Talking Life Technologies
Document Security Level: Maximum
Subject: Threat of Mutation

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Some of the results of the experiments carried out in our researching center showed it was possible the emergence of mutants within the sensing-bacteria population. Certain mutations in the sensor plasmid could result in a lack of production of the fluorescent protein that indicates drug concentration in the medium, which causes the software not to detect the protein and then the bacteria "lie". When most of the bacteria population was not *lying*, it did not affect the functionality of the device. However, at the moment at which the increasing number of cheater bacteria raised above a certain threshold the accuracy of the response was compromised.

Based these equations for normal bacteria (1) and cheater bacteria (2),

$$\textbf{(1)} \quad \dot{S} = \alpha S\left[(1-\gamma) - \frac{(1-\gamma)S + C}{K + sK}\right] - \mu S$$

$$\textbf{(2)} \quad \dot{C} = \alpha C\left[1 - \frac{(1-\gamma)S + C}{K + sK}\right] + \mu S.$$

whose parameters are (α, the maximum growth rate per time unit; γ, the metabolic burden; μ, the mutation rate and K, the carrying capacity of the environment) population evolution graphics were obtained (Figures 1-3). They differ in the metabolic burden.

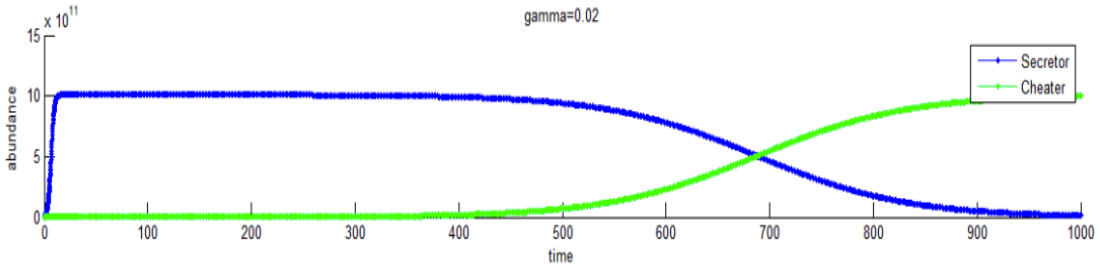

Figure 1. Population dynamics of secretor (or normal bacteria) versus cheater bacteria. This model uses the same parameters but γ= 0.02

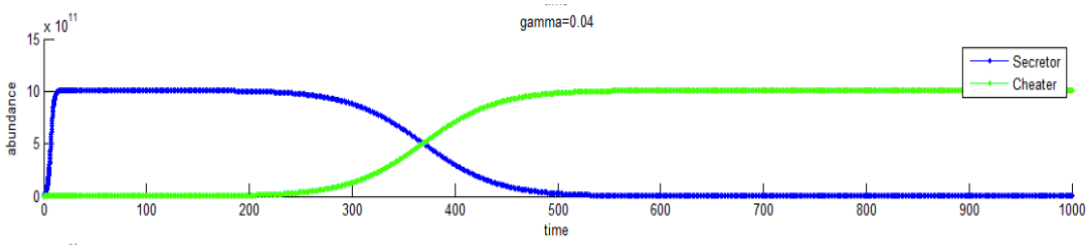

Figure 2. Population dynamics of secretor (or normal bacteria) versus cheater bacteria. This model uses the same parameters but γ= 0.04

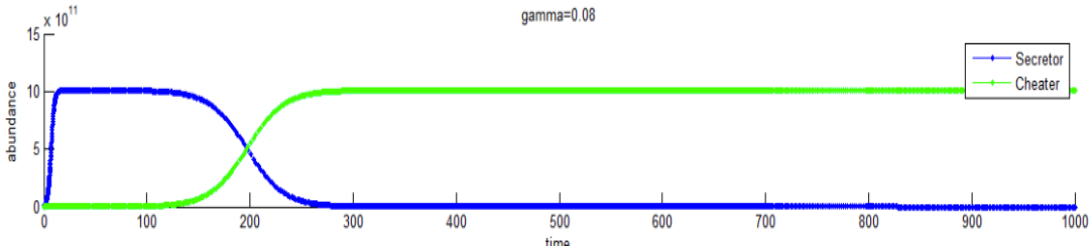

Figure 3. Population dynamics of secretor (or normal bacteria) versus cheater bacteria. This model uses the same parameters but γ= 0.08

From them it can be seen that after a certain time the cheater bacteria, i.e. those that do not have to produce the fluorescent sensing protein, are taking the control of the population (mainly due to the metabolic burden). Our scientific group has understood that depending on where the mutation occurs, the difference between the metabolic burden of cheater bacteria and normal bacteria will be different:

1. **Mutation within the coding sequence: expression of a truncated non-functional fluorescent protein, so the metabolic burden difference between the cheaters and normal bacteria is minimal.**

2. **Mutation in the promoter: no fluorescent protein expression whereby the metabolic burden is low.**

3. **Plasmid loss and gaining of resistance: plasmid is lost but antibiotic resistance that permits survival passes to the bacterial chromosome. In this case the difference regarding metabolic burden is maximal.**

These results could affect negatively the company's status. So they cannot be released out of the company. The Committee Board has decided to block this information and reinforce the antibiotic resistance in order to keep this situation under control.

Commitee Board of Talking Life Technologies