Growth assessment methods for *Helicobacter pylori* in liquid medium

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Helicobacter pylori is known to be associated with chronic gastritis, peptic ulcers and gastric cancer. The lack of physiological data has hampered the uncover of mechanisms associated with *H. pylori* infection and consequently, many aspects related with the appearance of diseases remain unclear.

It is well known that *H. pylori* can change cell morphology from spiral to coccoid form when exposed to adverse conditions. Some authors have reported the existence of a viable but nonculturable state of this bacterium. 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. As such, the purpose of this work was to study *H. pylori* growth in a chemically defined medium, compare different methods to assess the growth and observe the changes of morphology.

Cultures were grown at 37ºC under controlled conditions in Ham´s F-12 medium supplemented with fetal bovine serum. Samples were collected until 72 hours. For growth assessment, the following methods were used and compared: optical density, cultivable cell counts, total cell counts using DAPI staining, evaluation of viability with the Live/Dead viability kit and a PNA FISH probe which evaluates the content of stable rRNA. Cell counts and analysis of cell morphology were assessed using an epifluorescence microscope.

Under the conditions of atmospheric oxygen 6.5%, pH 7, and shaking speed 110 rpm, *H. pylori* was in exponential growth from 0 to 4 hours. In comparison to total counts, PNA FISH displayed, in general, lower counts, particularly after cells have reached the stationary phase. Changes in morphology and viability were observed. After 60 hours of culture cells were mainly coccoid and nonviable.

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