

ORIGINAL PAPER

Mycobacterium Tuberculosis growth model based on agents: proposal of a tool to aid in decision-making to “In Vitro” experiments

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Abstract

Even treatable and preventable with medication, tuberculosis (TB) continues to infect and cause deaths globally, especially in the poorest countries and in most vulnerable areas of the rich countries. Given this situation, the study of the growth curve of *Mycobacterium tuberculosis*, which causes tuberculosis, can be a strong ally against TB, helping in the development of new drugs or for the development of the theory. This paper describes a study of the *Mycobacterium tuberculosis* growth curve and it aims to test hypotheses on data obtained by agent-based simulation with the population mean observed in an experiment *in vitro*. The tested hypotheses compare the mean of experiments for the first hour of the day. The tests were performed using the Student distribution. We believe that our approach, that use agent-based model, could reproduce *Mycobacterium tuberculosis* growth curves.

Key words: Tuberculosis; Agent-based Simulation; Hypothesis test

Resumo

Mesmo tratável e evitável com medicação, a Tuberculose (TB) continua a infectar e causar mortes em todo o mundo, especialmente nos países mais pobres e nas áreas mais vulneráveis dos países ricos. Diante dessa situação, o estudo da curva de crescimento do *Mycobacterium tuberculosis*, causador da tuberculose, pode ser um forte aliado contra a doença, auxiliando no desenvolvimento de novos fármacos ou no desenvolvimento de novas teorias. Este artigo descreve um estudo da curva de crescimento *Mycobacterium tuberculosis* e tem como objetivo testar hipóteses sobre dados obtidos por simulação baseada em agentes com a média populacional observada em um experimento *in vitro*. As hipóteses testadas comparam a média de experimentos para a primeira hora do dia. Os testes foram realizados usando a distribuição de Student. Acreditamos que nossa abordagem, que usa o modelo baseado em agentes, reproduz as curvas de crescimento do *Mycobacterium tuberculosis*.

Palavras-Chave: Simulação baseada em agentes; Teste de hipótese; Tuberculosis

1 Introduction

Tuberculosis (TB) is a major public health problem, affecting predominantly low and middle-income countries, developing among immigrants, poorest and vulnerable parts of high-income countries

(Lönnroth et al.; 2015). According to Burgos and Pym (2002) *Mycobacterium tuberculosis*, which causes tuberculosis, is one of the most successful bacterial pathogens in the humanity history.

A report published in 2015 by the World Health Organization (WHO; 2015) estimates that in the year

2014 were 9.6 million new cases of tuberculosis (TB) and 1.5 million deaths, and together HIV virus, tuberculosis presents one of the biggest causes of deaths from infectious diseases.

Therefore, the study of *Mycobacterium tuberculosis* growth curve becomes extremely important, because this study will test behavior hypotheses in cases of environmental stress (Voskuil et al.; 2004), verify bacillus drug reactions and help to develop new ones (Andries et al.; 2005). For these objectives, growth curve are important not only to determine its dynamic but also sampling bacteria in different phase of growth to determine its behavior variation during the growth. However, the tuberculosis bacillus has a very slow rate of population growth, form clumps to grow and requires enrichment medium. Because this behavior, *in vitro* experiments are very costly and need the maximum of tools in order to rational design of the studies involving the growth curve and sampling of the bacteria.

Multi-agent systems, a field of artificial intelligence, enables, by means of their tools, to simulate behavioral rules of a system computationally. For Garcia and Sichman (2005), "Agents are computer characters that act according to the program set, directly or indirectly, by a user. They can act alone or in communities, trainees multi-agent systems".

Many measurable phenomena present in nature have probability distributions similar to some probabilistic models. Often, these models are used to represent the probability density function of random variables. Probabilistic models are useful in many real situations, to make the variable predictions study and assist in decision support. It is believed that the main variables that model the *Mycobacterium tuberculosis* bacillus growth curve also resemble a probabilistic model.

The main goal of this work is to model the tuberculosis growth curve, using agent-based simulations, where the values of some variables are drawn from probability distributions, thus making the system developed more similar to real systems. Finally, to test the similarity of the obtained growth curves, we performed hypothesis tests. The purpose of these tests is to provide a methodology for verifying whether sample data provide evidence supporting a formulated statistical hypothesis or not. The test was used to test the null hypothesis that the mean of the populations used to generate samples by agent-based simulation is equal to the population mean obtained in *in vitro* experiment, we used t test.

