Modeling Colonization by Growth-Promoting Rhizobacteria and the effect of Root Exudates on Root Length

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Abstract

Bacterial colonization of the root systems of plants occurs as a result of the newly established and growing rhizome coming in contact with bacterial populations and nutrients for older regions of the root system to exploit. Root exudates are a wide variety of compounds that roots use to signal to the rhizosphere and play a great role in root development, protection, and health. To create a simple simulation in Python 3 that models the dynamic between PGPR and the rhizome, a modified version of the peanut-associated bacterial isolates as characterized by Ankati et al. (2018) will be combined with Muci et al.'s (2012) agent-based model for bacterial colonization of a growing root. In our modified simulation, the root grows at a greatly reduced rate under low bacterial conditions, but as bacterial populations reach a threshold *theta* within the bottom row of the cellular automata, the root begins to rapidly grow and bacteria are able to colonize the new root growth. It is clear from Muci et al's paper that their simulation it is a robust and easily modifiable tool to examine bacterial colonization of a root and would be useful to any researcher looking to further examine their experimental results. As shown from Ankati et al's results and verified by our simulation, this variety of plant growth-promoting rhizobacteria can greatly increase the length of root.

Introduction

Bacterial colonization of the root systems of plants occurs as the root expands downwards. This is a result of the newly established and growing rhizome coming in contact with bacterial populations and nutrients for older regions of the root system to exploit. This exploitation of new nutrients and space also allows for more growth and thus the discovery of new resources. This dynamic was modeled by Muci et al. (2012) but for the sake of simplicity excluded important host-bacteria interactions that are vital to properly model the bacterial colonization of the rhizome. While root exudates (REs) are briefly touched on in Muci's paper, REs in reality are a wide variety of compounds that roots use to signal to the rhizosphere and play a greater role in root development than they took on in Muci et al.. These compounds include fatty acids, alcohols, organic acids, and carbohydrates and have a range of effects on their targets in the rhizome that in turn promote changes to root health. For this reason, disease-prone but highly profitable plants are often transplanted on the rootstock of heartier species that release more potent REs (Ling et al. 2013). Root exudates are highly specific to their targets and not only effect rhizobacteria but also other microorganisms such fungi in the plant's rhizosphere (Cao et al. 2016). One of the most useful industrial applications of REs is the natural protection from pathogens they can offer to the plant's root system by promoting bacteria that act antagonistically to the pathogens. Most relevant to positive reinforcement of bacterial colonization are a subclass of REs used to communicate directly to plant growth-promoting rhizobacteria (PGPR), which are bacteria that synthesize and secrete plant growth-promoting hormones that assist the root in nutrient uptake. Rhizobacteria their relationship with REs affect

root architecture in myriad different ways and their study is of great interest of botanists (Caffaro et al. 2011). By supplementing Muci's model of bacterial colonization of a growing root with PGPR, it is possible to create a general model to investigate the effect of PGPR on the rhizome.

To create a simple simulation that models the dynamic between PGPR and the rhizome, a modified version of the peanut-associated bacterial isolates as characterized by Ankati et al.(2018) will be combined with Muci's model. With this, we will be able to compare the results of the modified simulation to those of Ankati's *in vivo* experiments along with the original results of the Muci's model to assess the simulation's accuracy in depicting root-rhizobacteria interactions. A model such as this would allow for rapid *in silico* experiments that give reliable approximations of the efficacy of different PGPR on a variety of plant species. Depending on the ability to accurately simulate the interplay between REs and PGPR, this model could result in cheaper and shorter research and development cycles in the agricultural industry and could aid other research teams in their characterization of root exudates and their effects on transplanted and native rhizobacteria.

Methods

To model the relationship between PGPR and root growth, it was necessary to first model bacterial colonization of a root. For this, Muci et al.'s cellular automata and agent-based root surface colonization model was recreated in Python 3. This model relies on treating every individual bacterium as a unique entity that is able to perceive information from its environment and use it to decide to feed, reproduce, or enter a stasis period. Since this decision is informed by the bacteria's environment, it was necessary to construct matrices that store the nutrients in the soil, the pH of the soil, and the bacteria population count. Nutrients and pH were both evenly distributed along the space that the root could occupy. The growth of the root itself was governed by the equation $L(t) = R_0 * (1 - e^{U*t})$ where R_0 and U are constants determined by the plant species and *t* is the generation number. This root grew vertically along a rectangular 2D grid and fully spanned all columns (*y*) of each row (*x*) it occupied. The root was allowed to grow up until a certain length until it reached the maximum number of rows *n*. The columns of the root were governed by the constant *m*. For ease of explanation, the shape of the root matrix is referred to as *nm* and an individual cell within the grid being on row *x* and column *y*.

