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3D INDIVIDUAL BASED MODEL FOR BACTERIA GROWTH AND SPATIAL INTERACTIONS: APPLICATION TO THE CASE OF *LISTERIA MONOCYTOGENES* AND *CARNOBACTERIUM PISCICOLA*

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KEYWORDS

Predictive Microbiology, Bacterial Colony Growth Model, Spatialisation

ABSTRACT

By means of an interdisciplinary collaboration, we build a three dimensional individual-based model at the microscopic scale. This model is based on the cardinal model at the population scale, and aims at investigating the impact of spatialisation on the growth of bacterial colonies, in particular in the case of species in interaction. Our case of application is the influence of lactic acid bacteria on pathogens, which growth depends on pH evolution from lactic acid production and diffusion, according to carbohydrate concentration, temperature, water activity and ratio of both populations. We use our individual-based model to study and illustrate different and major effects of spatialisation on colonies growth. The last section presents some perspectives for the design of a continuous partial derivative equations-based companion model to our individual-based one.

INTRODUCTION

Nowadays, lactic acid bacteria (LAB) are not only considered for the production of fermented foods, but may be used as well to control the outgrowth of microbial pathogens – see (Matilla-Sandholm and Skyttä 1996) for an overview. Their presence can significantly modify a growth medium, by means of organic acids production which induces pH reduction, or through the increase of the medium solidity due to the presence of polysaccharides.

Recent numerical models in the area of food fermentation and biological preservation take into account the importance of these microbial metabolites, and not only the microbial growth. Moreover, they model the impact of these microbial metabolites on the growth medium in order to predict more accurately the microbial growth, but as well to enable medium-wise interactions between different species – see for example (Poschet et al. 2005). However, most of these models do not account for spatial effects, except in a few cases – see for instance (Kreft et al. 1998) for an individual based 2D model, or (Grimson and Barker 1994) for a continuous model.

In this paper, a model based on the cardinal model (Ellouze et al. 2008; Lobry et al. 1991) from literature is used to build a three dimensional individual-based model (IBM) at the microscopic scale, in order to investigate the impact of

spatialisation. The metabolite taken into consideration is lactic acid, since it is the main end-product of LAB metabolism.

Our work can be subdivided in two main parts. A first part describes the modelling process itself since it is a deeply interdisciplinary project and because we believe that the development methods may be as important as the resulting model itself. The resulting model describes the concomitant growth of lactic acid bacteria, namely *Carnobacterium piscicola*, and pathogens, namely *Listeria monocytogenes*; these growths depend on pH evolution from lactic acid production and diffusion, according to carbohydrate concentration, temperature, water activity and ratio of both populations. In a second part, we use our IBM in order to study and illustrate a possible effect of spatialisation. We mainly investigate around the growth rate of a colony, into a virtual Petri dish, in relation with the spatial distribution of *C. piscicola* individuals. Then, we show that the resulting variability may have dramatic consequences on the final size of *L. monocytogenes* colony. The last section presents some promising perspectives, since we are currently designing a continuous partial derivative equations-based companion model to our IBM.

A Few Words About Interdisciplinarity

Although it is not the main purpose of this work, we want to stress the point that this study takes place in the context of an interdisciplinary project. Indeed, microbiologists, biologists, mathematicians and computer scientists were involved, so that nontrivial issues had to be addressed, in particular the collaborative construction of a relevant model. The steps of the project progress are reported on Figure 1.

1. This project first started by interdisciplinary exchanges, the development of collaborative web environments, and shared objectives definitions.
2. Based on Virtual Reality principles (Tisseau 2001), the *in virtuo* modelling step used a virtual laboratory to co-design a model, understood by all the participants of the project. This enabled us to tackle interdisciplinary issues: implicit knowledge, mutual understanding, science integration, etc., and to build a shared IBM.
3. Once the project consortium agreed on a shared model, it was thus possible to optimise this IBM and scale it up. This highly technical step excluded non experts.
4. Finally, mathematicians translated the IBM into a continuous partial derivative equations model, to reduce computation time. This step is still in progress and is briefly exposed in our perspectives.

