Evolution of biomass distribution during bacterial lag phase through flow cytometry, particle analysis and Individual-based Modelling

C. Prats*[1], J. Ferrer [1], B. Flix [1], A. Giró [1], D. López [1], J. Vives-Rego [2]
[1]Departament de Física i Enginyeria Nuclear, Escola Superior d'Agricultura de Barcelona, Universitat Politècnica de Catalunya
[2]Departament de Microbiologia, Facultat de Biologia, Universitat de Barcelona
*clara.prats@upc.edu http://mie.esab.upc.es/mosimbio

INTRODUCTION

The bacterial lag phase has been widely studied in recent decades due its importance in basic microbiology, biothecnology, microbial ecology and, more recently, predictive and descriptive food microbiology. Several mathematical models have been proposed, developed and tested to explain the behaviours observed under controlled conditions [1]. However, the lag phase is still poorly understood, probably due to the complexity of the systems under study. The biological bases underlying the bacterial lag are complex, and there is no standard model to cover them all. A major difficulty is identifying and establishing the relative importance of each of the factors affecting this interim. Prats et al. [2] made progress to this end, with the application of Individual-based Modelling (IbM) to the study of the lag phase.

The aim of this work is to validate IbM simulation results [2] using experimental data obtained by flow cytometry and particle analyzer [3,4,5]. We focus on the evolution of the bacterial biomass (or size) distribution during the lag phase.

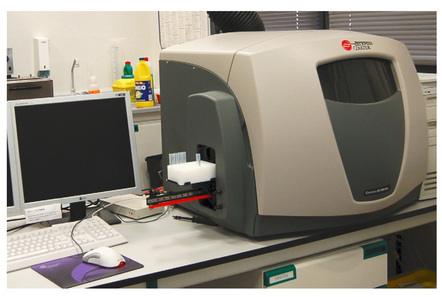
MATERIALS AND METHODS

Bacterial strain, media and culture conditions:

Experiments were performed with E. coli CECT 101 (Colección Española de Cultivos Tipo, Spain). Bacteria were grown in M9 medium consisting of part A) per liter, Na2HPO4, 6g, KH2PO4, 3g, NaCl, 0.5 g, NH4Cl, 1g; part B) a 1 M solution of MgSO4.7H2O; and part C) a 0.01 M solution of CaCl2. Parts A, B and C were autoclaved separately. 1 ml per liter of part B and 10 ml of part C were added to part A. Sterilized glucose by filtration was added at 0.5 g per liter. Final pH was 7.5. Cultures were incubated at 35°C with shaking at 150 r.p.m. Inoculums of 0.5% in volume were sampled from overnight cultures and added to fresh medium under the same conditions.

Experimental setup:

Flow cytometric experiments were carried out using a Beckman coulter FC cytomics 500 MPL flow cytometer (ref.: 20061121, Coulter Corp., Miami, Florida). A standard 488nm air-cooled argon-ion laser at 15mW power was used. Cell size distribution was determined with a Multisizer II electronic particle analyzer (Coulter Corp.).



