Is *Helicobacter pylori* a True Microaerophile?

Stephanie Bury-Moné,[*§] Nadeem O. Kaakoush,[†] Corinne Asencio,[‡] Francis Mégraud,[‡] Marie Thibonnier,[*] Hilde De Reuse[*] and George L. Mendz[†]

[*]Unité de Pathogénie Bactérienne des Muqueuses, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15, France, [†]School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales 2052, Australia, [‡]Laboratoire de Bactériologie, Université Victor Segalen Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux cedex, France

Keywords
Helicobacter, microaerophily, capnophily, oxygen tolerance, carbon dioxide, oxidoreductase.

Reprint requests to: Dr Hilde De Reuse, Unité de Pathogénie Bactérienne des Muqueuses, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15, France. Tel.: +33-1-40-61-36-41; Fax: +33-1-40-61-36-40; E-mail: hdereuse@pasteur.fr

Professor George L. Mendz, School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW 2052, Australia. Tel.: +61-2-9385-2042; Fax: +61-2-9385-1043; E-mail: G.Mendz@unsw.edu.au

[§]Present address: Laboratoire Signalisation et Réseaux de Régulation Bactériens, Université Paris-Sud, Orsay cedex 91405, France.

Abstract

Background: There is no general consensus about the specific oxygen and carbon dioxide requirements of the human pathogen *Helicobacter pylori*. This bacterium is considered a microaerophile and consequently, it is grown under atmospheres at oxygen tensions 5–19% and carbon dioxide tensions 5–10%, both for clinical and basic and applied research purposes. The current study compared the growth of *H. pylori* in vitro, under various gas atmospheres, and determined some specific changes in the physiology of bacteria grown under different oxygen partial pressures.

Methods: Measurements of bacterial growth under various conditions were carried out employing classical solid and liquid culture techniques. Enzymatic activities were measured using spectrophotometric assays.

Results: *H. pylori* and all the other *Helicobacter* spp. tested had an absolute requirement for elevated carbon dioxide concentrations in the growth atmosphere. In contrast with other *Helicobacter* spp., *H. pylori* can tolerate elevated oxygen tensions when grown at high bacterial concentrations. Under 5% CO_2, the bacterium showed similar growth in liquid cultures under oxygen tensions from microaerobic (< 5%) to fully aerobic (21%) at cell densities higher than 5×10^5 cfu/ml for media supplemented with horse serum and 5×10^7 cfu/ml for media supplemented with β-cyclodextrin. Evidence that changes occurred in the physiology of *H. pylori* was obtained by comparing the activities of ferredoxin:NADH (nicotinamide adenine dinucleotide) oxidoreductases of bacteria grown under microaerobic and aerobic atmospheres.

Conclusions: *H. pylori* is a capnophile able to grow equally well in vitro under microaerobic or aerobic conditions at high bacterial concentrations, and behaved like oxygen-sensitive microaerophiles at low cell densities. Some characteristics of *H. pylori* cells grown in vitro under microaerobic conditions appeared to mimic better the physiology of organisms grown in their natural niche in the human stomach.

Oxygen plays a critical role in the life of many microorganisms. Its high redox potential and ubiquity make it a very common electron acceptor in cellular metabolism. At the same time many products or by-products of oxygen reactions are toxic, and organisms have developed complex resistance mechanisms against them. Criteria on the requirement, use, and need for oxygen, as well as resistance to reactive oxygen species (ROS), have been employed to classify microorganisms into five major groups [1]. These are: 1, strict aerobes that need high O_2 tensions (pO_2) for growth, and grow and proliferate at pO_2 of 21%, e.g., some *Bacillus* spp.; 2, microaerophiles that need O_2 for growth but are sensitive to atmospheric oxygen tensions, and grow best at pO_2 of 5–10%, e.g., some *Campylobacter* spp.; 3, facultative anaerobes that use and tolerate O_2 but can grow without oxygen, e.g., some Enterobacteria; 4, aerotolerant anaerobes that do not use O_2, but tolerate it owing to resistance mechanisms to ROS, e.g., some lactic bacteria; and 5, strict anaerobes that do not use O_2, are unable to survive long exposures to oxygen, and grow only at a $pO_2 < 0.5–2\%$, e.g., *Clostridium* spp.

