

Networking Strategies in *Streptomyces coelicolor*

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Abstract: We are interested the soil dwelling bacteria *Streptomyces coelicolor* because its cells grow end to end in a line. New branches have the potential to extend from any point along this line and the result is a network of branches and connections. This is a novel form of colonisation in the bacterial world and it is advantageous for spreading through an environment resourcefully. Networking protocols for communication technologies have similar pressures to be resourceful in terms of time, computing power, and energy. In this preliminary investigation we design a computer model of the biological system to understand its limitations and strategies for survival. The decentralised capacity for organisation of both the bacterial system and the model reflects well on the now-popular conventions for path finding and ad hoc network building in human technologies. The project will ultimately become a comparison of strategies between nature and the man-made.

Keywords: computer networks, *Streptomyces coelicolor*, mycelia, branching, agent based modeling, systems biology

1. Understanding Biological Systems

Biological systems found in nature comprise of finely evolved strategies for competing for resources and regenerating. These strategies are a reflection of the information coded in the DNA of each individual organism, combined with the physical constraints of the environment. When we think of a system we are considering the behaviour of the whole as a product of all the individuals that make up that whole. Interpreting a system in a bottom-up fashion is key to a Systems Biology approach and aids a complete understanding by establishing what is happening at the lowest levels. There is a tendency that the lower the level of representation, the more simple the rules are to follow. Famous examples in the literature include computational modeling of swarming, shoaling, and flocking that you can find in social insects, fish, and birds respectively [15,16]. In each of these types of systems the processing required at the level of the individual is relatively simple. For example, each bird in a flock only needs to align itself with a few of its neighbours and respond to the usual environmental queues but the degree of organisation and coordination at the top-most level is immense [15]. The alternative idea of a central leader conducting the group is far less feasible due the resultant complexity of the group behaviour being far greater than just the sum of its parts [1,2].

The simple logic decisions that are made by the individuals in these systems lend themselves well to computational modeling not only because computers are intrinsically logic gates but also because they are iterative machines that can store and traverse through virtual representations of the

individuals in a system. This is called agent based modeling. Parameters and variables are applied from the context of the individuals in a system and tuned in an attempt to simulate the observed collective patterns. This can lead to a robust form of modeling because small errors at the individual level are amplified in the overall behaviour. Most importantly, agent based modeling will help us to uncover some of the information hidden in the genetic make up of the organism in question in regards to what choice the individual makes under specific circumstances

1.1 *Streptomyces coelicolor*

Our preliminary studies take inspiration from a biological system simpler than birds or fish. *Streptomyces coelicolor* is a naturally occurring filamentous bacterium in soil that is a popular research choice for bacteriologists because of its ability to produce clinically useful antibiotics [6,7,8]. Most interesting to us is the bacteria's less than usual manner of dividing which is more reminiscent of filamentous fungi [11]. During the first growth phase of *Streptomyces coelicolor*, cells grow in a line with new cells being added at the apex [10]. Machinery associated with extending the cell wall is transported to the tip of the apical cell and hyphal cross walls are formed further down to complete the process of cell division [10]. This is in contrast to rod-shaped *E.coli* for example, that elongate by adding cell wall content to their lateral walls [12]. Although only the tips are actively dividing, a cell anywhere along the line can create a new tip by lateral branching [10]. This results in growth of a network that resembles the branching of a plant root system or the venation found in leaves. These branches however, called mycelia, are a single cell in width and thus transport through them is limited compared to multi-cellular systems. The second stage in the lifecycle of *Streptomyces coelicolor* is a cessation in lateral growth, giving rise to a period of skywards vertical growth of aerial mycelium. The apical cells of aerial mycelium become spores, capable of surviving and spawning a new colony [13].