The paper is structured in 6 sections. In Sections 2 and 3, respectively, there are a theoretical basis for the work and some studies on bacterial growth. In section 4 is presented the proposal of the work: a modeling of the growth curve for *Mycobacterium tuberculosis* using agents. The Section 5 presents the graphical and numerical comparison using *in vitro* experiment data for comparison. Finally, in Section 6, we present the main contributions and the further works

2 Background Literature

2.1 Growth Curve Bacterial

When a bacterium is inoculated in a medium that contains all the nutrients necessary for its survival, the bacteria tend to duplicate. Initially, it adjusts to the new environment (lag phase) until it can begin the process of division regularly (exponential phase/log phase). When growth become limited, the cells stop dividing (stationary phase), until finally they die for the saturation of environment (Todar; 2013). These phases as shown in Fig. 1.

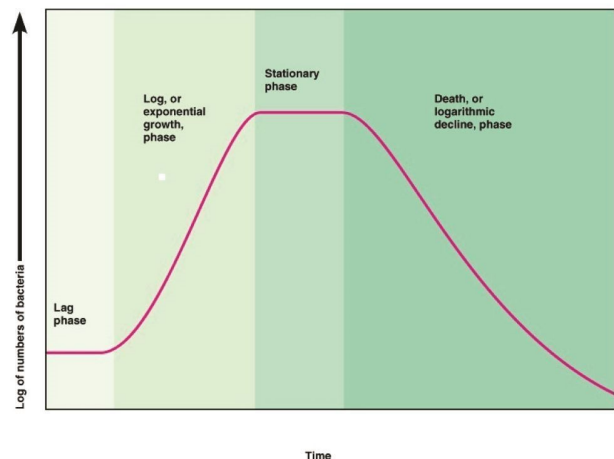


Figure 1: Growth curve and its phases (Todar; 2013)

- **Latency phase**: Immediately after inoculation, the population temporarily remains unchanged. Although there is no apparent cell division, the bacteria can be grown in mass or volume, synthesizing enzymes, proteins and RNA;
- **Exponential phase**: After the adaptation, they rapid growth and consume the maximum amount of nutrients from the environment, also in this stage release wastes and chemical signaling molecules in the environment;
- **Stationary phase**: After increasing population growth, the bacteria begin to decrease due to saturation of available nutrients, waste accumulation in the environment and the lack of space. In this stage, they enter dormancy stage where saving energy to increase the time survival;
- **Decrease phase (death)**: The last phase of the curve is the death, where they begin to die for lack of nutrients.

2.2 Growth Curve to *Mycobacterium Tuberculosis*

Von Groll et al. (2010) standardizes a method for determining the growth curve based on a system that obtains the bacterial growth by monitoring the liquid medium by means of an oxygen sensor which emits fluorescence. Growth curves were generated by the MGIT (*Mycobacteria Growth Indicator Tube*). The data are for the strains that originate in different geographical regions and they are resistant to different drugs, according to Tab. 1.

Table 1: Used strains and their source

Strains identification	Source
GC 01-2522	Georgia
GC 02-2761	Bangladesh
GC 03-0850	Georgia
GC 03-2922	Georgia
H37Rv	ATCC

For each of the strains was made one experiment with the same solubility. The monitoring was conducted for 25 days and every time the equipment bacterial growth medium, the growth expressed as growth units (GU).

However, this method does not allow to know how long the bacteria remained in the stationary phase or the rate of population decline in the death phase. This is because the measurement comes from the metabolic activity of the bacteria, and therefore can only be monitored if there is population growth.

In this paper, we have as hypotheses that, for each time, the simulated model can be useful to represent the model of [Von Groll et al. \(2010\)](#), i.e., the null hypothesis is that the population mean of simulated model is equal to the value observed in the *in vitro* tests. To do these analyses, we have used just one *in vitro* experiment, because this procedure has a high cost and demands a long temp to be completed. In this way, it is not possible to do more observations.

2.3 Agent-based systems

The conventional simulation is one of the more viable tools available to project, plan, control and evaluate new alternatives/changes to real world. To use the computational simulation, we need software's to represent the functions of the real world ([Rebonatto; 2000](#)).

According to [Azevedo and Menezes \(2006\)](#), the use of computational simulations is very appropriate to describe real systems, because the simulation tries to reproduce a real situation artificially, where hypothesis could be verify without risks.

One type of simulation is the agent-based simulation. In this type of simulation is possible recreate a population of a real system, where each individual of this population is represented by an agent and each agent has a set of specific rules to define its behavior, its interactions with other agents and the environment where it is inserted ([Colella et al.; 2001](#)).