Microorganisms such as *Neisseria* spp. that need an atmosphere with an elevated carbon dioxide partial pressure of 5–10% for growth are defined as capnophilic or capneic. Microaerophilic microorganisms are generally capnophilic and their sensitivity to high pO_2 is caused by oxygen-dependent inactivation of some vital cellular components such as enzymes involved in redox reactions [2].

Microaerophiles are widely spread in the environment and in living hosts and present a broad metabolic diversity. It is possible to group them into three categories: 1, obligate microaerophiles that require low oxygen tensions for growth, such as some deep-sea marine bacteria; 2, microaerophiles that can grow aerobically only under certain conditions, such as the nitrogen-fixing bacteria *Azospirillum* spp. or *Rhizobium* spp.; and 3, facultative microaerophiles that have anaerobic metabolism and phenotype but can grow at low pO_2, such as sulfur-reducing bacteria [3] and some anaerobic phototrophes [4]. Some obligate microaerophiles live at the limit between oxic and anoxic environments, because they use O_2 as an electron acceptor and products of anaerobic metabolism as electron donors, for example, aquatic chemolithotroph and chemoorganotroph bacteria such as *Gallionella ferruginea* and *Aquaspirillum magnetotacticum,* respectively [1,2]. Microaerophiles are found also among pathogenic microorganisms including protozoans such as *Treponema pallidum*, *Plasmodium falciparum*, *Giardia* spp., and bacteria such as *Borrelia* spp., *Campylobacter* spp. and *Helicobacter* spp. [2,5,6]. An important step to combat these infections is to understand their physiology.

The Gram-negative bacterium *Helicobacter pylori* colonizes the human stomach and its presence is associated with the development of gastritis, ulcers, and gastric cancers. Since its discovery in 1983 [7], it has been considered a microaerophilic microorganism [6].

The sensitivity of *H. pylori* to oxygen has been reported in a number of studies and has been debated for considerable time [8,9]. The bacterium is equipped against oxidative stress with enzymes such as superoxide dismutase, peroxidases, and a highly active catalase [10,11]. At the same time, published data suggest that *H. pylori* may not be too sensitive to high pO_2. In several laboratories *H. pylori* strains are grown in regular 5–10% CO_2-air incubators, in atmospheres containing between 19 and 20% O_2 [12–15]. The bacterial viability of *H. pylori* cocultured with macrophages in a CO_2-air incubator was not affected after 24 h incubation [16]. Similarly, there are several reports of *H. pylori* strains cultivated under or adapted to high oxygen tensions [8,9,17–21].

Analyses of *H. pylori* enzymes and metabolic pathways reveal interesting relations between *H. pylori* and oxygen tolerance. The susceptibility to metronidazole, the presence of fumarate reductase, menaquinones, POR, and OOR oxoacid:acceptor oxidoreductases, a terminal cbb_3-type cytochrome *c* oxidase, as well as an unusual citric acid cycle, point to aspects of the physiology of *H. pylori* that are in common with organisms of reduced tolerance for oxygen. Also, the bacterium can readily modulate the expression of oxygen-resistance factors as a compensatory response to the loss of a major oxidative-stress resistance component [22].

H. pylori is susceptible to metronidazole, an antibiotic active against anaerobic microorganisms. This susceptibility is associated with an NADPH nitroreductase (RdxA) and NAD(P)H:flavin oxidoreductase (FrxA) [23–25], suggesting mechanisms different from those described in anaerobes. The fact that *H. pylori* is not able to grow under anoxic conditions ([26,27] and our unpublished observations) even in the presence of additional fumarate [28] suggests that anaerobic respiration and fermentation are not sufficient to support the growth of the bacterium despite of its active fumarate reductase [29]. The POR and OOR oxidoreductases are oxygen-sensitive essential enzymes of *H. pylori* that usually are found in anaerobic metabolism, but able to function under oxic conditions provided they have sufficient protection from ROS [30]. The cbb_3-type cytochrome *c* oxidase of *H. pylori* belongs to a family of terminal oxidases often associated with microaerophilic bacteria. However, the affinity of the *H. pylori* enzyme (K_M = 0.4 µmol/l) for O_2 is significantly lower [31] than that of bacteria requiring microoxic conditions that have K_M in the nanomolar range [1,11,32]. Previous data confirmed that the citric acid cycle of *H. pylori* is atypical and may function oxidatively like that of *Escherichia coli* under oxic conditions [33].