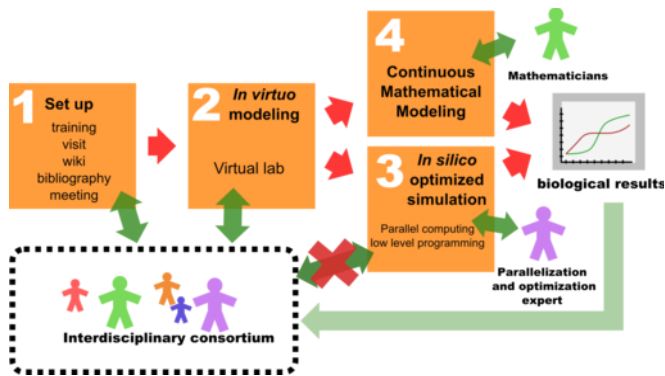


Figure 1: Four Step Interdisciplinary Project

INDIVIDUAL BASED MODEL

We built models of *C. piscicola* and *L. monocytogenes* interacting on a Petri dish. Bacteria shapes are 3D capsules that have mechanical interactions. Bacteria feed on glucose – and produce lactic acid –, and divide over time, depending on their local environment. The substrate was modelled as a 3D discrete reaction-diffusion system in which glucose and lactate diffuse. We made use of Unity3D to handle the graphical user interface that made it possible to see, experiment and modify the model during the simulation. Based on this *in virtuo* modelling, we used the TransProg C library (Harrouet 2012), so as to develop an optimised and parallel IBM, from the cardinal populational data and model. Spherical shapes for bacteria constitute the only simplification made to enable the simulation of hundred millions of individuals.

Development Methods

Two software tools were used to simulate the IBM.

RéISCOP for Interdisciplinary Co-Construction.

During the *in virtuo* experimentation step, we used the RéISCOP software (Figure 2), which is both a meta-model and a simulation engine (Desmeulles et al. 2009). It relies on the multi-interaction modelling paradigm which is dual and similar to the multi-agent paradigm. Multi-interaction model description is focused on interactions between components rather than on components themselves. This dual perspective is suitable for modelling complex and multi-model systems. RéISCOP 2.0 was written with the C# language for this project, and was interfaced with the VR software Unity3D, to provide a graphical user interface (GUI).

TransProg for Optimisation and Parallelisation.

In order to achieve high computational loads we used the TransProg library (Harrouet 2012) to develop an optimised and parallel model. TransProg is a set of facilities (multi-platform and written in C language) for a programmer to harness the full potential of modern general purpose computers. It was designed with individual-based simulations in mind, and consequently makes use of multiple cores and processors, as well as graphical processing units for both rendering and computing. It is dedicated to interactive simulations of highly dynamic systems where entities can move, change, appear, disappear and interact with each other and the user at any time.

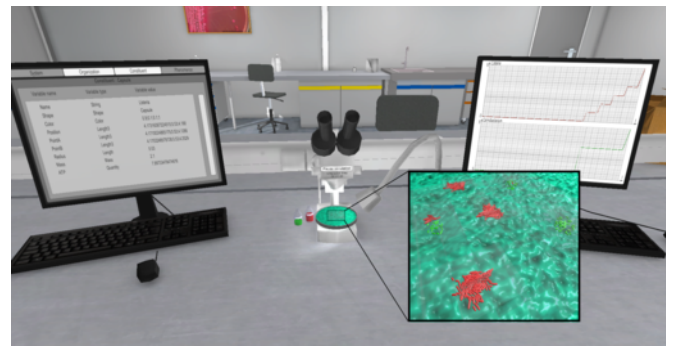


Figure 2: RéISCOP Based Virtual Laboratory

Model

The optimised IBM we used to produce our results is depicted on Figure 3. It consists in a diffusive substrate that simulates an agar-agar medium containing glucose and lactate, some pathogenic bacteria (*L. monocytogenes*) and some lactic acid bacteria (*C. piscicola*) – parameters are given in Tables 1 and 2. Each bacterium is modelled as an autonomous individual with its own behaviour.

