Universidade do Minho
Escola de Engenharia

Semana da Escola de Engenharia
October 24 - 27, 2011

GROWTH ASSESSMENT OF *HELICOBACTER PYLORI* IN LIQUID MEDIUM – EFFECT OF AGGREGATION

Daniela M. Correia[1], Lucinda J. Bessa[2], Nuno F. Azevedo[3], Maria João Vieira[1], Isabel Rocha[1]
[1] Centre of Biological Engineering, University of Minho; [2]Department of Biomedical Sciences, University "G. d'Annunzio", Chieti-Pescara, Italy; [3] LEPAE, Department of Chemical Engineering, Faculty of Engineering, University of Porto
E-mail: danielacorreia@deb.uminho.pt

KEYWORDS

Helicobacter pylori; growth, aggregation

ABSTRACT

Helicobacter pylori is a pathogenic organism associated with gastric diseases. It is described that *H. pylori* can change morphology when exposed to adverse conditions and *H. pylori* cells can aggregate in clusters when in liquid culture. Such phenomenon makes it difficult to assess growth using the conventional methods. The development of robust methods to assess growth in a more reliable way is needed. In the present work a method that allows efficient cell disaggregation was developed.

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).

The discovery of mechanisms associated with *H. pylori* infection has been hampered by the lack of physiological data and consequently, many aspects related with the appearance of diseases remain unclear.

It is described that *H. pylori* can change cell morphology from spiral to coccoid form when exposed to adverse conditions, such as: aerobiosis, alkaline pH, high temperature, extended incubation, or treatment with antibiotics (Mizoguchi et al. 1998). *H. pylori* cell-to-cell aggregation in liquid culture in complex and defined media was described by some authors (Donelli et al. 1998; Williams et al. 2008). It is reported that this phenomenon is more noticeable when the oxygen is scarce (Donelli et al. 1998). The formation of large cell aggregates in Ham's F-12 medium supplemented with 1% of fetal bovine serum was reported (Williams et al. 2008).

The development of robust methods to grow this bacterium and reliable methods for the assessment of growth are needed for a better characterization of its physiology. In previous experiments, the cell-to-cell aggregation phenomenon was observed on Ham's F-12 nutrient mixture, making difficult to assess the growth of *H. pylori* using the conventional methods such as cultivable cell counts and total cell counts using fluorochromes. As such, the purpose of this work was to study *H. pylori* growth in this chemically defined medium, giving special relevance to clusters formation and to the effort of developing a method that allows to disaggregate cell clusters in order to achieve a more reliable assessment of growth.

METHODS

H. pylori was grown in 1L Erlenmeyer flasks with 150 mL of Ham's F-12 nutrient mixture (defined medium) supplemented with fetal bovine serum. The experiments were performed at 37ºC under microaerophilic conditions. Samples were collected until 72 hours. At each time, after collecting the sample, 1 mL of culture was vortexed during 5 minutes in an eppendorf tube with 8-10 glass beads. During method development different number of glass beads and times of shaking were tested.

For the assessment of growth, optical density at 600 nm, cultivable cell counts and total cell counts using 4',6-diamidino-2-phenylindole (DAPI) staining were performed. Cell counts were assessed using an

epifluorescence microscope. To evaluate the efficiency of the method developed to disaggregate cells, samples were observed by phase contrast microscopy.

RESULTS

The results obtained for the growth of *H.pylori* in Ham's F-12 with the growth conditions used, before and after the application of the disaggregation method developed, are showed in Figure 1.

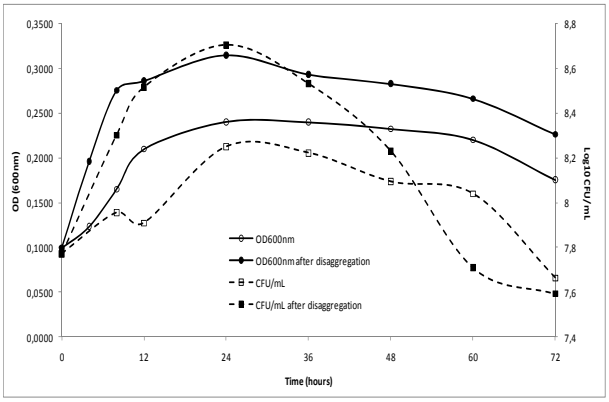

Figure 1: Growth of *H. pylori* along the time. Optical density at 600nm and cultivable cell counts (CFU/mL).

The formation of cell clusters affects the assessment of growth, as shown by the increase of optical density and cultivable cell counts after cells disaggregation (Figure1).

The total cell counts were not possible to perform without cell disaggregation, as the clusters increase the difficulty of observing and identifying individual cells. The number and size of clusters increased along the time, and is more evident when the cells are in coccoid form, as reported by Donelli in 1998.

Different methods described in the literature for cells disaggregation (mechanical and chemical) were tested, but all tested methods failed to disaggregate cells or led to a high percentage of viability loss.

CONCLUSIONS

In the present work a method that allows assessing *Helicobacter pylori* growth in liquid culture in a more robust way using the conventional methods to evaluate growth was developed.

REFERENCES

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

Donelli, G.; P. Matarrese; C. Fiorentini; B. Dainelli; T. Taraborelli; E. Di Campli; S. Di Bartolomeo and L. Cellini. 1998. "The effect of oxygen on growth and cell morphology of *Helicobacter pylori*." *FEMS Microbiology Letters* 168, 9-15.

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.

Misoguchi, H.; T. Fujioja; K. Kishi; A. Nishizono; R. Kodama and M. Nasu. 1998. "Diversity in protein synthesis and viability of *Helicobacter pylori* coccoid forms in response to various stimuli." *Infection and Immunity* 66, No.11, 5555-5560.

Williams, J.C.; K.A. McInnis and T.L. Testerman. 2008. "Adherence of *Helicobacter pylori* to Abiotic Surfaces is influenced by Serum" *Applied and Environmental Microbiology* 74, No. 4, 1255-1258.

ACKNOWLEDGEMENTS

This work was supported by Fundação para a Ciência e Tecnologia (FCT) within Daniela Correia PhD Grant (SFRH/BD/47596/2008) and HeliSysBio Project (PTDC/EBB-EBI/104235/2008).

AUTHOR BIOGRAPHY

DANIELA M. CORREIA was born in Santo Tirso, Portugal. She went to Instituto Politécnico de Bragança where she studied Biotechnological Engineering and obtained her degree in 2003. Between 2003 and 2008, she worked in the same institution in Research Projects in the areas of Analytical Chemistry and Fermentations. In 2008 obtained her master degree in Quality and Food Safety. In 2009 she moved to the University of Minho where she started a PhD in Chemical and Biological Engineering. Her e-mail address is: danielacorreia@deb.uminho.pt