The present study addresses the status of *H. pylori* as a microaerophile by comparing the growth of several strains in various media under microoxic and oxic atmospheres enriched with CO_2, and by measuring the kinetics of NADH (nicotinamide adenine dinucleotide):ferredoxin oxidoreductase of bacteria grown under the same microoxic and oxic atmospheres.

Methods

Materials

Blood Agar Base no. 2, defibrinated horse blood, and brain heart infusion (BHI) broth were from Oxoid (Heilderberg, Victoria, Australia), and vancomycin from Eli Lilly (North Ryde, NSW, Australia). Gas generating Campigen and CO_2Gen packs were from Oxoid. Polymyxin B, trimethoprim, amphotericin, bovine serum albumin, β-cyclodextrin, NADH, and spinach ferredoxin were purchased from Sigma (St Louis, MO, USA). All other reagents were of analytical grade.

Table 1 The different atmospheres employed in this study

Atmospheric condition[a]	% O_2	% CO_2
atm.1	20–19	5–10
atm.2	6	10
atm.3	15	6
atm.4	21	5
atm.5	5	5

[a]The gas balance was achieved using N_2 gas.

Bacterial Strains and Growth Conditions

H. pylori strains 26695, J99, N6, NCTC 11639, and SS1, and low-passage isolates BLE 107, LC 11, and LC 20 from patients with gastritis are from the University of NSW culture collection, and CAS 015, HER 126, and RIG 117 from the Université Bordeaux II collection, were grown at 37 °C under the following atmospheres: atm.1: 5 or 10% CO_2 in air, in regular CO_2 incubators; atm.2: 10% CO_2, 6% O_2, in jars with gas generating Campigen packs (Oxoid, Thebarton, Australia); atm.3: 6% CO_2, 15% O_2 in jars with gas generating CO_2Gen packs; atm.4: 5% CO_2, 21% O_2 in a Sanyo Tri-Carb incubator (Quantum Scientific, Paddington, Australia); or atm.5: 5% CO_2, 5% O_2 in a Sanyo Tri-Carb incubator (Table 1). The low-passage isolates used in this work have been grown always under microaerobic conditions. Some cultures of the laboratory adapted strains have been grown always under microaerobic conditions, and some under both aerobic and microaerobic atmospheres.

The *H. pylori* strains mentioned above as well as *Helicobacter bilis* strain ATCC 51630 and *Helicobacter hepaticus* strain ATCC 51449 were grown under microaerobic or aerobic atmospheres on plates for 24 h at 37 °C on Campylobacter Selective Agar consisting of Blood Agar Base no. 2, containing 5% defibrinated horse blood, 5 μg/l vancomycin, 2.5 IU/ml; polymyxin B, 2.5 μg/l; trimethoprim, and 2 μg/l amphotericin.

For growth experiments in liquid cultures, bacterial cells were harvested from plates and inoculated into BHI broth supplemented with either 0.2% β-cyclodextrin [34] or 10% horse serum [12]. The growth media were dispensed on shallow layers in vented culture flasks and allowed to equilibrate with the appropriate atmosphere by gentle shaking overnight. Bacterial growth in cultures shaken gently was determined at different time points up to 60 hours by either measuring the optical density of the cell suspensions at 600 nm, or counting the number of colony forming units (cfu/ml) by the method of Miles and Misra [35]. Bacteria were grown also in 24-well cell-culture plates with cultures separated into two compartments using Nunc 25 mm transwell inserts with 0.2 μm anapore membranes (Medos, Mt Waverley, Victoria, Australia).

Enzyme Assays

The kinetics of NADH:ferredoxin oxidoreductase for several strains were determined in cell-free extracts by measuring spectrophotometrically at 340 nm and 25 °C the rates of decrease of NADH levels in the presence of ferredoxin. The assay mixture consisted of Tris-HCl (20 mmol/l, pH 7.4), 0.15 mmol/l NADH, and ferredoxin at concentrations between 2.2 and 130 μg/ml. The method was validated by establishing that no oxidation of NADH took place in the absence of cell-free extracts. NADH oxidation was observed in suspensions of extracts in the absence of ferredoxin. Ferredoxin oxidoreductase rates were calculated for each sample by subtracting the rate of NADH oxidation in the absence of ferredoxin from the value measured in the presence of ferredoxin.