FC cytomics 500

Multisizer II

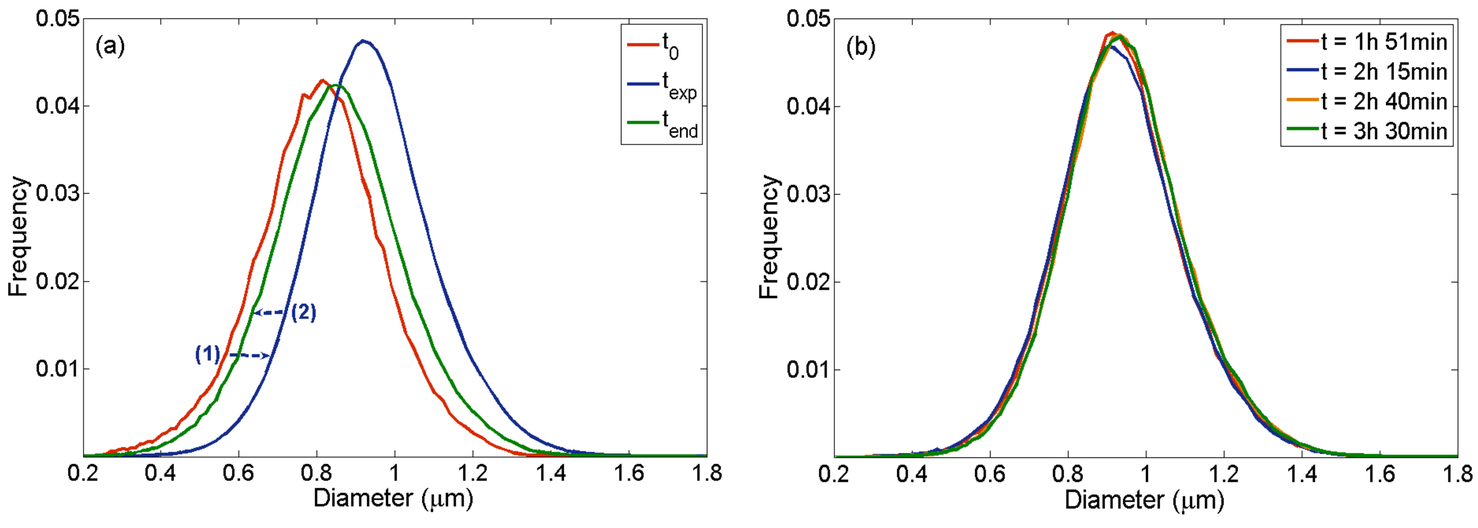
Model and simulations:

INDividual DIScrete SIMulation (INDISIM), the model and simulations used to address the lag phase, was presented in Prats et al. [2]. A more generic version of this IbM may be found in Ginovart et al. [6]. In the current model explicit genetic and biochemichal diversity is excluded. However, randomness is introduced when setting individual variables to implicitly take diversity into account. Individual size is assumed to be poorly affected by the genetic modifications occurring during the evolution of the culture. Experimental results are compared to the simulations of Prats et al.[2].

RESULTS AND DISCUSSION

EVOLUTION OF THE SIZE DISTRIBUTIONS

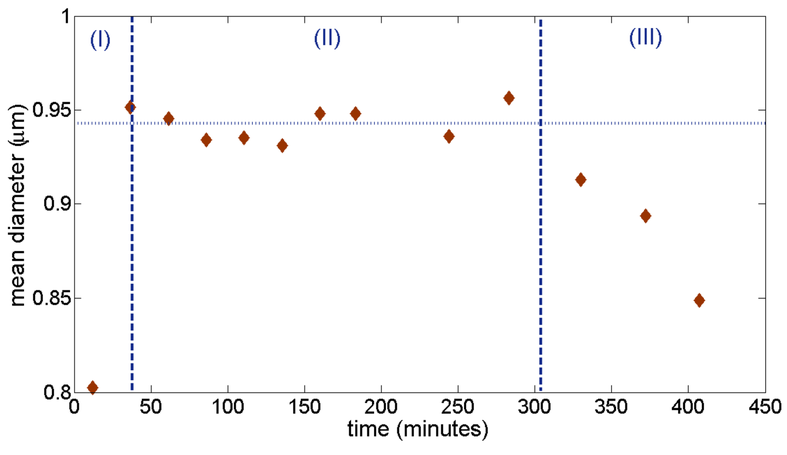
Lag, exponential and stationary phases are detected. During the lag phase, an upward shift of size distributions is observed. Lag phase lasts 38 minutes. The exponential phase is characterized by a set of nearly unchanging size distributions, showing the characteristics of a balanced growth. A downward shift in the size distribution is found as the culture enters the stationary phase.



(a) Sample of the inoculation time (t_0), mean distribution of the exponential phase (t_{exp}) and sample of the end of growth (t_{end}). The arrows indicate the displacements (1) from lag to exponential and (2) from exponential to stationary phases. (b) Four samples of the exponential phase.

EVOLUTION OF THE MEAN DIAMETER

The initial mean diameter is quite small, since the inoculum is taken from an overnight culture (stationary phase). Then, it increases and remains more or less constant during the exponential phase. During the stationary phase, there is a decrease in this value. Therefore, it will increase again when a sample of the culture is taken and inoculated into fresh medium before reaching the exponential growth.



Mean diameter evolution, from FS measurements. Dashed lines indicate the lag (I), exponential (II) and stationary (III) phases.

