\mathcal{A}}(s) \int_{\mathcal{S}} V^*_{k+1}(s_{k+1}) T_s(ds_{k+1}|s, u).$$

Having set up the above mathematical machinery, we are now ready to apply it to the problem under study. The result obtained in Thm. 1 is practically implemented via a dynamic programming algorithm. This procedure, along with the optimal safety level $p^{\mu^*}_s(\mathcal{A})$ associated to any point in the state space, yields also the optimal state-dependent, time-varying policies μ^*. This second output is really the focus of our attention, as it shall represent the synthesized control functions for the relation (1), to be interpreted as the activation thresholds for the production of subtilin.

5. NUMERICAL RESULTS

In this section we report the outcomes of the simulations for the above experimental setup. The parameters have been chosen to be the following: $r = 0.8, k_1 = 2, k_2 = 4, k_3 = 2.5, k_5 = 0.8, \lambda_1 = 0.5, \lambda_3 = 0.2, \Delta G_{rk}/RT = 1.1$. The discrete time horizon has been set to $N = 40$.

In Fig. 3, the plots of the maximal probabilistic safe level sets, with safety level $\epsilon = 1$, are shown for different time samples. The colors have been added only to enhance the perspective. All the points in the plots above the curves are considered to be "almost surely" safe. Notice that, as expected, the safe set shrinks as we proceed backwards in time (this in fact translates to a longer safety requirement for the trajectories of the system). For the sake of visualization, we plotted the results corresponding to a fixed value of the sigma factor $[SigH] = 1$. All the plots refer to the discrete state being in the OFF mode.

Fig. 4 represents couples of plots referring to maximal probabilistic safe sets and corresponding optimal actions. More precisely, the green plots (second and fourth row) represent the regions in the state space that are associated with a *switching action*, and are to be matched with the safety level sets plotted directly above them. It is interesting to realize that the optimal control functions, associated with the activation thresholds, have a characteristic "onion layer" shape, varying along time. It can be thus claimed that the optimal actions single out switching surfaces corresponding to certain safety levels. These surfaces have profiles that match the variation in safety probability (or, according to our interpretation, in survival likelihood) for the species, as appears by comparing the control plots with the

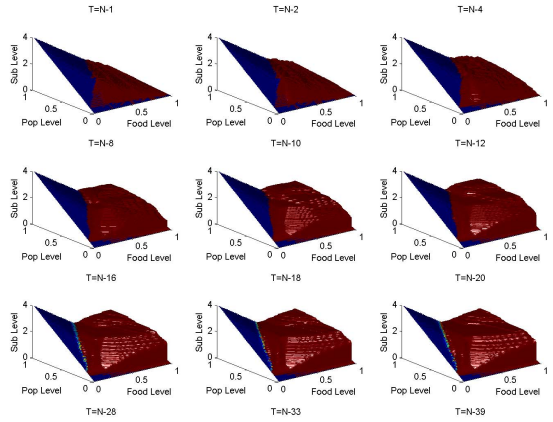

Fig. 3. Maximal probabilistic safety level set corresponding to $\epsilon = 1$, backwards in time.

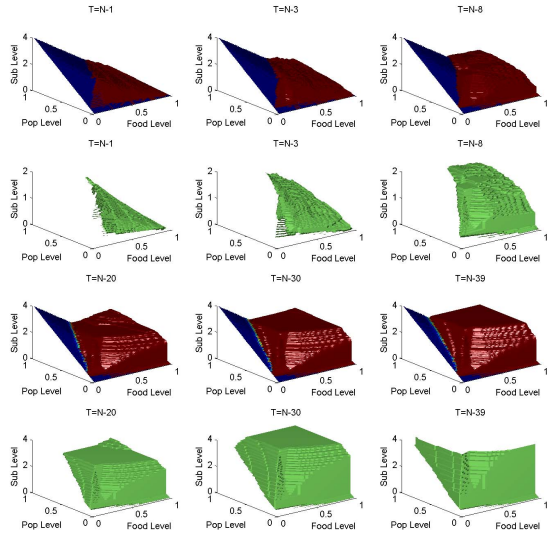

Fig. 4. Maximal probabilistic safety level sets and optimal switching control, backwards in time.

safety level sets. In general, these surfaces are not hyper-rectangular, as the previous research efforts that sought to identify thresholds related to them assumed. Instead, they are rather nonlinear functions of the state space, showing a manifest but non-explicit dependence with the change in safety level of the single species.

It would be consequently instructive to understand what is the *critical* safety level that corresponds to the activation of the production of subtilin Our computations hint at showing that, for the particular case under study, the level is a safety probability value close to one. More accurate testing needs to be performed on this aspect.

6. CONCLUSIONS AND FUTURE WORK

This methodological study suggests to interpret the problem of identifying production thresholds in the subtilin pathway as an outcome of a survival study. The adopted modeling framework is that of stochastic hybrid systems. Survival is encoded in a fitness function and interpreted as a (probabilistic) safety problem. Verification techniques based on optimal control (A. Abate *et al.* 2006) are then used to mathematically translate and compute this quantity, which also yields the desired activation thresholds. We argue that the functioning of a genetic network can be viewed as an optimization problem where the objective for the individual is to maximize its probability of survival. The dependence of the outcome of the technique on the choice of the fitness function is currently studied.

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