Protein Assays

Protein concentrations were determined by the bicinchoninic acid method based on a microtitre plate protocol (Pierce Chemical Co., IL, USA) using bovine serum albumin as a standard.

Results

Five strains and six isolates of *H. pylori* showed similar growth on blood agar plates after 24 h incubation under the aerobic and microaerobic conditions atm.1 and atm.2, respectively (Table 1). In contrast, *H. bilis* and *H. hepaticus* grew only under microaerobic conditions (data not shown). *H. pylori* strains and isolates were grown at 37 °C in BHI broth supplemented with 10% horse serum under conditions atm.4 and atm.5. Bacterial growth in the cultures was determined at 0, 4, 8, 12, 18, and 24 hours by counting the number of cfus at each time point. The strains and isolates grew similarly under both atmospheres; and in the absence of CO_2 no growth was observed under the same oxygen tensions (data not shown). The experiments were repeated three times with qualitatively the same results. Figure 1 shows the data corresponding to one such experiment for strains J99, LC11, and LC20.

H. pylori strains 26695, SS1, and N6 were grown at 37 °C in BHI liquid medium supplemented with 0.2% β-cyclodextrin in jars under the conditions atm.2 and atm.3. Cell growth in the cultures was determined at 0, 5, 10, 15, 24, and 50 h, by measuring the optical density of the cell suspensions at 600 nm. The experiments were repeated at least three times with independent cell cultures. Similar growth curves were obtained for each strain under the two different atmospheres when cultures were inoculated at high density (0.1–0.2 OD at 600 nm, approximately to 5×10^7–10^8 cfu/ml) (Fig. 2). The cells from cultures under

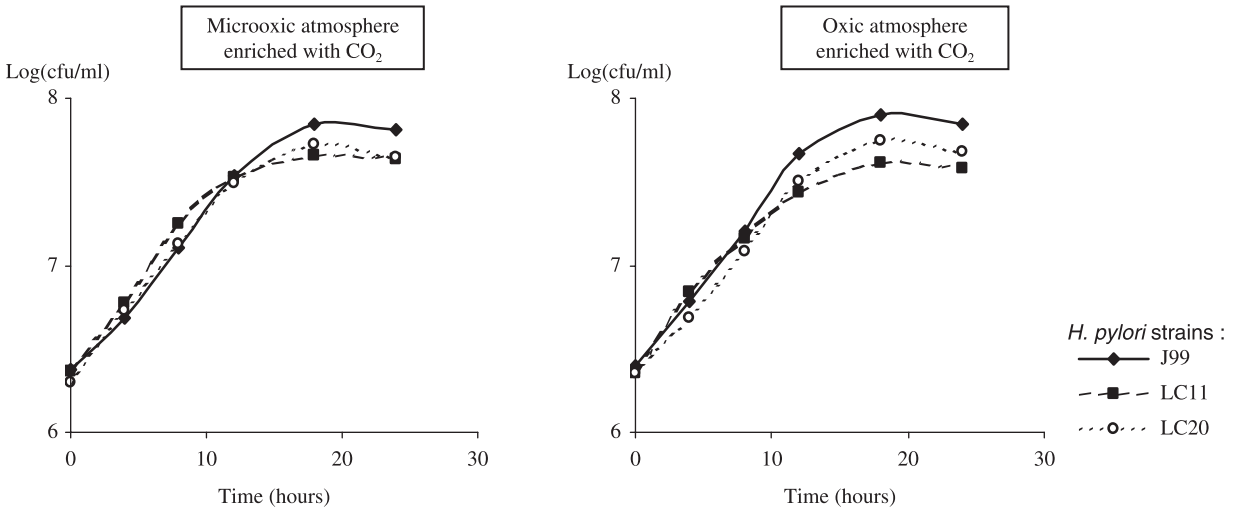

Figure 1 Growth curves of *H. pylori* J99 strain and LC11 and LC20 clinical isolates cultured under microaerophilic or aerobic atmospheres with CO_2 in liquid medium supplemented with horse serum.
Bacteria were incubated at 37 °C in BHI broth with 10% horse serum in a Sanyo Tri-Carb incubator under aerobic (5% CO_2, 21% O_2) and microaerobic conditions (5% CO_2, 5% O_2) as described in the Material and Methods section.