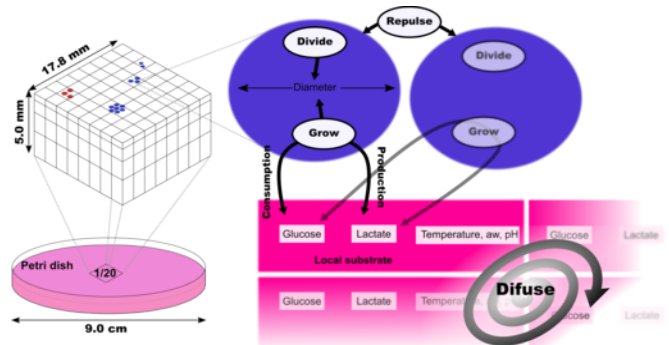


Figure 3: Individual Based Model Description

Substrate.

As shown on Figure 3, the simulated substrate represents $1/20$ of the surface of a Petri dish and its full thickness (about 5 mm). It consists in a 3D mesh of spatial cells maintaining concentrations of glucose and lactate. Although the horizontal grid step is regular, the vertical one uses a geometric progression in order limit the number of spatial cells while keeping a sufficient volume for a buffer effect. Because bacteria only interact with the top of the substrate, the coarse grained cells used at the bottom don't harm so much. We had to adapt the computation of Fick's second law with the Crank-Nicolson integration scheme for accuracy (Hairer et al. 1996), and its resolution with the Jacobi method, to this non isotropic 3D grid pattern.

Global substrate parameters are temperature and water activity. Local pH is calculated from local lactate concentration according to an empirical polynomial law:

$$pH = pH_0 + pH_1 \cdot [Lac] + pH_2 \cdot [Lac]^2$$

Growth, Lactic Acid Production.

The cardinal model (Ellouze et al. 2008) for colony growth μ was turned into individual bacteria behaviour based on individual generation time (t_g) and local parameters:

$$t_g = \ln(2)/\mu$$

We estimate empirically the amount of glucose an individual consumes during a generation time. Thus, a bacterium, according to its local perception of the substrate, is able to determine its generation time and the corresponding amount of glucose to consume during the current simulation time step. Although bacteria metabolism is not modelled, this consumption yields a lactate production and raises the individual volume. Both phenomena are taken into account in our model.

Division.

When the radius of a bacterium reaches a limit ($1.5 \mu\text{m}$ for *L. monocytogenes*, see Table 2), it splits into two halved volume individuals. One of them draws a new random perturbation for its optimal growth factor in order to desynchronise later divisions, otherwise the number of individual would always be a power-of-two.

Mechanics.

Because we planed to simulate millions of individuals in a 3D environment, collision detection and response are very critical considering computation time. As shown on Figure 4, we simplified this problem by turning bacillus shapes into spheres and we introduced a local density based stacking algorithm (not physically exact) to organise bacteria in distinct layers in which collision detection occurs.

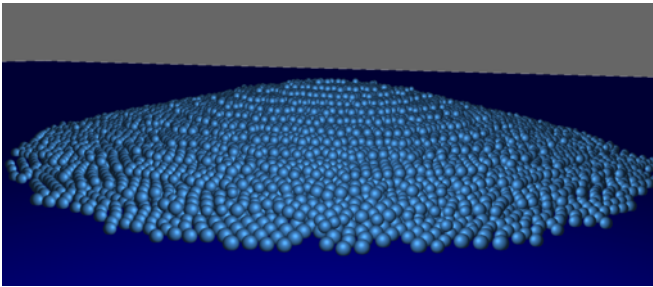


Figure 4: Multi-Layered Colony Shape

Validation

We validated our IBM on real data, provided by the ADRIA laboratory, Quimper, France. This validation was presented in (Desmeulles et al. 2015): the IBM parameters were adjusted by interpolating simulated data with real ones.

BENEFIT OF SPACIALISATION

As already explained in the introduction of this paper, cardinal models cannot account for the variations of bacteria spatial distribution observed on real food, or on Petri dishes. The main interest of our individual based simulation is its ability to achieve more realistic simulations, thanks to its 3D-spatialisation. In this section, we present two concrete cases which enlighten this ability.

