# Pantoea Model description

To describe the model, we use the ODD protocol (“Overview, Design concepts, and Details”) which helps to ensure that the model explanation is complete (Grimm et al., 2010).

## Purpose

To develop a computational model to study the growth of *Pantoea* sp. YR343, a bacteria interesting due to its several plant growth-promoting characteristics. *Pantoea* is grown in batch culture under aerobic growing conditions, to reproduce the agar plate experimental protocol and to explore the consequence of different priorities in the individual use of nutrients on the system dynamics.

## Entities, State Variables, and Scales

Our model has two types of entities: individuals and square patches of culture medium. Each individual represents a unique bacterium of *Pantoea* and has the variables: unique identification number, size, location (XY grid cell coordinates of where it is), mass, reproduction mass, counter for each reproduction cycle, individual variables for each nutrient uptake, viability and reproduction time.

The model assumes that the smallest individual represents a bacterium with a diameter of ~ 0.55 μm and the largest one a bacterium with a diameter of ~ 1.1 μm. To characterize the composition of the microbial cells, the model uses the empirical formula C4.17H8O1.75N so that each bacterium is assumed to have this elementary cell composition.

A two-dimensional lattice of 251 x 251 grid cells represents the agar plate that contains the culture medium; each grid-cell represents a volume that can be tuned by changing the world dimensions, depth, and length. A volume was calculated using a portion of the wells in the experimental setup with dimensions of 605 μm × 605 μm × depth, where depth ranges from 10 μm to 50 μm. The spatial cell variables are unique position identifier in XY coordinates, total amount of each nutrient: glucose, oxygen, ammonium, bicarbonate, carbon dioxide, water, and counters to stablish the number of bacteria on it.

All microbial and culture medium processes are discretized in time steps. One time step represents 30 seconds; for the current work the simulations were run at least for 4000-time steps (32 h) but the user can choose the max time to run the model. Using High Performance Computing the model could run until all the bacteria die. With these units, graphical and numerical model outputs are the molar concentration of nutrients and metabolic products expressed in millimolar (mM) and the growth curve of *Pantoea*.

## Process Overview and Scheduling

The initial configuration of our model has two parts: the first one for the system and the second one for the entities (culture medium and bacteria). The initial system setup sets the world size and topology, and the time scaled factor (time step). The topology of the world is programmed using a box mode (world with vertical and horizontal limits). The initial culture medium concentrations and the initial bacterial population are established using random variables, normal probability distributions with mean values that are established by the user following experimental procedures.

At each time step a group of individuals are controlled using a set of time-dependent variables for each bacterium. All individuals perform the following processes: nutrient uptake, cellular maintenance, biomass synthesis, and bipartition. Culture medium processes are different depending on the agar plate protocol and experimental conditions, but in any case, the culture medium is randomly diffused to simulate the mass transfer process in solid phase.

For each time step the time-dependent variables of microorganisms and culture medium are calculated, updating the graphics and digital outputs according to the time scale proposed. The model also controls the whole carbon and nitrogen mass inside and outside of the system to ensure the carbon and nitrogen are balanced. During the simulation processes the bacteria are called in a different random order in each time step and the state variables changes are immediately assigned generating an asynchronous update.

## Design Concepts

## Basic Principles

The model has two kinds of behavior-rules, rules for the individuals (bacteria) and different rules for the environment (culture medium). The set of individuals and the environment is called the system (agar plate). All the rules are used at the level of the sub-models, and they are explained in the corresponding section. The individual rules are: i) nutrient uptake, ii) cellular maintenance, iii) cellular growth when a micro-organism executes any of the metabolic reactions adjusted by TEEM, and iv) cellular division following binary fission. The system rules are those that mimic the general agar plate procedures.

## Emergence

The system dynamics emerge as the result of the interaction between bacteria and the culture medium that they find inside of the agar plate. The model outputs are simulated volume (nl), simulated time (hours), the biomass over the time evolution, nutrient uptake rate consumption and all nutrients’ concentrations on the agar plate. All the model outputs appear at the system level consequently, and from, the individual bacterial activity.

## Adaptation

All the individuals (bacteria) are programmed with the same rules; some of these rules will be executed and others not, depending on the internal changes of the individuals and/or the characteristics of their local environments. Individuals act one after another, not in parallel. Hence, after one individual carries out all of its actions the composition of the spatial cell where it lives changes, and the next individual meets a different medium composition in relation to the previous acting or post-acting individuals.