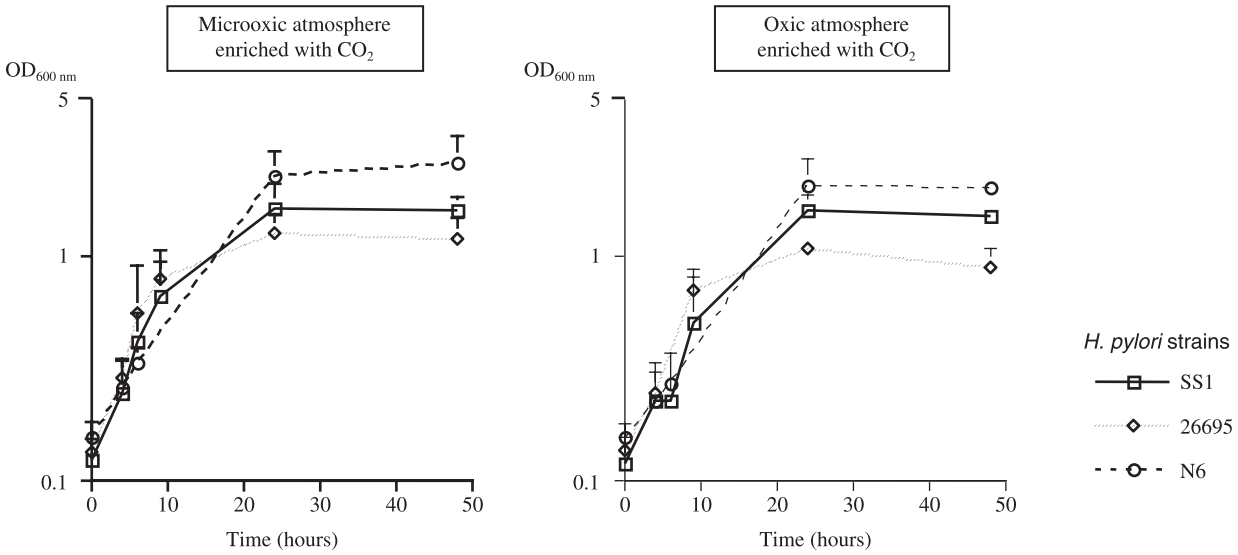

Figure 2 Growth curves of *H. pylori* laboratory strains SS1, 26695, and N6 cultured under microaerophilic or aerobic atmospheres with CO_2 in liquid medium supplemented with β-cyclodextrin.
Strains were cultivated at neutral pH in BHI liquid medium containing 0.2% β-cyclodextrin. The inoculums of the cultures were started at 0.1–0.2 OD at 600 nm (corresponding to approximately to 5×10^7–10^8 cfu/ml). The microaerophilic atmosphere was generated with Campigen gas packs (10% CO_2, 7% O_2). The aerobic atmosphere with CO_2 was generated with CO_2Gen gas packs (6% CO_2, 15% O_2). Errors are shown as standard deviations of the measurements. Curves are representative of five experiments for SS1, three experiments for 26695 and two experiments for N6.

different atmospheres did not show differences in the bacterial morphology or in the evolution towards coccoid forms as determined by optic or electron microscopy. Strain 26695 was grown also in BHI medium adjusted to pH 5 under the conditions atm.2 and atm.3, and comparable growth was observed under both conditions (data not shown).

The growth of *H. pylori* strain 26695 at lower cell densities was investigated in BHI broth cultures supplemented with 0.2% β-cyclodextrin. Cultures were inoculated to cell densities of approximately 10^7 cfu/ml (0.03 OD at 600 nm in this medium), and grown under the conditions atm.2 and atm.3. The bacteria grew well microaerobically but did