In agent-based systems, a real phenomenon is decomposed in a set of elements and their interactions. Each element is modeled as an agent and a general model is the results of all interactions between the agents. Strack (1984) apud [Adamatti \(2011\)](#) reports that the simulation could be divided in three steps:

- i. Modelling Step: build the phenomenon model;
- ii. Experiment Step: apply variation in the built model, changing parameters that influence in the resolution process;
- iii. Validation Step: compare the simulated data with real data, to analyses the results.

To simulate computationally a real problem, it is necessary a rigorous study to be able to abstract all variables and relationships that define the model. This study is usually done through observation and analysis of the real phenomenon.

Capturing all of the components of the simulation model is not an easy task and the higher the number of variables and more detailed the model to be simulated, the greater the computational work.

After defining the model and done the simulations, the results are compared with those observed in natural phenomena, in order to evaluate their equivalence, i.e., the similarity with the reality.

The choice of agent-based simulation to model of *Mycobacterium tuberculosis* growth curve was done because the need for integration of different behaviors among the agents, the dynamism that this kind of modeling can enable, such as the interaction between agents, the interaction between agents and environment, and the flexibility to do modifications and extensions in the model.

3 Related Works

With computational advances it became possible to analyze social and economic systems through simulation studies, including the agent-based modeling. [Terano \(2011\)](#) says that the simulation method based on agents is very important, since it can produce results without assumptions unlike conventional approaches.

Since most of the time it is not feasible to experimentally test all possible hypotheses, modeling and simulation can reduce laboratory time-consuming work and assist in property research. In this way, a wide variety of agent-based structures is currently available. Each one of these provides a different set of features in order to allow efficient simulation of certain types of systems.

[Gopalakrishnan et al. \(2013\)](#) used an agent-based model to investigate the role of potential bacterial virulence in surgical site infections (SSI). For this author, the dynamic representation of knowledge through computational modeling and simulation can increase traditional research studies, generating and instantiating new hypotheses, integrating information and filling gaps in the current knowledge base. In his work the author creates an environment that reproduces the dynamics of healing in surgeries (Muscle Wound agent-based model - MWABM). The developed environment simulates the interaction between the cellular and molecular mechanisms of wound healing and contaminating bacteria. To study SSI, the simulated bacteria were added to the MWABM base. The rules that govern many types of cellular agents were created, according the literature. The execution of MWABM involved repeated iterations ("stages") where the computational agents interact with other agents and their environment. MWABM was used to identify threshold zones that marked the phase transition between healing and non-healing and abscess formation, with a specific emphasis on the difference between healing, the presence of avirulent bacteria and the presence of bacteria with virulence potential.

Until now, the focus of agent-based approaches

to the study of bacterial population growth has to develop models that can accurately replicate known results and understand how these are affected by different behaviors within a bacterial population (Gorochowski et al.; 2012). One of the first studies in this area was developed by (Kreft et al.; 1998), where they created a simulator based on individuals to represent the bacterial growth dynamics, the Bacsim. The authors created agents with generic behavioral rules for bacterial cultures, with the intention that the model presented serve as a basis for the study of growth in different bacterial colonies. The agents present in the model absorb nutrients and thereby gain cell mass as well as produce waste in the environment.

When they reach a certain threshold, agents reproduce (mimicking the process of cell division). Each agent spends a fixed amount of energy in order to maintain its metabolic activities, and if the nutrient intake is less than necessary for this maintenance, the agent will lose cell mass. After a certain threshold, the agent dies, and its cell mass is reintegrated into the environment with a percentage of utilization as part of the substrate.

Other work that used agent-based simulation to recreate bacterial growth was that of Werlang et al. (2013). The author describes a model to growth curves of *Mycobacterium tuberculosis* based multi-agent systems. For the author, the study of this bacterium is very important as it allows the study of characteristics and the development of new drugs. The performance of experimental tests with this bacillus are slow, taking at least three weeks to show some result, and often fail because of contamination or dehydration means.

In the model of Werlang et al. (2013), agents that represent bacteria have differences characteristics, and these are extremely important for agents to represent their roles in the environment and to interacting with it, similarly as to *Mycobacterium tuberculosis* bacteria would interact in their natural living environment. The author reports that the results were very satisfactory and the curves found reached a very close similarity to the real curves. Finally, the author also points out that the model is useful as it enables the testing of hypotheses in some hours in opposition to those carried out *in vitro* which would take days.