1.2 Networking in Biological Systems

The ability to network brings advantages to this species in an environment that is naturally patchy (soil) in regards to finding energy resources for colonisation because it can cover greater areas with relatively small cell counts. To be competitive a colony favours production of many cells, leading to production of many aerial mycelium, and ultimately many spores. This helps ensure the gene pool survives and spreads. In a barren local environment however, the colony cannot afford for tips to extend, new branches to emerge, and spores to be grown. On the other hand, extending more branches will increase the likelihood that

more desirable environments will be encountered eventually. This is a considerable limitation against colonisation and we are interested in the strategies that *Streptomyces* has evolved to optimise its growth in an unpredictable environment.

Networking as a method for optimisation is predominant in the human world too. This is particularly true in communication technologies such as peer-to-peer data transfer over the Internet, and ad hoc wireless networks between portable devices. In a similar fashion to the biological systems discussed, these mechanical systems are subject to their own limitations and strive to be efficient in regards to energy, cost, and time. Wireless transmissions are generally more effective over short distances. Over long distances they require unreasonable amounts of energy so, networks should favour multiple short hops between 2 devices as opposed to a direct, but long link between them [14]. This resembles the high bifurcation incidence of the *Streptomyces* system in high nutrient areas, which are analogous to a high density of nodes in an ad-hoc system. Mobile nodes change their position frequently and the network structure that connects them has to be torn down and re-established in real time. Eventually we would like to exploit these parallels between the different systems to see how a *Streptomyces*-inspired protocol fares in a model of a communications network.

Communication systems may also lend themselves well to a decentralised structure as seen in the biological systems that we have discussed. The nodes that are looking to form the connections are computers (in some form or another) and thus have a capacity to process information. Since the ability to form networks quickly and efficiently is favoured, sharing the processing among the nodes is advantageous. This is especially true if it means (as in the biological systems we discussed) that simplifying the process would simultaneously increase performance and complexity beyond what is easy for a central processor to do [5].

This means that agent based modeling not only suits Biological systems but it can also be used for simulating communication networks. In this case, designs for a simulation can be literally applied to the technology itself to improve its performance. The classic example of a crossover between biological networking and networking in computer sciences is the series of ant colony optimisation models that have been successfully applied to routing data packets over a network. Ants have limited processing capacity (compared to birds or fish for example) but collectively they can accomplish tasks like building nests, and transporting food sources back to the nest along the shortest path [3]. There are clear evolutionary pressures for finding the shortest path between food sources and the ant nest and the strategies undertaken by each individual colony member have been discovered not least by representing the ants as objects in a computer program and putting lots of them together in a virtual environment[3,4]. This is effectively the same as we are doing with *Streptomyces*.

2. Making a Computer Model of *Streptomyces coelicolor*

This study is the first step towards developing an agent-based model of *Streptomyces coelicolor* during the first growth stage. The primary goal of the investigation is to establish its characteristics for growth in light of assumed physical and environmental limitations. This includes average trajectory of mycelium, frequency of bifurcation/branching, average angle of branching, distance between branches, life time of cells, capacity of mycelium to extend, capacity for resources to be transported between cells, and how these characteristics change over time and over variation in the environment. The secondary goal is to modify the model once it is optimised for survival to generate networks in a communications context as a comparison to human-formulated models. This report is preliminary however, and our short term goal for this study was simply to achieve a basic representation of the system and discuss our approach.

The bacteria can be imaged growing from individual germinating spores on standard agar with time-lapse photography. Many of the attributes of the model will be based on simple observations of the true system growing over time under the microscope in agar mediums at a range of nutrient concentrations. There are also plans in the coming months to combine blocks of agar at different nutrient concentrations to create gradients to assess the bacteria's chemotactic ability. For now, we have constructed a model based on the first set of observations of *Streptomyces coelicolor* growth on standard LB medium. Mycelium extend because the apical cell undergoes mitosis and splits into 2 [10], thus the 'individual' in this context is a single bacterial cell. Java programming language was used to create an object representing just that, a bacterial cell, and an extended child object with extra functionality to represent a cell that is active (undergoing mitosis). Agents have a radius, an $X(<1.0), Y(<1.0)$ coordinate, and the capacity to become active according to probability P . These features reflect a growing mycelium being viewed in a finite microscopic field that can extend a new branch from any cell in its lineage.

