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SYSTEM ANALYSIS OF METABOLISM IN *HELICOBACTER PYLORI*

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ABSTRACT

Systems biology integrates different levels of information for understanding biological systems. The availability of the genome sequence of *Helicobacter pylori* has allowed the construction of a genome-scale metabolic model for this organism.

In order to study the behaviour of *H. pylori* and understand the mechanisms associated with infection using systems biology tools and controlled cultivation conditions, fermentations in a chemically defined medium were performed and several conditions were tested. The experimental data obtained were compared with simulated data generated by the existing model.

The simultaneous use of both approaches allows to correct the *in* silico model and, on the other hand, to rationally adjust the medium components. The improvement of the genome-scale metabolic model will allow the identification of potential targets in order to design more effective drugs for the inactivation of *H. pylori.*

INTRODUCTION

Helicobacter pylori is a pathogenic organism associated with chronic gastritis, peptic ulcers and gastric cancer (Blaser and Atherton 2004). More than 50% of the global population is infected with this bacterium (Kusters et al. 2006).

Systems biology is a new discipline which integrates different levels of information for understanding biological systems (Kitano 2002). Genomic information, combined with biochemical knowledge made possible the development of metabolic models for several organisms, such as *H. pylori* (Thiele et al. 2005). This model can be used to assess the organism's metabolic capabilities and fitness. However, the quality of the predictions obtained with this model has been hampered by the lack of physiological data for this organism.

OptFlux is an open-source software platform for metabolic engineering applications, which allows the use of stoichiometric models of microbial metabolism for simulation and optimization. For that purposes, OptFlux includes several tools and algorithms such as Flux Balance Analysis (FBA), among others (Rocha et al. 2010) FBA is based on the principles of constraints-based analysis and can make quantitative predictions even in the absence of detailed kinetic information (Edwards et al. 2002).

The main aims of this work are to design reproducible and reliable protocols for the controlled growth of *H. pylori* and to obtain a reliable genome-scale metabolic model of this organism.

METHODS

Growth Conditions

Initially several conditions were tested, such as: volume of medium/flask volume, concentration of supplement (fetal bovine serum), antibiotics and type of agitation. After selection of the appropriate conditions, *H. pylori* was grown in Erlenmeyer flasks with Ham's F-12 nutrient mixture (defined medium) supplemented with fetal bovine serum. All growth experiments were performed at 37ºC under microaerophilic conditions. For assessing the growth optical density at 600 nm, counting of CFU/mL and cell dry weight assays were performed.

Model Simulation

The model simulation was based on a metabolic reconstruction made by Thiele *et al.* with 341 genes, 476 intracellular reactions, 78 exchange reactions and 485 metabolites. The biomass equation in this model was adapted from the biomass composition of *Escherichia coli.* OptFlux was employed to simulate the wild type behaviour of *H. pylori* using FBA in different *in silico* media and in the conditions used *in vivo*. The simulation results were compared with experimental data.

RESULTS

The results obtained for the growth of *H.pylori* with Ham's F-12 in the selected growth conditions are showed in Figure 1.

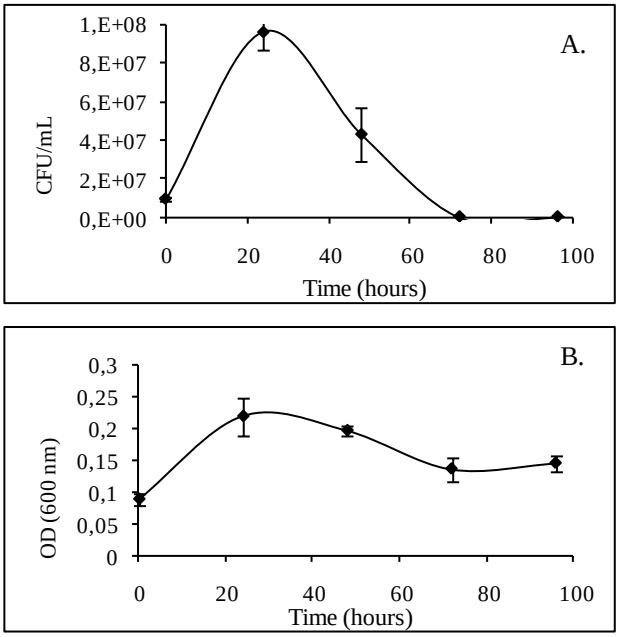

Figure 1: Growth of *H. pylori* in Ham's F-12, under selected conditions. A. Optical density measured at 600nm along the time; B.Colony- forming units per milliliter along the time.

The results obtained by simulation of the wild type strain using the existing model reveals no bacterial growth in F-12 medium, contrasting with experimental data (Table 1 and Figure1). Analyzing the composition of the medium used in *in vivo* and *in silico* experiments, pimelate is lacking in Ham's F-12, although this medium has biotin which is synthesized from pimeloyl-CoA, which sequentially is synthesized from pimelate in some microorganisms (Streit and Entcheva 2003). In Gram-negative bacteria, the precursors of pimeloyl-CoA are not known. When pimelate is added to the *in silico* model, the specific growth rate value is set to 2.43h^{-1} (Table 1).

Table 1: Simulation of wild type behaviour of *H. pylori* in Ham's F-12 with the genome-scale metabolic model reconstructed by Thiele *et al.*

	Simulations with the medium used *in vivo*		Experimental data
	Without the flux of pimelate	With the flux of pimelate	
Biomass (h^{-1})	0.00	2.43	0.037

CONCLUSIONS

The comparison of the simulation output with the experimental data of bacterial growth in Ham's F-12, reveals that the metabolic model used does not mimic the *in vivo* conditions.

In the future work data from analysis of exometabolome in GC/MS will be used to help to adjust the components of the medium.

In order to improve the quality of the *in silico* predictions, possible "unrealistic" reactions in the metabolic model should be identified and modified.

The aim of our future work is not only to improve the genome-scale metabolic model, but also to identify potential targets for designing more effective drugs for the inactivation of *H. pylori.*

REFERENCES

Blaser, M.J. and J.C. Atherton. 2004. "Helicobacter pylori persistence: biology and disease." *Journal of Clinical Investigation* 113, 321-333.

Edwards, J.S.; M. Covert; and B. Palsson. 2002. "Metabolic modelling of microbes: the flux-balance approach." *Environmental Microbiology* 4, 133-140.

Kitano, H. 2002. "Systems biology: A brief overview." *Science* 295, 1662-1664.

Kusters, J.G.; A.H.M. Van Vliet; and E.J. Kuipers. 2006. "Pathogenesis of *Helicobacter pylori* Infection." *Clinical Microbiology Reviews* 19, No.3, 449-490.

Rocha, I.; P. Maia; P. Evangelista; P. Vilaça; S. Soares; J.P. Pinto; J. Nielsen; K.R. Patil; E.C. Ferreira; and M. Rocha. 2010. "OptFlux: an open-source software platform for *in silico* metabolic engineering." *BMC Systems Biology* 2010, 4-45.

Streit, W. R. and P. Entcheva. 2003. "Biotin in microbes, the genes involved in its biosynthesis, its biochemical role and perspectives for biotechnological production." *Applied Microbiology and Biotechnol*ogy 61, 21-31.

Thiele, I.; T.D. Vo; and B.O. Palsson. 2005. "Expanded metabolic reconstruction of Helicobacter pylori (iIT341 GSM/GPR): an *in silico* genome-scale characterization of single- and double-deletion mutants." *Journal of Bacteriology* 187, 5818-5830.

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