4 Growth Model Curve Of *Mycobacterium Tuberculosis*

When the growth curves are obtained by MGIT, the information that these curves present are the product of several factors of population dynamics. Therefore, it is not possible to extract isolated information, such as: how much they consume or how much fail to consume after reaching environmental saturation, or the proportion of signaling molecules are required to enact the saturation.

Considering these circumstances, it is necessary to infer how many are the variables that affect the population growth, using just the observation of the results.

To simulate the population dynamics we used Netlogo (Wilensky; 1999) programming environment.

The agents based model implemented simulates an environment where agents represent *Mycobacterium tuberculosis*.

The model agents have specific rules of behavior, which are modeled as variables of the agents. These rules are essential for them to represent their role in the environment and how they interact.

The simulation has the time division: the tick. Every tick, the agents perform one or more actions. These actions are modeled by functions set out in the model, as follows: feed, continue signals and reproduce.

As many measurable phenomena present in our everyday lives tend to be distributed according to some probabilistic theoretical models, there is a possibility that the main variables that growth curve of *Mycobacterium tuberculosis* model can also be distributed as normal model.

How many random variables biological fit a normal distribution (Callegari-Jacques; 2003), i.e., the central values are more frequent and extreme rarer (very low values as infrequent as very high). It was assumed that the model variables also are distributed normally.

Agents are inserted in an environment that is shared by all. In this environment, they find the nutrients needed to survive and deposit the waste from their metabolism.

Each space in the environment is called patch. In each patch, there is a number of nutrients and waste. Nutrients are used by agents throughout the simulation to keep their vital functions active and accumulate energy. The waste is deposited by the agents after the metabolism of nutrients.

As *in vitro* experiments, that are performed in the laboratory, where a number of bacteria are inoculated into a container, the model initialization is done with a number of agents in the environment.

Agents receive different values for each variable. The set of possible values have different probabilities of occurrence, a characteristic of normal distribution. When an agent is generated, its variables receive simulated values of the distribution used.

Fig. 2 shows the agent's life cycle, clarifying the actions and decisions they must take every tick.

After the start of the simulation, the agents begin to move in the environment to search for nutrients. However, in the beginning, they only main is survival, which they are adapting to the environment, and therefore unable to reproduce. This same behavior is observed *in vitro* experiments that the bacteria need a certain time to adjust their metabolic functions to the new habitat.

Later this adaptation time, the agent starts to perform normally all its functions, including reproduction. It consumes nutrients and then transform them into energy, which will be deposited in its reservation to indicate how healthy it is and to keep active vital functions.

Each agent of the model has a different time to reproduce. This time indicates the amount of energy that the agent should have available in its energy reservation to perform the reproduction function. In this way, the energy is a limiting factor for reproduction.

There is an amount of energy to maintain the agent alive at each cycle. If it consume less than necessary

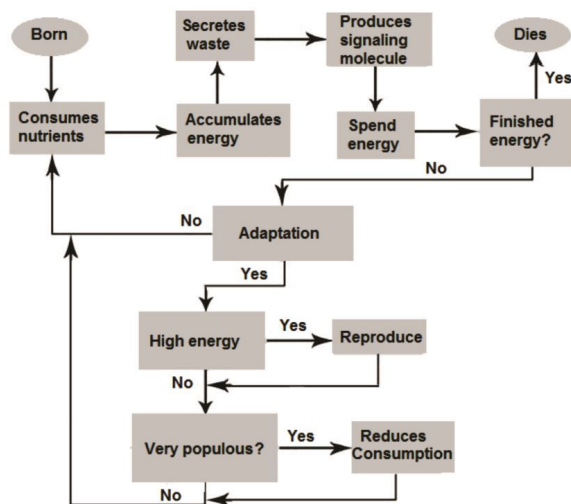


Figure 2: Flowchart of agents lifecycle

to maintain its metabolism, it will start spending power of its reserve in order to survive and the end of this reserve will take its death.

When there is accumulation of waste in patches, the agents have difficulty absorbing nutrients, because it becomes more difficult to agents survive in a very saturated environment.

Another aspect in the curve modeling is the bacteria sensor. This variable determines how many agents in the environment will reach the saturation situation. When this threshold is reached, the agents release a signal molecule, called *quorum sensing*, that warn others that the environment is full. According to Whitehead et al. (2001), this process occurs when there is awareness of high cell density, allowing the entire population initiate an action, once the critical concentration was achieved.