Another observation we have made from imaging is that each spore spawns 2 (or more) mycelia that grow outwards from of opposite ends. Also, the tip's trajectory is not straight but appears to be subject to noise. In recognition of this erratic trajectory, active agents are subject to a noise function in our current model. This assumption is based on the idea that tip extension through a tough medium like agar occurs by the turgor pressure that results from fresh cell wall matter being incorporated at the tip [9]. At this stage, it is not clear how a cell could easily change its direction and it is liable to opt for a low-energy straight-line course. Considering this difficulty to change direction, *Streptomyces coelicolor* may not rely on chemotaxis to ensure that it grows towards sustainable regions opting instead to rely on chance encounters of food by throwing out new branches when it can. Using our model, we will eventually be able to determine if chemotaxis is even necessary to help the species colonise more competitively.

Each cell is connected to 2 other cells, one basally and one apically. The cytoplasm of these cells is connected as well so there is opportunity for transport of materials between cells [9]. This may not happen to the extent that cells can distribute their resources across the whole network as mycelia are a single cell's width in thickness. Again we can use our model

to eventually establish the effect of redistribution of resources and compare this with the real life system to provide clues about how much redistribution is occurring and whether it is a useful feature or not in regards to colonisation.

Preliminary evidence suggests that branching frequency is higher when the medium is saturated with nutrients, apparent when we grew in an LB agar medium versus plain agar. We aim to measure and scale this response in the near future. This is probably the bacteria's primary option for exploiting resources considering it may not transport them far, or able to locate them by chemotaxis. If a tip's sole option is to grow in a straight line, it could pass through areas of high nutrients. The second-generation cells left behind can afford to produce a lineage of their own more than those that are further away from the food patch. These are soil dwelling bacteria and soil by nature is patchy/non continuous. From these preliminary imaging experiments, it also looks like spores come with sufficient energy to develop the beginnings of a colony without needing to take up nutrients from the environment. This maternal energy is dispersed relatively well so that cells anywhere in the lineage can become active.

In these experiments we look at the effect of changing the P value of a model of single virtual spore placed in the centre of a uniform virtual environment beginning with 2 active agents extending outwards. The P value is the probability that each cell will become active at each time step. One time step is defined as the time it takes for an active agent to extend and leave a new agent in its wake. So every time step, each mycelium will grow by one cell. In these experiments we also assess the effect of having zones in the environment that represent nutrient dense zones where agents can better afford to divide and thus have a higher P value. These preliminary experiments will demonstrate how feasible it is to use agent based modeling to represent *Streptomyces coelicolor* by comparing simulation results with the first set of images we have taken. In the short term, the model will increase in relevance as more and more image data is collected.

3. Findings

The task ahead is to create a program that builds networks. The motivation for this line of research is a demand for efficient networking protocols: The performance of a network is entirely dependent on the structure of that network. When the environment remains relatively static for a moment in time, applying a network can allow quick access between one point in that environment and another, but apply the wrong network and the necessary connection between two points will be long and complicated, or not existent. We are looking for decentralized ways that are quick and undemanding in terms of processing, but take advantage of the many individual entities that make up particular systems. Finding parallels between man-made systems and biological systems enables us to apply strategies that have evolved to be optimal for the purpose.

Currently, the virtual cells do not consume resources from the environment to obtain energy for growth. Therefore, there unlimited potential for an active cell to keep dividing but in all these tests they are restricted by timing.