Root growth at each time resulted in a matrix with a cell count of the number of rows occupied multiplied by the number of columns occupied. To approximate the cylindrical shape of the root, the columns on opposite ends of rows were allowed to pull environmental information from each other. To allow for a cell to identify its neighbors within the cellular grid, 3 cases were needed to prevent a cell *xy* from drawing environmental data from inappropriate neighbors. The general case was used by all cells not at the first or final occupied rows and gave a cell 8 neighbors The first special case occurred at the first occupied row of cells and prevented vertical wrapping, which stopped the cell from identifying the bottom row as its neighbor, resulting in 5 possible neighbors. The second special case occurred at the bottom occupied row of cells and prevented vertical wrapping but also included a special neighbor *across* to simulate

the conical shape of the root tip which was determined by the closest number to $y + \frac{m}{2}$, resulting in 6 possible neighbors.

From these neighbors, a candidate cell could be chosen to populate if a bacteria's fitness allowed for it to reproduce. To determine an individual bacterium's fitness, the nutrient to bacteria ratio (*NB*) of a cell *xy* was calculated as a for loop iterated through every bacterium (*Bi*) within the cell. If the NB was found to be above a constant alpha, fitness of that bacterium would change by +4 to represent eating. If *NB* was below a constant *beta*, fitness would decrease by -4 to represent starvation and if *NB* was between *alpha* and *beta*, fitness would not change. If fitness fell below the starvation threshold, that bacterium entered stasis and if it remained in the stasis state for 10 generations, it would die. If the fitness was between *gamma*, the reproduction threshold, and the starvation threshold the cell would feed and the nutrients of the cell would be decreased by the consumption potential, which was determined by taking the minimum value between NB and the maximum consumption rate. If the fitness of the bacterium was above a constant *gamma*, the bacterium could reproduce. This resulted in the placement of a daughter cell in the best candidate cell and would halve the value of the bacterium's fitness to represent the partitioning of resources between the daughter cells.

The best candidate cell was determined as being the cell with the best *NB* and having a pH value between *pHmax* and *pHmin.* If both of these conditions were satisfied, the candidate cell had its bacteria population increased by 1. If more than 4 bacteria were able to reproduce within a cell *xy* during one generation, the pH of cell *xy* decreased by .1 to simulate the environmental degradation from overpopulation and rapid consumption of resources.

This model is directly copied from Muci's with slight modifications. The root matrix was shortened to reduce the length of the simulation, \ and the agent-based system was redesigned due to Python's fencepost indexing resulting in arrays beginning at zero. The number of generations that the model was allowed to run for was also shortened as the full simulation took around one hour to run completely and greatly impacted the performance of our systems while it was running. The changes in root shape and bacteria per cell decrease the total possible bacteria that can occupy the root and result in the numerical differences between areas of low and high bacteria concentration are less stark.

Three scenarios were tested by Muci: one in which bacteria colonize a growing root, a second in which bacteria colonize an already established root, and a third scenario in which the root initially grows under low nutrient conditions and receives an influx of nutrients every 18 generations.