The main action in the proposed growth model is the decision to reduce consumption. Once the agent perceives the situation of saturation, and release a signal molecule, it enters reduced power state, which consumes less nutrients, and it generates less waste and reproduce less.

Reduced consumption is a *boolean* variable that can only receive two values, true or false. Once the real consumption is reduced, the bacteria reduces the quantity of nutrients that will absorb the environment by tick and also decreases proportionally to the amount of energy required to keep it alive.

In the model initialization, all agents have false value for reduced consumption and it just gets true value when the agent detects a significant number of agents or signals in the environment. This information is stored into the variables, sensor signals or sensor bacteria.

Reducing consumption of nutrients and energy to maintain vital functions agent aim to make it grow less, and so can survive longer in the environment.

4.1 Model Implementation

After defining the population dynamics, the next step was to implement the model in NetLogo (Wilensky;

1999). Through user intervention, it is possible configure some environmental parameters, such as the initial number of agents and the amount of available nutrients. Fig. 3 shows the model interface created and its variables.

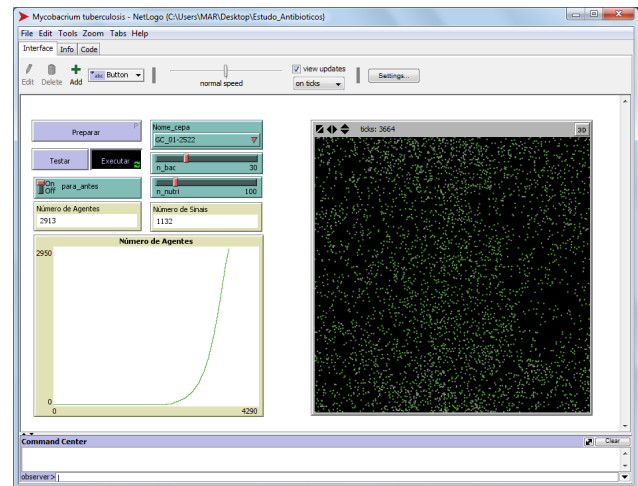


Figure 3: Interface of developed model

Each of the simulated strains have different times to start its phases. Thus, each receiving different values for the variables. To not create five simulations for each of them, a device in which the five simulations can be run on the same code. Just choose the strain to be simulated and start it in Cepa_name button.

The results obtained by Von Groll et al. (2010) represent the growth curves of *Mycobacterium tuberculosis* in only two stages, the adaptation and the exponential phase. In order to facilitate comparisons between real growth and simulated, a key has been created that enables the user to simulate curves with the same stages of growth, i.e., it is detected that the curve is not growing, the simulation is terminated. See more details about this model in Moraes et al. (2017).

4.2 Values interpretation

The curves from the MGIT express the result in units growth (GU). Therefore, an arbitrary measure of equivalence becomes necessary GU relates to the number of simulation agents. In the model developed was used an agent to represent every two GUs.

The growth curves of Fig. 4 were obtained by Von Groll et al. (2010), using MGIT, with growth in GUs and time in days. However, in the figure, growth was expressed in number of agents. The equivalence between GUs and agents was 20.000 GUs for 10.000 agents.

Using the same idea, an equivalence was also obtained for the growing time. As the experimental curve expresses the results by the day, and as seen in the simulation proposed model is temporal division ticks, the equivalence of 260 ticks for each day simulation was used.

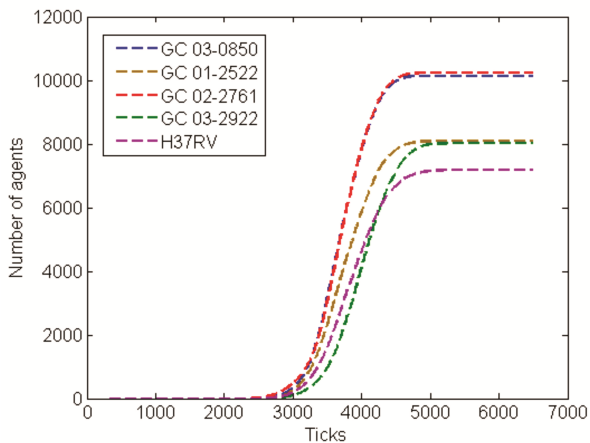


Figure 4: Real growths represented by agents

5 Results

This section presents the results obtained after the implementation of the proposed model. Initially, it presents the curves generated by the model with the real data (*in vitro*). In a second step, the validation showing some data and hypothesis tests.