3.1 Surface Area Coverage as a function of Bifurcation

Figure 1 demonstrates an obvious relationship between bifurcation rate and surface area coverage. The Java program was a given a module to visualize the distribution of agents. The red outline of a circle in each case marks the furthest point away from the central spore that has been reached by an agent. This is to demonstrate the distance covered in relation to area covered and figure 3 represents these values as proportions. Each picture in figure one shows a separate simulation that has run a course of 500 time steps, the only difference in each experiment is that P changes. With a relatively low incidence of bifurcation, the cell count can rocket after enough time. This is evident in the image data too: Growth on agar can be viewed realistically for about 6-8 hours after a spore first starts to grow. Beyond this point it is difficult to distinguish individual mycelia because they begin to climb over the top of each other as their total area exceeds the amount of corresponding surface area (figure 2).

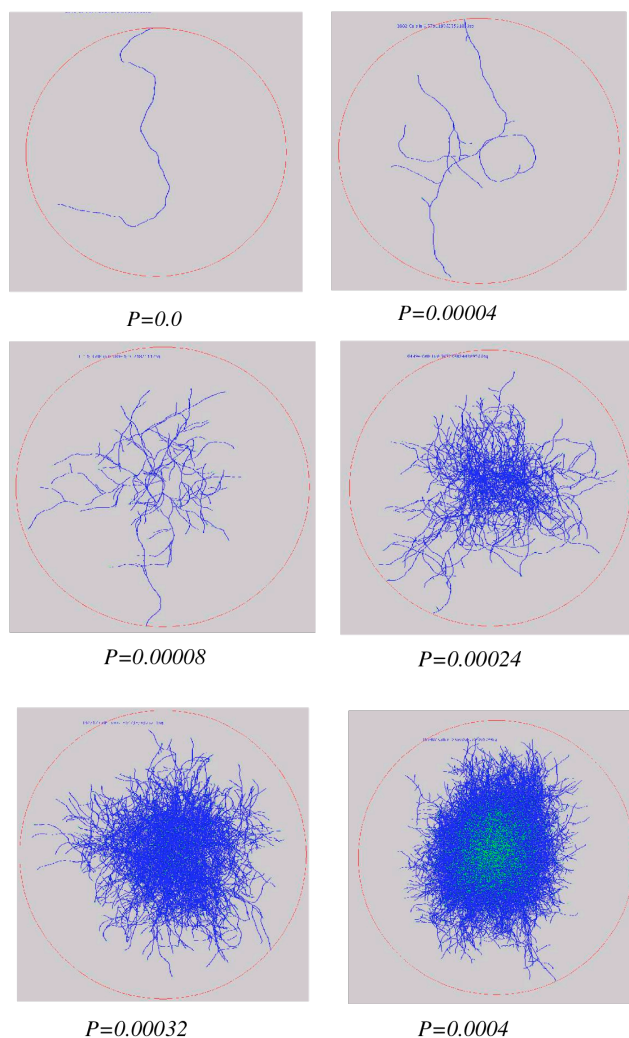


Figure 1
Sample images from model output after 500 time steps with increasing P value.

In figure 3 each progressive bar represents average surface area coverage over 20 runs, with the P value increasing by 0.00004 per bar (starting with 0.0 on the far left side). The

snap-shots from the 2 highest P values in figure 1 look like the colony has grown out from every angle as a tightly packed mass. This is reminiscent of most other colonising prokaryotes but we know that *Streptomyces coelicolor* achieves this effect with extensive one-dimensional branching. We may expect *Streptomyces coelicolor* therefore to be better at colonising patchy environments as it can spread far before generating much mass simply by lowering its frequency of bifurcation.