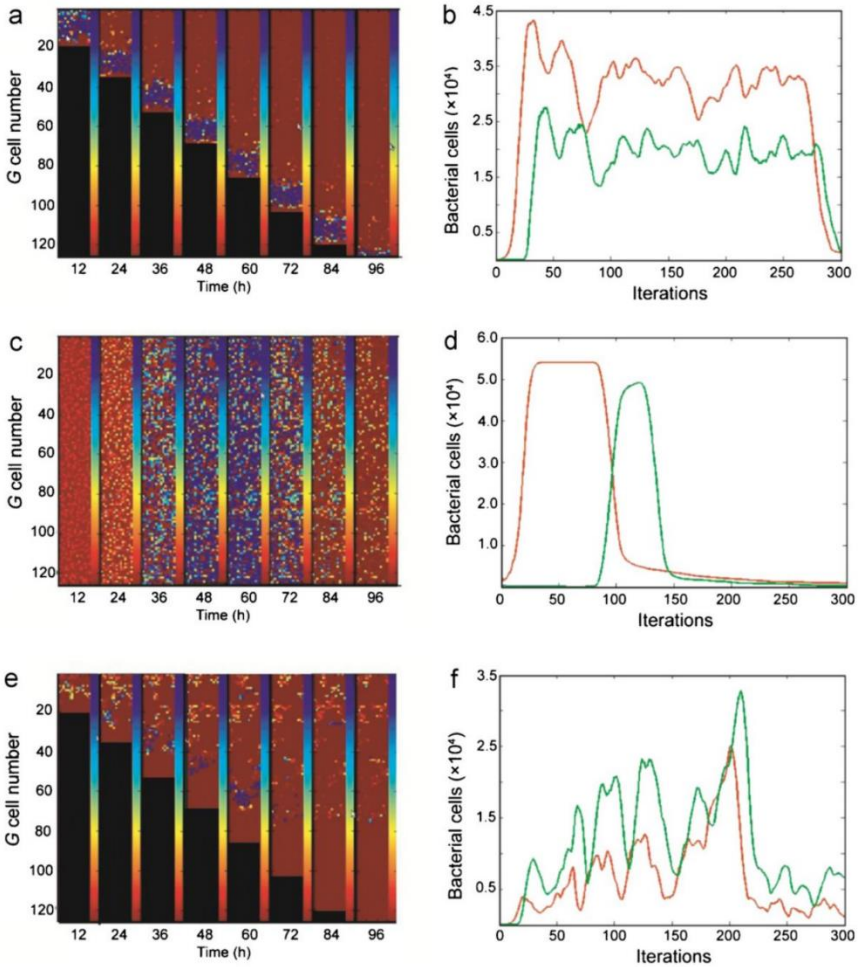
The experiment performed using data from Ankati et al's PGPR can be thought of as a modification on the third scenario. Instead of the root exudate taking the form of nutrients being introduced every 18 generations, the root will constantly exude nutrients that are consumed by the bacteria at a rate that allows for bacteria to never enter the starvation plane. The root grows at a greatly reduced rate under low bacterial conditions, but as bacterial populations reach a threshold *theta* within the bottom row, the root begins to rapidly grow and bacteria are able to colonize the new root growth. Since it is assumed that bacteria will be constantly reproducing

and thus synthesizing high quantities of the plant growth hormone in the bacterialized root it will grow at a constant rate of R1 and the non-bacterialized root's growth will be modeled by R1*x, the number of rows occupied.

This experiment will have two trials: one in which the root lacks any of the PGPR bacteria and is allowed to grow at its natural rate and another with a bacterialized root. Both roots begin with the same initial length and are given 24 generations to grow. Both bacterialized and non bacterialized root will be given equal amounts of plant growth hormone (PN) but only the bacterialized root would have the ablity to synthesize more PN and thus will grow at a greater rate than the non bacterialized root for the duration of the experiment.

Results

The outcomes of our modified approximation of the model of bacterial colonization of a growing root and those of Muci et al's model are published below.

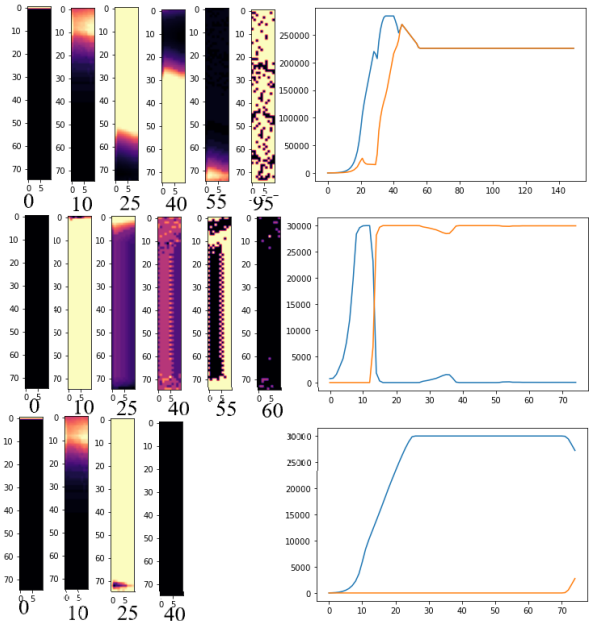


In the figure to the left (figure 1), the results of trials 1, 2 and 3 are displayed as a comparison of bacterial concentration on the root at various points during the simulation in the left column and as a graph of the active and starving bacteria counts in the right column.