Fig. 5 shows the results generated for all strains. Generated values were obtained by the averaging of 50 simulations for each of the strains (continuous line). The gray shaded area represents the standard deviation.

The standard deviation is an indicator of greater flexibility with respect to possible curves to be generated, noting that the higher standard deviation the lowest the robustness level (stability) of the model. The graphs of Fig. 5 are composed from two growth curves: the dotted line represents the real curve data and continuous curve is the simulated data curve.

5.1 Model Validation

The proposed model has been tested in order to be validated. The graphic results and numeric results presented in the previous section were compared with real data obtained by (Von Groll et al.; 2010). To do the comparison it was necessary to convert the simulated data. The ticks were converted to days and the agents number for Growth Units. Remember that each day is equals to 260 ticks, and each agent represents two growth units.

Various parameters settings were tested. These adjustments allowed the behavior of the simulated growth curves to be reproduced with a relatively similarity to the real growth curves.

By analyzing the obtained data, it is clear that some of them represent better the beginning of growth; others the growth medium; and most of them can represent faithfully the end of growth. In this way, we cannot conclude that got better representation viewing only graphics.

To do a comparison more precise between the real growth curves and the simulated curves, we did some hypothesis tests using Student's test (t-test).

The tests were executed in R software, through the

t.test function, at a significance level of 1%. The level of significance is the maximum allowed probability for the test statistic to fall in the critical region when the null hypothesis is actually true. The function returns the value of the t-statistic, the number of degrees of freedom, the corresponding p-value, and the confidence interval. The p-value is the probability of obtaining a test statistic value that is, at least, as extreme as that representing the sample data, assuming that the null hypothesis is true.

The t-tests were performed for every first hour of the day from the tenth day. From the first to nine days, it was not possible to perform them due to the fact that no experiment showed population growth, which characterizes a similar behavior in these 9 first days, considering the adaptation phase, presented in *in vitro* experiments.

Tables 2, 3, 4 and 5 show the null hypotheses (μ in GU's) evaluated for each of these days and the respective values for the sample mean (without GU's), sample standard deviation (without GU's), test statistic, p-value and confidence interval (in GU's) of 99%.

For GC 02-2761 and GC 03-0850 strains, from the 10th to the 13th day, p-values lower than the level and significance were adopted (0.01), indicating an evidence contrary to the null hypothesis, since these results suggest that the observed result is very uncommon to happen when the null hypothesis is true. On the tenth day, for the GC 02-2761 strain, the result of the sample mean was lower than the population mean indicated in the null hypothesis, the same did not happen on the 11th, 12th and 13th days. From day 14, however, GC 02-2761 and GC 03-0850 strains have p-value results higher than the level of significance adopted, so for those days there is no evidence that contradicts the hypothesis Null.

The GC 03-2922 strain began its growth one day after the others. In this way, the analyzes were performed from the 11th day. The p-values results were higher than the level of significance from the 15th to the 20th day, and therefore, there is no evidence to contradict the null hypothesis for those days. In the 16th day, the p-value was very close to the level of significance (0.01).

For H37Rv strain, from the 10th to the 13th, and from the 15th to the 17th day, evidence was found that contradicts the null hypothesis. For the 14th and the 18th to the 21st day there is no evidence to contradict the null hypothesis, since the p-value is greater than the level of significance. However, it is important to highlight to the fact that the p-value found for the 14th day was very close to the level of significance adopted (0.01).