Impact of Spatialisation on One Species Growth

We first simulate the temporal response of *C. piscicola* bacteria in a Petri dish for two distinct spatial distributions. Bacteria colonies grow on a $c * c * 5 \text{ mm}^3$ medium (sub-box of a virtual Petri dish), with $c = 17.836$. The environmental conditions are given in Table 1 and bacteria parameters in

Table 2. All these parameters come from the actual experiments that were conducted by the ADRIA laboratory (see web references).

Table 1: Substrate Parameters

parameters	values	units
substrate size	17.836 x 17.836 x 5	mm
discretisation	89 x 89 x 8 (1.3 depth progr.)	–
substrate step	200	μm
diffusion time step	10	s
initial glucose	2	g/l
initial pH	7.2	–
pH ₀	7.4	–
pH ₁	-0.0758	–
pH ₂	0.0004	–
glucose diffusivity	$6.7 \cdot 10^{-10}$	m^2/s
lactate diffusivity	$2.35 \cdot 10^{-10}$	m^2/s
temperature	8.0	$^{\circ}\text{C}$
water activity	0.997	–

Table 2: Bacteria Parameters

parameters	Listeria	Carnobacterium	units
shoving factor	1.3	1.3	–
opt. growth rate (μ_{opt})	1.12	0.898	h^{-1}
division radius	$1.5 \cdot 10^{-6}$	$1.0 \cdot 10^{-6}$	m
div. glu. consum.	$13.5 \cdot 10^{-15}$	$13.5 \cdot 10^{-15}$	Mole
lactate yield	0.18	1.8	–
pH _{min}	4.21	5.12	–
pH _{opt}	7.21	7.27	–
pH _{max}	10.07	10.24	–
t _{min}	0.6	-5.42	$^{\circ}\text{C}$
t _{opt}	37.4	31.6	$^{\circ}\text{C}$
t _{max}	45.3	36.5	$^{\circ}\text{C}$
aw _{min}	0.922	0.924	–
aw _{opt}	0.997	0.997	–
gamma pH exponent	1.68	7.77	–
Min inhib. concent.	6.4	0.48	mMole/l

Comparison of Two Spatial Distributions.

- **Case A:** we initialise our simulation with a packed configuration of 375 *C. piscicola* individuals. As depicted on Figure 5-A, all bacteria are uniformly distributed in a disk with diameter $c/10$ (the size of a bacterium is approximately $c * 10^{-4}$).
- **Case B:** we initialise our simulation with a homogeneous distribution of 375 *C. piscicola* individuals. As depicted on Figure 5-B, all bacteria are uniformly distributed on the virtual Petri dish sub-box with dimensions $c * c * 5 \text{ mm}^3$.

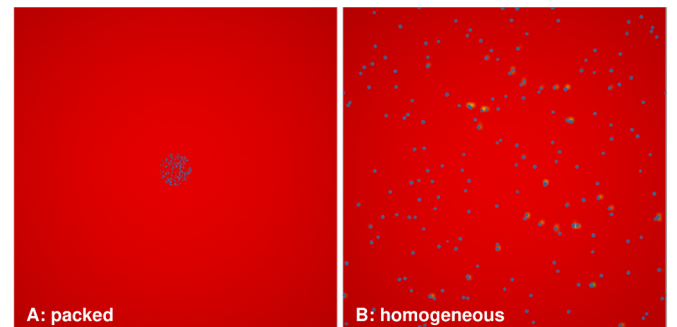


Figure 5: Two Spatial Distributions of *C. Piscicola*

Notice that the choice of 375 individuals on the sub-box considered here corresponds to 7500 CFU on a Petri dish which surface is about 20 times larger: these were the conditions of real data provided by the ADRIA laboratory.

Results.

These distinct spatial distributions highlight the importance of the spatial dimension for colony growth. Figure 6 shows how the same colony grows in drastically different ways, depending on its spatial distribution. These results are natural, since a high concentration of lactic bacteria induces a drastic decrease of pH, consequently a high self-inhibition.