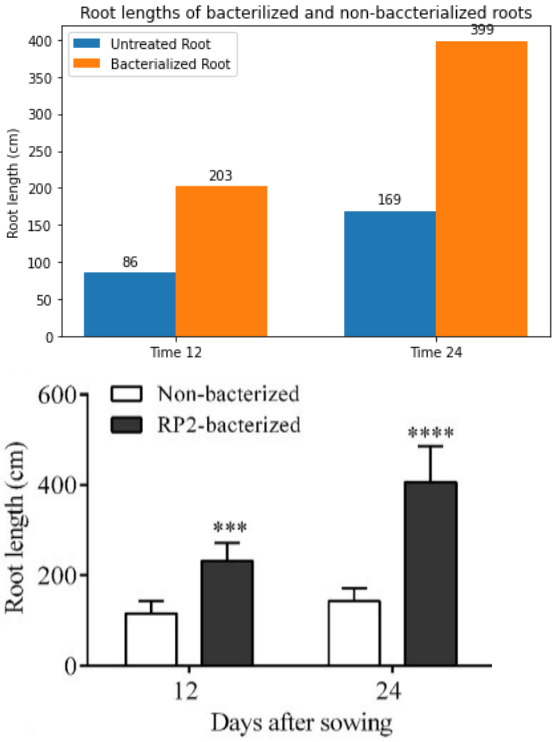
In the left column, black indicates root-less soil, red indicates root presence of root at that cell, purple indicates high concentration, and other colors indicate concentrations between high and low. The specific values of these colors were not given in the paper.

In the right column the number of active (fitness > starvation) and the number of starving cells were tracked in orange and green respectively.

Row one of the figure to the left is trial 1, row two is trial 2, and row three is trial 3.



The figure to the left is our team's recreation of Muci's data (figure 2). In the measures of activity versus starvation, blue represents active and orange represents starving cells.



Above (figure 3) is our approximation of root length of bacterialized root and non-bacterialized root growths (3a, left) and Ankati's experimental data (3b, right).

Discussion

As described by Muci, the early rows of the root experience rapid growth due to the greater length of time at which they are able to reproduce and this growth results in likewise rapid decreases in *NB* and environmental *pH*, making further reproduction impossible. As the root continues to grow, most increases in bacterial population and depletion of nutrients will occur in the younger regions of the root. As bacteria enter the starvation plane and begin to die, *NB* will increase and reproduction once again becomes possible in the earlier rows, resulting in periodic rises and falls in bacteria populations. Unfortunately, it is impossible to compare the progress of the cellular automata of the two models as the videos of their simulation running have since been removed from the drop box links.

Our re-creation of Muci's model had significant issues. Greatest among these were the starvation and fitness systems and the equations that governed both. As is evident in the comparison of our Active vs Starving graphs to Muci's, our team was not able to capture similar

trends to those observed by the original paper. The greatest impediment in developing the starvation and fitness systems was our lack of experience with agent-based models and with Python itself. Color mapping was also a great issue when it came to implement a visual representation of the 2D cellular automata. Due to the way that Matplotlib's color mapping works, rather than individual numbers being given a unique color, the lowest number in the array is given the darkest color (black) and the highest number is given the brightest color (beige) with all values between these numbers being a color in the map that ranges between these two values. This leads to cells with low bacterial counts being given the color purple and cells with moderate scaling from pink to orange. Because the lowest value in the array is constantly changing, there are situations such as the case of trial 3 where the end state of the simulation misleadingly portrays a population of zero in all cells while in reality all cells were completely saturated at Bmax. Despite the difference in our implementation Muci's model compared to their published results, it was easy to find conditions under which to run our function modelling PGPR and the results of our simulation fall within the standard deviation of Ankati's data.

It is clear from Muci et al's paper that if their simulation is implemented correctly it is a robust and easily modifiable tool to examine bacterial colonization of a root and would be useful to any researcher looking to further examine their experimental results. As shown from Ankati et al's results and verified by our simulation, this variety of PGPR can greatly increase the length, diameter, and mass of a root.

The characterization of rhizobacteria is a worthwhile topic for further study. The relationship between plants and their bacteria have great effects on the outcome of plant health and deeper knowledge would create a more comprehensive understanding of the interactions within the rhizosphere. Likewise, the simulation of rhizobacterial growth encourages synthesis of many fundamental concepts in computational biology and provides interesting challenges to any biologists hoping to increase their knowledge of agent-based systems, 2D automata, and functions governing bacterial growth. As mentioned in Caffaro et al. horizontal root growth is an important component of root architecture development, a feature which is absent from Muci's model. Implementing a method to track horizontal growth would contribute greatly to increasing the accuracy of the simulation.

Works Cited

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