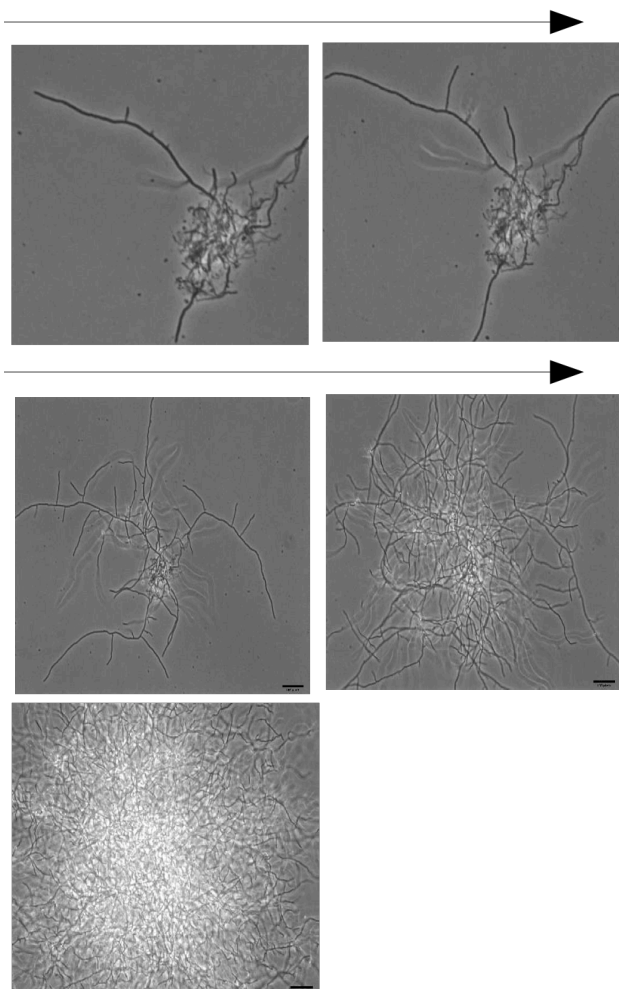


Figure 2

Stills of *Streptomyces* mycelia. These stills are from a time lapse. Note how early on the density resembles the computer model at low bifurcation and several hours later it resembles the high bifurcation simulation (part A). Thus a relatively low bifurcation rate over a much longer relative period of time is equivalent to a high bifurcation rate in a short amount of time. This “spread out first and fill in later” strategy probably enables a quick and rough scan of the environment before committing to much growth

The photographs in figure 2 show a time series of a single spore growth. They resemble the progressive surface area coverage of the computer simulations shown in figure 1 but the difference is that figure 1 is not a time series, it is 6 separate simulations all at the end of their 500 time-step course. This suggests that *Streptomyces* grows over a longer time scale than the current model and has a bifurcation frequency to match the first half of simulations in figure 1. This theory may change in the future when we grow the bacteria under different conditions but future simulations may be better applied by increasing the time scale and keeping bifurcation rates lower. As evident in figure 2, high

degrees of surface coverage still occur but not before the earliest generation of active agents have covered a lot more ground. In the interest of analyzing networks before they become too complicated and over-run, it may also be necessary to allow tips to grow outside the field of focus and disappear from the simulation. Especially since we cannot track every mycelium on agar over long time periods.

The furthest distance traveled from the original spore was comparable in all simulation tests and independent of bifurcation rate. This feature is almost certainly more to do with the noise function, which has not been analysed at this stage. It is also notable that although the increase in bifurcation is linearly applied in figure 2, the increase in cellular area after 500 time steps does not appear linear. This is suggestive of a threshold, above which, P values may be associated with an 'exploit' mode suited to resourceful conditions and below which, may suit foraging modes suited to less desirable conditions.

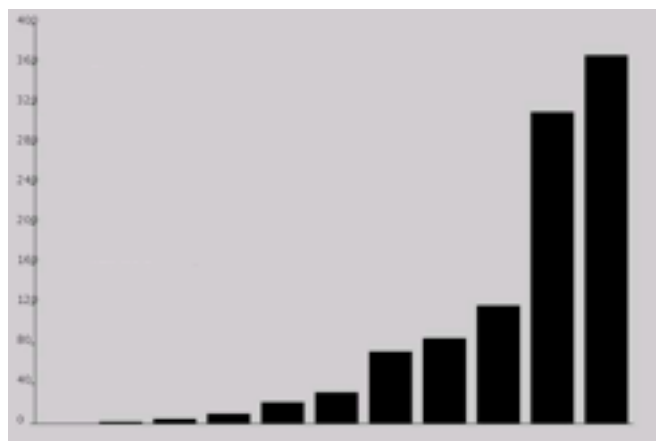


Figure 3

See text for explanation. This chart shows the results from the experiment featured in figure 1.