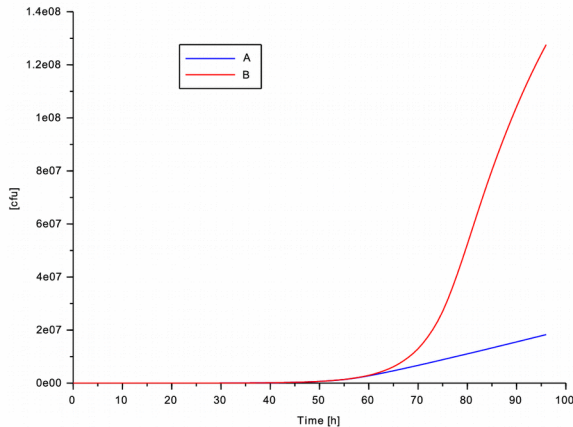


Figure 6: IBM Simulation of *C. Piscicola* Growth for Two Different Spatial Distributions (A: packed; B: homogeneous)

Impact of Spatialisation on Interacting Species

Comparison of Three Spatial Distributions.

- **Case A:** we initialise our simulation with a packed configuration of 375 *C. piscicola* individuals, and one *L. monocytogenes* individual. As depicted on Figure 7-A, *C. piscicola* bacteria are uniformly distributed in a disk with diameter $c/4$; in the middle of this disk stands a *L. monocytogenes* bacterium.
- **Case B:** we initialise our simulation with a packed distribution of 375 *C. piscicola* individuals, and one isolated *L. monocytogenes* individual. As depicted on Figure 7-B, *C. piscicola* bacteria are uniformly distributed in a disk with diameter $c/10$; this disk is placed in a corner of our virtual Petri dish subbox, in the opposite corner is placed a *L. monocytogenes* individual.
- **Case C:** our simulation is initialised with a homogeneous distribution of 375 *C. piscicola* individuals. As depicted on Figure 7-C, one *L. monocytogenes* individual is placed in the middle of the virtual Petri dish sub-box.

Results.

The results exposed for lactic acid bacteria without pathogens have emphasized the effect of spatial distribution on the self-inhibition mechanism. Figure 8 now illustrates how the inhibition of pathogens growth by lactic acid bacteria depends heavily on the spatial distributions of the different colonies. These results are once again natural: in case A, the inhibition is maximal, since the *L. monocytogenes* individuals stands where the lactic acid

concentration is maximal, whereas in case B, the situation is reversed, and case C is intermediate. Note that these results strongly depend on the lactic acid diffusion coefficient. In case B, if the acid produced by *C. piscicola* had been more fastly diffused, the *L. monocytogenes* colony would obviously have grown slower.

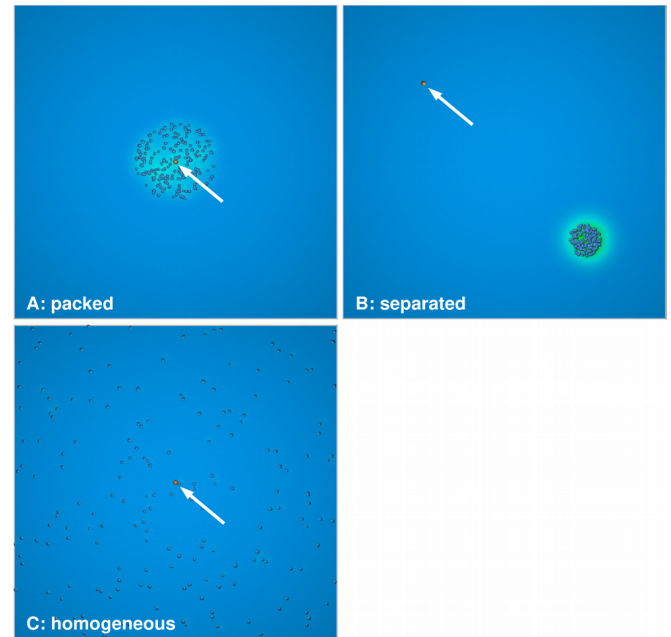


Figure 7: Three Spatial Distributions of *C. Piscicola* Around *L. Monocytogenes*