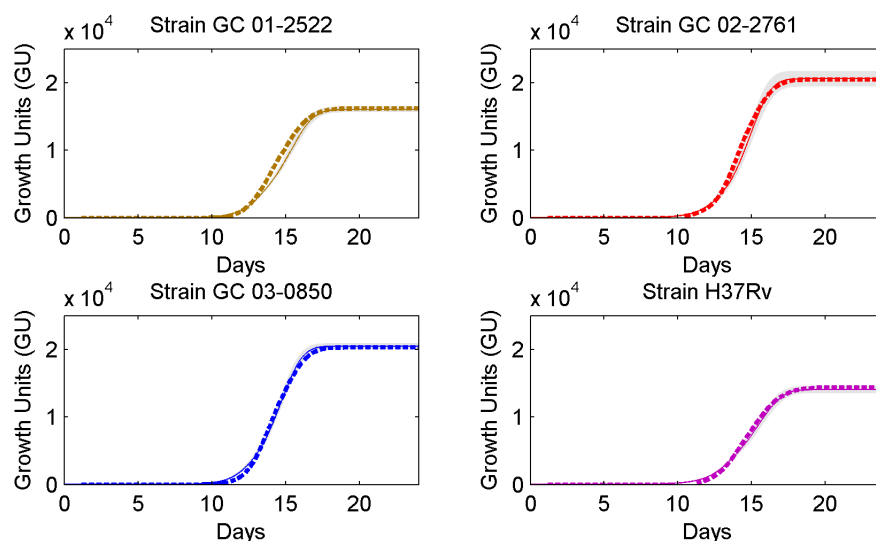


Figure 5: Simulate Curves of all strain with real data

Table 2: T-test for each day of GC 02-2761 strain

Day	Null hypothesis	Sample mean	Sample standard deviation	T-statistic	P-value	Confidence interval
10 ^o	$\mu=109$	102,60	10,40	-4,23	0,0001097	98,53 a 106,66
11 ^o	$\mu=262$	337,64	45,60	11,80	1,636e-15	320,41 a 354,86
12 ^o	$\mu=750$	983,30	156,44	9,26	4,427e-12	915,60 a 1050,99
13 ^o	$\mu=2094$	2274,00	403,49	3,26	0,002071	2125,86 a 2422,14
14 ^o	$\mu=4491$	4396,47	760,84	-0,87	0,3869	4105,71 a 4687,22
15 ^o	$\mu=7038$	6954,66	1023,29	-0,57	0,5682	6565,13 a 7344,19
16 ^o	$\mu=8936$	9083,15	967,85	1,07	0,2898	8713,95 a 9452,34
17 ^o	$\mu=9890$	10034,32	764,72	1,32	0,1925	9741,09 a 10327,54
18 ^o	$\mu=10194$	10235,15	682,02	0,42	0,675	9973,13 a 10497, 17
19 ^o	$\mu=10217$	10253,74	669,92	0,386	0,703	9996,39 a 10511,09

Table 3: T-test for each day of GC 03-0850 strain

Day	Null hypothesis	Sample mean	Sample standard deviation	T-statistic	P-value	Confidence interval
10 ^o	$\mu=29$	142,91	13,55	57,27	2,2e-16	137,57 a 148,26
11 ^o	$\mu=155$	315,32	42,64	25,59	2,2e-16	298,48 a 332,15
12 ^o	$\mu=628$	1088,76	226,95	13,92	2,2e-16	999,81 a 1177,72
13 ^o	$\mu=2056$	2408,30	464,15	5,20	4,425e-06	2226,38 a 2590,22
14 ^o	$\mu=4552$	4432,38	863,39	-0,9498	0,3472	4093,98 a 4770,78
15 ^o	$\mu=7014$	6990,78	1174,60	-0,1349	0,8933	6528,47 a 7453,09
16 ^o	$\mu=8897$	9080,29	1111,67	1,13	0,2642	8644,58 a 9516,01
17 ^o	$\mu=9826$	9932,32	888,29	0,8205	0,4161	9584,16 a 10280,47
18 ^o	$\mu=10111$	10140,11	774,17	0,2578	0,7977	9836,67 a 10443,54
19 ^o	$\mu=10139$	10163,91	754,25	0,2265	0,8218	9868,29 a 10459,53

6 Conclusion and Further Works

Tuberculosis is one of the oldest diseases, with wide geographical distribution, constituting a serious public health problem worldwide. The study of growth curve of *Mycobacterium tuberculosis*, which causes tuberculosis, enables the understanding of various behaviors of bacilli, such as response to different chemical agents and environment conditions. In spite of the behavior of bacillus face to different conditions still no predict by in silicum models. Modeling growth curve is a tool allows

better designing studies involving growth curve from normal condition of growth and maximizing the analyses.

This work shows development of a growth curve model of *Mycobacterium tuberculosis*, able to reproduce the real curves given as input. The proposal was inspired by the work of Werlang (2013), which created a model based on multi-agent capable of representing the real curves of *Mycobacterium tuberculosis* with a certain degree of similarity (Von Groll et al.; 2010), which has standardized a method to measure growth population bacillus when placed in a medium. The