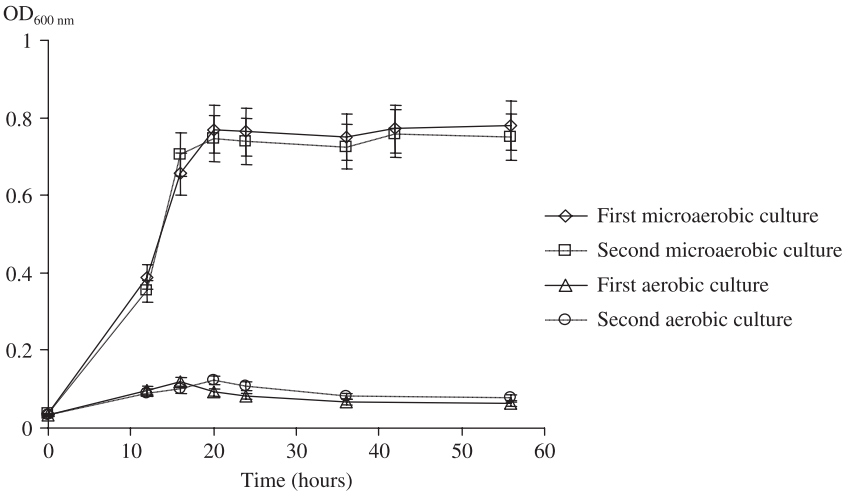

Figure 3 Growth curves of *H. pylori* strain 26695 cultured under microaerophilic or aerobic atmospheres with CO_2 in liquid medium supplemented with β-cyclodextrin. Bacteria were cultivated at neutral pH in BHI liquid medium containing 0.2% β-cyclodextrin. The inoculums of the cultures were started at 0.03–0.035 OD at 600 nm (corresponding to approximately to 10^7 cfu/ml). The microaerophilic atmosphere was generated with Campigen gas packs (10% CO_2, 7% O_2). The aerobic atmosphere with CO_2 was generated with CO_2Gen gas packs (6% CO_2, 15% O_2). Errors are shown as standard deviations of the measurements.

not grow under aerobic conditions (Fig. 3). In media supplemented with 10% horse serum instead of 0.2% β-cyclodextrin, bacterial cells grew under the atm.1 aerobic conditions at cells densities of approximately 5×10^5 cfu/ml but were unable to grow under this atmosphere at lower densities (data not shown).

To identify potential factors involved in producing the bactericidal effects observed under aerobic conditions, *H. pylori* cells were grown in two compartments in liquid contact separated by a membrane that stopped bacteria moving between them. The compartments were culture inserts placed into wells, and the membrane allowed the exchange between them of metabolites, chemical compounds, and macromolecules. Suspensions of bacteria at densities of 10^8 and 10^3 cfu/ml were inoculated in the inner and outer compartments, respectively. Independent control cultures were carried out at both bacterial densities. Cells grew well under the microaerobic atm.5 conditions in all compartments and cultures. Under the aerobic atm.1 conditions, bacteria grew well in the compartments and cultures with high bacterial densities, but did not grow in the compartments and cultures with low bacterial densities.

To circumvent potential artifacts owing to the presence of membranes separating the cultures, *H. pylori* was grown in broth constituted by adding fresh nutrients to media in which bacteria had already been grown. Cells grown under the atm.1 conditions for 48 hours at densities of 10^8 cfu/ml in BHI with 10% horse serum were removed from the media by centrifuging the cultures, collecting the supernatant, and filtering it through a 0.22 µm membrane. The cell-free supernatant was supplemented with fresh media (3: 1, v/v) constituted at four times the normal concentration, such that the final concentration of nutrients was at least equal to that of standard cultures. Bacteria were inoculated into this "recycled media" at densities of 10^3 or 10^8 cfu/ml and grown under the microaerobic atm.5 or aerobic atm.1 conditions. To control for potential artifacts arising from employing used media, bacteria were grown also in media constituted in a similar way as the "recycled media", but in which the fraction corresponding to cell-free supernatants was substituted by fresh media. Cells grew in all cultures incubated under microaerobic conditions and the recycled media did not have any effect on growth. Under aerobic conditions, bacteria grew at high density cell cultures, and did not grow at low cell density cultures.

To investigate potential effects on the physiology of *H. pylori* grown aerobically or microaerobically, the reduction of ferredoxin by bacteria grown under both conditions was measured. For strains 26695, J99, N6, NCTC 11639, and SS1, and low-passage isolates CAS 015, HER 126, RIG 117, LC11, and LC20, reduction of ferredoxin in the presence of NADH was observed in extracts from cells grown under microaerobic condition atm.5, but not from cells grown under the high oxygen levels of conditions, atm.1 or atm.4. The K_M measured for the NADH:ferredoxin oxidoreductases of various *H. pylori* strains and isolates is given in Table 2.