X axis = 0 – 400 % surface area covered by cells

Y axis = Incrementing P values from 0 to 0.0004

One bar = average over 20 replicates

Observation: At high bifurcation rates cells take up more space than the area they have spread to and occupy multiple layers.

3.2 Surface Area Coverage in a Patchy Environment

Figure 4 shows what *Streptomyces coelicolor* might look like in a patchy environment. In part A and part B you can see the same circle outlines drawn in but only in part B do those circles apply a higher bifurcation rate to resident cells for comparison purposes. There is no capacity for cells to choose the angle for their bifurcation (which is a random function). Nor is there opportunity for the active cells to choose their trajectory (they go in a straight line subjected to noise). All patches featured in Part B through Part D apply a bifurcation rate to their resident cells that is 10 fold higher than the surrounding medium. Between Part A and Part B there is a clear affect to the growth pattern when the environment becomes patchy. There is an explosion of growth helping to define at least 3 of the surrounding patches. This effect is more significant when the patches are larger- allowing tips to remain in a patch for longer periods of time, as shown in part C. Part D features smaller patches again, but this time there are more of them in 2 layers surrounding the centre. The non-

patch bifurcation rate is low and many of the patches have been missed. Thanks to the second layer, the colony manages to encounter and exploit one patch before time is up. This undoubtedly increases the chances that some more of the patches will be encountered if the simulation was left to run for longer because there is an increased proportion of active agents travelling in new directions. This may compliment the strategy that we encountered in the previous section to ensure a good coverage of the surface area: Low bifurcation frequency means that cells on average will sit a round for longer before becoming active resulting in proportionally fewer extending mycelium in the short term but also ensuring that most cells retain their resources for entry into the second growth phase: production of a new generation of spores. If one of the few mycelia encounters a favorable patch, an explosion of new mycelia comes into existence at a higher rate from outside the patch and a host of new trajectories are set up. This increases the chance that new patches are encountered in areas that were missed out during the earlier stages of colonisation. And, the agents living in barren conditions can mostly be preserved by keeping their bifurcation rates low. This will become clearer after a series of experiments in the lab to determine the change in bifurcation rate over increasingly desirable agar conditions.

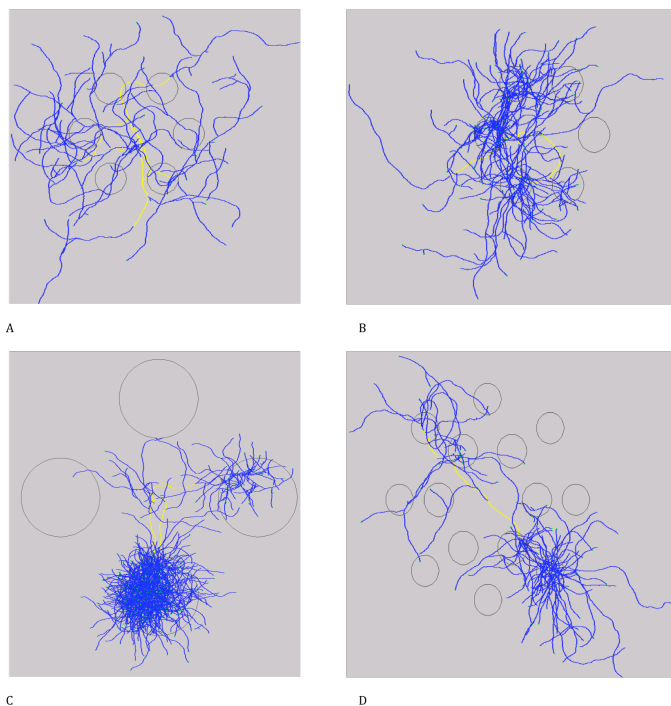


Figure 3:
Part A shows an even distribution of patches around the central spore, without any effect on colony behaviour. Part B shows the same patch distribution as A, with any cell in the patching having a 10 fold higher likelihood of bifurcating. Part C and part D also demonstrates 10-fold patches: 4 large ones and 2 layers of small ones respectively