Table 4: T-test for each day of GC 03-2922 strain

Day	Null hypothesis	Sample mean	Sample standard deviation	T-statistic	P-value	Confidence interval
11 ^o	$\mu=73,5$	147,64	10,44	47,64	2, 2e-16	143,45 a 151,83
12 ^o	$\mu=198,5$	337,63	42, 65	-4, 09	2,2e-16	353, 85 a 388,09
13 ^o	$\mu=610,75$	917,97	151,12	13,63	2,2e-16	857,32 a 978,63
14 ^o	$\mu=1621$	1997,02	327,34	7,70	1,071e-09	1865,64 a 2128,40
15 ^o	$\mu=3347$	3343,31	485,91	-0,0509	0,9596	3148,29 a 3538,33
16 ^o	$\mu=5329$	5002,33	668,69	-3,27	0,012052	4733,96 a 5343,70
17 ^o	$\mu=6789$	6597,97	724,96	-1,76	0,08407	6307,02 a 6888,93
18 ^o	$\mu=7655$	7629,53	621,26	-0,275	0,7846	7380,19 a 7878,87
19 ^o	$\mu=7966$	8009,80	509,75	0,5764	0,5673	7805,21 a 8214,38
20 ^o	$\mu=8014$	8079,62	467,70	0,9412	0,3517	7891,91 a 8267,33

Table 5: T-test for each day of GC 03-H37Rv strain

Day	Null hypothesis	Sample mean	Sample standard deviation	T-statistic	P-value	Confidence interval
10 ^o	$\mu=8$	89,31	5,79	98,26	2.2e-16	87,09 a 91,52
11 ^o	$\mu=172,5$	206,59	20,17	11,82	7,822e-16	198,86 a 214,32
12 ^o	$\mu=352$	501,12	65,26	15,99	2,2e-16	476,17 a 526,13
13 ^o	$\mu= 1070$	1162,69	198,74	3,26	0,002023	1086,54 a 1238,84
14 ^o	$\mu= 2449$	2305,92	396,11	-2,53	0,0148	2154,14 a 2457,69
15 ^o	$\mu= 4053$	3575,12	573,15	-5,84	4,453e-07	3355,51 a 3794,74
16 ^o	$\mu= 5535$	4947,53	751, 22	-5, 47	1,577e-06	4659,68 a 5235,38
17 ^o	$\mu= 6485$	6144,73	766,56	3,11	0,003169	5851,01 a 6438,46
18 ^o	$\mu= 6972$	6848,73	671,36	-1,28	0,2049	6591,49 a 7105,98
19 ^o	$\mu= 7135$	7090,55	592,60	-0,525	0,602	6863,48 a 7317,62
20 ^o	$\mu= 7176$	7125,96	570,70	-0,6138	0,5423	6907,28 a 7344,63
21 ^o	$\mu= 7179$	7126,55	570,08	-0,644	0,5226	6908,11 a 7344,99

results obtained by Von Groll et al. (2010) enabled to verify the model with more fidelity to reproduce the real curves.

The proposed growth curve was modeled using simulation based agents. Wooldridge (2009) says that multi-agent systems are a powerful and flexible tool for modeling environmental/social systems, because this type of system allows to analyze the behavior of each individual, rather than an average behavior of them.

To make the proposed model more similar to real growth model, we proposed that possible values of the variables were stochastically distributed, thus, treating the individuality of each agent. We have as hypotheses estimate the best possible probability density function of the variables, with the final product an identical curve, or minimum error when compared to real data.

In order to compare data from agent-based simulation with data obtained from an *in vitro* experiment, hypothesis tests were performed, because we believed that the developed model could reproduce *Mycobacterium tuberculosis* growth curves similar to those obtained in an *in vitro* experiment. In order to do this verification, Student's t-test was used, where the hypothesis to be tested was that the average for each day should not be different from the average obtained in the *in vitro* experiment.

The agent-based model presented satisfactory results for most days, and those that do not contradict the null hypothesis are considered satisfactory. In our analysis, satisfactory results means that were found on almost 80% of the days for strains GC 02-2761 and GC 03-0850 and 75% of days for strains GC 03-2922 and H37Rv. Also, most of the unsatisfactory results occurred in the first 4 days analyzed (0th to

13th or 11th to 14th).

Considering the results found in the statistical tests carried out, it is believed to have obtained sufficient numerical arguments for the use of the agent-based model developed to simulate growth curves of *Mycobacterium tuberculosis*. Therefore, it can be very useful for checking hypotheses and aiding real experiments.

In this work, we have used data about just one real experiment. However, it is important, when possible, to do more studies with real samples and to define a bigger number of observations to realize the comparison, to maximize the results.

In the proposal of this work, we simulate the growth of *Mycobacterium tuberculosis* considering normal growth conditions. Future studies are intended to introduce into the model situations that occur "in vitro" experiments, such as the use of antibiotics and the bacillus resistance at different concentrations.

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