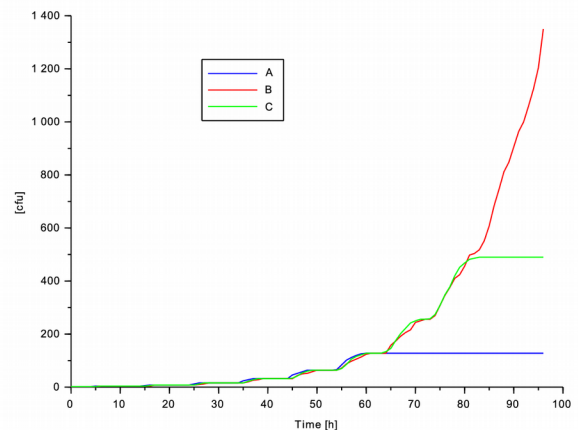


Figure 8: IBM Simulation of *L. Monocytogenes* Growth for Three Different Spatial Conditions Relative to *C. Piscicola* (A: packed; B: separated; C: homogeneous)

PERSPECTIVES

As described *supra*, the IBM we have developed is able to account for effects of spatialisation, such as diffusion, which the Cardinal models can obviously not consider. However, this IBM has a major flaw: its cost in terms of computation time can be huge when it comes to simulating a hundreds of millions of bacteria, particularly because of the collision process between individuals.

In order to supplement the IBM in cases where large colonies are formed and can be considered as “super

individuals”, but also in order to be provided with a tool for parameters estimation, we have built a partial derivative equation model (PDE), which is briefly described here: the validation process for this model is not completed yet, so that precise developments will be exposed in a future work.

To understand our PDE model, we give a simplified version, in which only one population of bacteria is considered. We denote by $\rho(x, y, t)$ its density at the point (x, y) and at time t , γ_0 the threshold, for the norm of the density gradient, beyond which the population starts to move. Therefore, we name *truncated gradient* the vector field:

$$\overline{\nabla\rho} = \begin{cases} \nabla\rho & \text{if } \|\nabla\rho\| \geq \gamma_0 \\ 0 & \text{if not} \end{cases}$$

The density flow is given by $\mathcal{O} = \rho V$ where V is the velocity field. We choose to consider that this velocity is proportional to the truncated gradient, rather than to the gradient, as usually used:

$$V = -K\overline{\nabla\rho}$$

This choice leads to a *modified transport equation*, that we complete by taking into account the population growth, here with a simple factor μ , so that we get:

$$\frac{\partial\rho}{\partial t} - K\nabla \cdot (\overline{\nabla\rho\rho}) = \mu\rho$$

We first checked that our continuous model could account for growth in layers, realistically, that is, in accordance with actual experiments. This verification was based on the work of (Su et al. 2012). We used the results exposed in this article to deduce our threshold for the truncation of the gradient norm: indeed, this threshold can simply be observed on the slope of a bacterial colony. We also used the same initial conditions as (Su et al. 2012), for instance sizes of bacteria and initial density.

Of course, this fitting with real growth-in-layers observations will have to be improved, and performed in our case of *C. piscicola* and *L. monocytogenes* colonies. However, the modest results exposed here seem rather promising.

CONCLUSION

We have presented an individual based model for bacteria colonies growth and inhibition by means of lactic acid production. This model enables a high level of description and a detailed co-construction of simulations by researchers from different domains. Even if we optimised the computing time of this IBM, when it comes to simulating hundreds of millions of bacteria, a partial derivative continuous model would enable faster simulations, but would suffer from a lack of accuracy in the description of local interactions. As a consequence, our aim is to make use of both IBM and PDE models. Although we have already built our PDE model, which gives in particular a realistic description of the layers which are observed in real growing colonies, we just briefly exposed it in this paper, since we have not completed its validation process.

Our next goal will be to make our IBM and PDE models involved in the same simulations, and interact in real time: the IBM will provide local precise results, transfer these results to the partial derivative equations model, which will fast give global results: these results will in turn be taken into account by the IBM.

Finally, the benefit of spatialisation which is provided by this IBM approach is currently exploited to investigate around the conditions leading to branched colony shapes in relation with local nutrient depletion.

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(provides simulation source code and data)