4. Conclusions

At this early stage we have created a computer model to represent a branching bacterial system to help us identify optimised strategies for exploiting its environment. The model tries to take into account the limitations of the true system, such as: very limited processing capabilities per

individual. These single celled organisms have a short amount of DNA, and this is the extent of their decision-making. On the other hand, Bacteria are renowned for the speed that they can evolve and with enough selective pressures they are liable to demonstrate some clever lessons. In this model we have assumed that the cells, in line with many other bacterial species, can take up resources from the environment. They respond with an increase or decrease in the likelihood of entering into repetitive mitosis. An accurate measure of this behaviour will follow further experimentation. Other limitations however, are not so easy to measure. For example, the amount of re-distribution that occurs across (laterally between cells in a mycelial line) a network is hard to determine. Re-distributing resources from areas of high nutrient value to lower nutrient value could prove useful, or may not be necessary at all in terms of achieving the highest possible spore count after the initial growth phase is over. Similarly, communication via chemical signaling between cells in a network is more feasible if there is transport occurring across the network, but again, may not be necessary for achieving the ultimate goal. The goal is, as with any biological organism, competitive success. This means as wide spread surface coverage as possible, and as much passing on of genetic make up as possible (highest spore count as possible).

Another variable of the model that will have a strong influence on the pattern of growth and consequently the success of colonisation is the trajectory of the mycelia. Previously we stated that this is most likely to be a direct consequence of the growth medium providing resistance but increasing the noise function of the model had dramatic effects on the surface coverage. Increasing noise decreased the furthest distance achieved by any agent from the central spore after 500 time steps and inevitably increased the coverage of that zone. These results are not shown because it wasn't considered as a variable that the organism has any control over but if it did have, it may use this ability to increase the time spent in a patch enabling it to lay down more cells, for example. For future tests, it will be interesting to see if we can fine tune the noise variable to increase the competitive success of the model. By using the model to find methods that work well for the system, or that help the model to mimic the real life system, we can indirectly suggest mechanisms that might have evolved in *Streptomyces coelicolor* but that are not easy to prove in the lab. This is one of the strengths of a Systems Biology approach to the task, one of inference.

The next stage for the model is to find a way of ranking its success in the face of varying the different variables present in the system. The first step will be to represent the environmental resources more effectively. Currently an extending mycelium will continue dividing indefinitely, but an active cell needs the right materials if it is going to divide continuously, not least a source of carbon for energy. By incorporating at least one limiting factor for growth into the model, a colony's success is immediately measured by how many time steps it can exist before it runs out of steam and ceases to grow. One thing we can't do in the lab is create an identical environment of patches with each experiment. For the model however, the environment can remain static while the colony tries to grow under various conditions. These

conditions include variable patch-related bifurcation frequencies, and the new variables discussed: patch-variable noise, and distance of resource sharing permitted. The next step is to include a statistics module into the program so that characteristics of the networks generated by the model and those in the images can be directly compared in a quantitative fashion. This will be followed by a fine-tuning of the variables so that the model statistics mimic the averages calculated in real-life networks. Experimental work will be carried out alongside the model implementation to compliment the model and help identify certain truths. Future versions of the program, if needed, will look into higher and more accurate representations of *Streptomyces coelicolor* and its environment.

Following these leads will help to uncover many attributes of *Streptomyces coelicolor*. With this information we can enhance our understanding of networking in nature and begin to apply it to other areas in both biology and computer science.

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