Discussion

Microaerophilic microorganisms have oxygen-dependent growth, but under aerobic conditions cannot grow, or grow very poorly. The existence of microaerophiles has been known for some time, but the study of their specific characteristics has been neglected owing to the inclination to regard them as aerobes, facultative anaerobes, or aerotolerant anaerobes. This lack of understanding of the fundamental properties that define microaerophily has left

Table 2 Michaelis constant (K_M) of ferredoxin reduction by NADH in *H. pylori* cytosolic fractions measured at 340 nm
Measurements (n = 3) were carried at 25 °C and in Tris-HCl (20 mmol/l, pH 7.4) buffer and 0.15 mmol/l NADH concentration

	Growth condition	
H. pylori strain	Aerobic	Microaerobic
26695	No activity	14.6 ± 1.6 μg/ml
J99	No activity	28.9 ± 2.8 μg/ml
LC20	No activity	1.6 ± 0.3 μg/ml
NCTC 11639	No activity	4.6 ± 1.0 μg/ml

significant gaps in our knowledge of microorganisms that have widespread habitats and are important in several scientific and technological fields.

Examples of microearophiles include pathogenic bacteria such as *Campylobacter, Borrelia, Helicobacter*, and *Treponema*; and parasites such as *Entamoeba, Giardia, Plasmodium*, and *Trichomonas.* Understanding their physiology is important to combat these infections, since redox mechanisms are employed by bacteria to protect themselves against oxidative host responses, and many antibiotics act by producing toxic redox products.

The conditions employed to grow in vitro the human pathogen *H. pylori* vary significantly between laboratories. There is no general consensus about the specific oxygen and carbon dioxide requirements of the organism. The bacterium is considered a microaerophile and, consequently, it is grown under atmospheres at oxygen tensions 5–19% and carbon dioxide tensions 5–10%, both for clinical, and basic and applied research purposes. To examine the microaerophilic requirements of *H. pylori*, growth experiments with laboratory strains and low passage isolates were conducted under controlled conditions. The results indicated that the bacterium is a capnophile that had an absolute requirement for elevated carbon dioxide concentrations. At cells densities at and above 5×10^7 cfu/ml for media supplemented with β-cyclodextrin, and cells densities at and above 5×10^5 cfu/ml for media supplemented with horse serum, *H. pylori* grew similarly in liquid cultures under oxygen tensions ranging from microoxic (5%) to fully aerobic (21%). At lower cell densities, it grew microaerobically but not under aerobic conditions. The data suggested that *H. pylori* is a microaerophile that can grow under aerobic atmospheres at high cell densities, probably by modulating the oxygen concentration in the liquid cultures.

This conclusion is similar to the interpretation given by Henriksen *et al*. to the results of their study on the effects of atmospheric conditions on the growth of *H. pylori* on plates [9]. The study concluded that in atmospheres at 10% CO_2, growth of *H. pylori* was significantly better under microaerobic conditions than at normal oxygen tension. However, important methodological differences exist between the study by Henriksen et al. and this one. First, those authors assessed growth by measuring colony diameters of bacteria grown on solid media. It should be noted that colonies grown on solid media are highly heterogenous owing to the different ages of the cells, the various environments found by bacteria in the colony, etc. Second, besides specific concentrations of O_2 and CO_2, both studies used different gas mixtures. Henriksen and coworkers obtained very high concentrations of H_2 gas in some of the atmospheres they employed by not including a catalyst in the jars. The problem with this approach is that in the absence of a catalyst, the oxygen present was not reduced by hydrogen, and thus, it cannot be assumed that the atmosphere resulting under those conditions was microoxic. In the present study the gas balance was achieved by N_2 and not by H_2, and the oxygen concentrations were controlled. Table 1 of Henriksen et al. shows the importance of the effects of H_2 gas, also in the presence of O_2 gas as discussed above. Their data, albeit not their interpretation, show significant differences in bacteria grown under conditions with or without hydrogen, and no significant differences between cultures grown without H_2 gas either microaerobically or aerobically.

In contrast to *H. pylori*, other *Helicobacter* species, such as *H. bilis* and *H. hepaticus*, were unable to grow at oxygen tensions above 10% O_2 in the presence of 10% CO_2. Thus, there are interesting differences within the genus *Helicobacter* between more strict microaerophiles such as the latter two species and *H. pylori.* A systematic investigation of the oxygen phenotypes of other gastric and enterohepatic *Helicobacter* species will serve to obtain an understanding of the requirements and use of oxygen by the different species of this genus.