The first individual rule is how to execute cellular maintenance which follows the energy reaction written using TEEM. Each bacterium according to its biomass calculate the nutrient quantity to accomplish its maintenance; if the cellular maintenance is not fulfilled the bacteria cannot execute the next individual rules. The second individual rule is performing biomass synthesis for growth. This rule is executed only when the amounts of nutrients taken in the uptake are enough to accomplish the maintenance requirement, and after updated amounts also allow execution of the corresponding synthesis reaction in the aerobic phase. The third individual rule is whether to divide or not, depending on whether it has reached the minimum reproduction mass. The reproduction mass (mR) is the mass the bacterium must reach to start the bipartition (mR is obtained from a normal random distribution with a mean value of 75% of the larger bacterium size). Additionally, the model includes an individual rule to control and avoid the overlay of bacterium on its surrounding culture medium.

## Interaction

*Pantoea* is the only bacteria species in the virtual culture chamber. The microorganisms interact with the culture medium; therefore, there is an indirect interaction in which nutrient competition takes place among the bacteria that share the same spatial cell.

## Collective

The simulated bacteria do not form aggregates, each individual acts uniquely.

## Stochasticity

Several processes are modelled on criteria of randomness: i) the reproduction sub-model, ii) a portion of the uptake sub-model, iii) some parameters involved in the culture chamber management or operating protocol, and iv) a part of the initial system configuration.

Inside the reproduction sub-model, we consider that the reproduction threshold biomass for each bacterium is determined using a value from a normal random distribution. For the physical separation of the two bacteria, the original mass is separated into two new bacteria with masses according to a value from the normal random distribution with mean value 0.5 and standard deviation 0.075. Thus, the mass of the original bacterium does not divide exactly in the proportion 50-50.

Inside the uptake sub-model, we consider that at each time step, each individual nutrient uptake-rate for each nutrient is established from a normal random distribution with the mean value calculated from stoichiometric coefficients using TEEM and the individual mass of each bacterium with a standard deviation of 10% of this value.

Regarding the agar plate management: At the beginning of each simulation the model randomly distributes each bacterium on the system (setup procedure). For the initial system configuration, we consider that the initial culture medium composition, the initial population biomasses are established from normal distributions with mean values determined by the experimental procedure and standard deviations of 10% of these values. To represent the small reactor with constant agitation, we introduce a redistribution of nutrients and metabolic products in random time steps.

## Observation

The graphical and numerical outputs of the model are the concentration (mmol·l-1) of each culture medium component (glucose, ammonium, oxygen, bicarbonate, carbo dioxide and water) and the time evolution of the microbial biomass (mg·ml-1). The user can obtain all simulated data in the output file with the extension “.txt”).

## Initialization

The user can adjust: i) the culture medium composition (mmol·l-1) of glucose and ammonium, ii) initial amount of viable micro-organisms (bacteria), iii) step time (min), iv) the size of the culture chamber fixing the values of depth (um) and total length world (um), v) the individual max time availability (min), vi) the maintenance energy requirement for aerobic phase (gCdonor·gCmic-1·h-1), vii) thermodynamic efficiency to simulate microbial metabolic reactions, viii) the maximum growth rate for the bacteria population (h-1), ix) the diffusion coefficient for the nutrients, and x) the maximum number of bacterium on each culture cells.

## Sub models

The bipartition reproduction process is a sub model that is taken from INDISIM (Ginovart et al., 2002). The uptake, cellular maintenance and biomass synthesis sub models are obtained from INDISIM-Paracoccus (Araujo Granda et al., 2016) and INDISIM-Denitrification (Araujo-Granda et al., 2020). Thus, we only describe the individual sub-models that we designed particularly for the *Pantoea* model.

## Agar Plate operation

The sub-models related to the culture medium on the agar plate are: i) Nutrient diffusion: We use the Netlogo primitive called “diffuse” to simulate the mass diffusion process in solid-phase linked with an input parameter called diffusion-coefficient, ii) Reproduction cycle on the culture medium: if the number of bacteria in the spatial cell of the system is higher than a maximum threshold the bacterium cannot execute de bipartition procedure until exist and empty space around it.

## References

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