SIMULATION PREDICTIONS AND EXPERIMENTAL RESULTS

A new system variable was defined and presented by Prats et al. [2], in order to assess the evolution of biomass distribution:

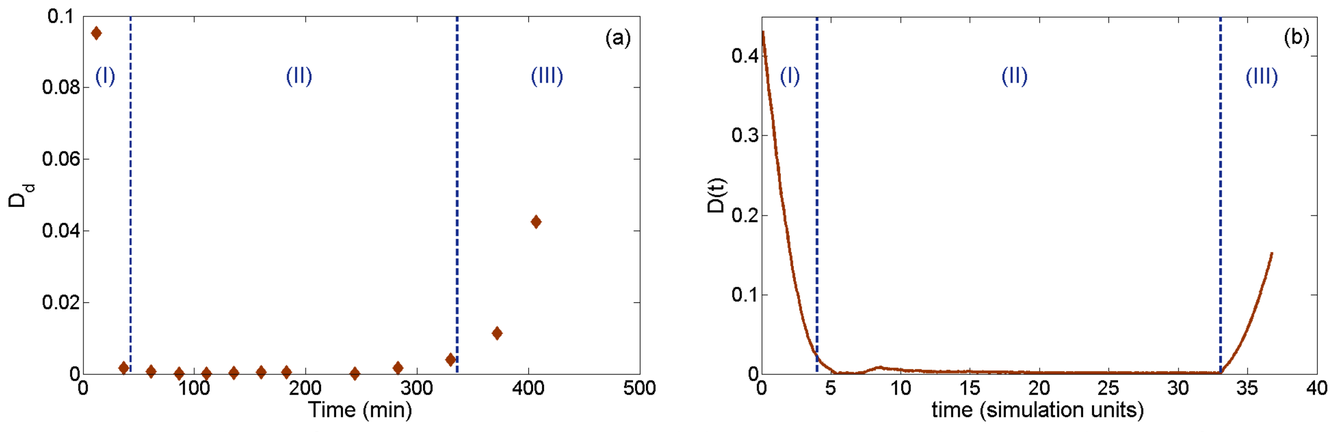
$$D_P(t) = \frac{\left|\bar{d}(t) - \bar{d}_{\exp}\right|}{\bar{d}_{\exp}} \cdot \sum_{k=1}^{N} \left|p_k(t) - p_{k,\exp}\right|$$

$\bar{d}$ Mean bacterial diameter of the culture

p_k Frequency of the diameter interval k, from the diameter distribution.

(t=instantaneous, exp=exponential phase)

This variable quantifies the evolution of size distributions toward the characteristic stable distribution of the exponential phase. The closer the distributions, the lower the distance values.



(a) D_P evolution for FS measurements, and (b) INDISIM simulation prediction of the D_P evolution. Dashed lines indicate the lag (I), exponential (II) and stationary (III) phases

These results reflect the dynamic behaviour of the culture during the different growth phases. The stability of the distribution during the exponential phase shows that it is in balanced growth. Any displacement of the distribution is an indicator of some changes in the culture's evolution. Therefore, it is a good tool to study transitory states like the lag phase.

REFERENCES

[1] Swinnen et al., 2004. *Int. J. Food Microbiol.* **94**, 137-159.
[2] Prats et al., 2006. *J. Theor Biol.* **241**, 939-953.
[3] Julià et al., 2000. *J. Microbiol. Meth.* **40**, 57-61.
[4] Vives-Rego et al., 2003. *J. Microbiol. Meth.* **53**, 43-50.
[5] Julià et al., 2005. *Microbiology* **151**, 749-755.
[6] Ginovart et al., 2002. *J. Theor. Biol.* **214**, 305-319.

5th International Conference Predictive Modelling in Foods

September 16-19, 2007 (ATHENS-GREECE)

ACKNOWLEDGEMENTS

We are extremely grateful to Rosa Carbó (UPC), Jaume Comas-Riu, Ricard López and Chary González Flores (Scientific and Technical Services of the University of Barcelona) for their helpful technical assistance. We gratefully acknowledge the financial support of the European Social Fund and AGAUR-Generalitat de Catalunya 2007FIC-00941, and the Plan Nacional I+D+i of the Ministerio de Educación y Ciencia CGL 2004-01144.

UNIVERSITAT DE BARCELONA
Parc Científic de Barcelona
UPC