The dependence of *H. pylori* on CO_2 is better known than its O_2 sensitivity. The molecular bases for this requirement have not been established, but several enzymes using CO_2 or HCO_3^- have been proposed to be implicated in the CO_2 dependence of *H. pylori* including carbonic anhydrases (Bury-Moné et al., submitted), carbamoyl phosphate synthetase [36], and acetylCoA carboxylase [37].

Although *H. pylori* can be grown in atmospheres with very different oxygen contents, there is evidence that significant changes occur in the physiology of the bacterium. In vivo *H. pylori* effects on the inflamed gastric mucosa were mimicked by using in vitro cocultures of the bacterium with epithelial cells kept under a 5% O_2, 5% CO_2 atmosphere [38]. These effects were not observed in systems that kept bacteria under aerobic conditions [39]. Moreover, the growth atmosphere of the bacterium has also an impact on its haemolytic activity [18] and metronidazole sensitivity [40].

Ferredoxin oxidoreductases participate in many electron-transfer pathways in plants and bacteria including photosynthesis, nitrogen fixation, redox signalling, etc. The genome of *H. pylori* encodes the three subunits of a ferredoxin oxidoreductase (genes *hp0589* to *hp0591,* in strain 26695). The role of this enzyme in biological oxidation–reduction systems suggested that it may be involved in changes in the *H. pylori* physiology grown under different atmospheres. Comparison of ferredoxin oxidoreductase activity in bacteria grown under oxic and microoxic conditions showed that the former were unable to reduce exogenous ferredoxin. These observations underline the importance of understanding the molecular mechanisms involved in the physiology of bacteria grown under oxic or microoxic conditions.

Thus, it has been demonstrated that *H. pylori* is a capnophile and a microaerophile, which under elevated CO_2 conditions and high cell densities is able to grow in vitro in atmospheres with oxygen concentrations between 5 and 21%. At the same time, the physiologic differences that arise from growing *H. pylori* under various oxygen atmospheres remain to be fully understood. It is important to be aware that differences are induced in *H. pylori* grown under aerobic or microaerobic conditions since these may influence the outcome of experiments. Considering the relatively small genome of *H. pylori*, it is of great interest to elucidate how this bacterium manages to adapt to the different oxygen tensions it may encounter until it reaches the mucosa of the human stomach.

The authors thank the Australian Research Council for its support, and Dr Kerstin Stingl for helpful discussions and critical reading of the manuscript.

References

1 Unden G. Aerobic respiration and regulation of aerobic/anaerobic metabolism. In: Lengeler JW, Drews G, Schlegel HG, eds. Biology of the Prokaryotes. New-York: Thieme Stuttgart, Blackwell Science, 1999.

2 Krieg NR, Hoffman PS. Microaerophily and oxygen toxicity. *Annu Rev Microbiol* 1986;40:107–30.

3 Finster K, Liesack W, Tindall BJ. *Sulfurospirillum arcachonense* sp. nov., a new microaerophilic sulfur-reducing bacterium. *Int J Syst Bacteriol* 1997;47:1212–7.

4 Romagnoli S, packer HL, Armitage JP. Tactic response to oxygen in the phototrophic bacterium *Rhodobacter sphaeroides* WS8N. *J Bacteriol* 2002;184:5590–8.

5 Lloyd D, Harris JC, Maroulis S, Biagini GA, Wadley RB, Turner MP, Edwards MR. The microaerophilic flagellate *Giardia intestinalis*: oxygen and its reaction products collapse membrane potential and cause cytotoxicity. *Microbiology* 2000;146:3109–18.

6 Andersen LP, Wadström T. Basic bacteriology and culture. In: Mobley HLT, Mendz GL, Hazell SL, eds. *Helicobacter pylori:* Physiology and Genetics. Washington: ASM Press, 2001;27–52.

7 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;1:1311–5.

8 Kangatharalingam N, Amy PS. *Helicobacter pylori* comb. nov. exhibits facultative acidophilism and obligate microaerophilism. *Appl Environ Microbiol* 1994;60:2176–9.

9 Henriksen TH, Lia A, Schoyen R, Thoresen T, Berstad A. Assessment of optimal atmospheric conditions for growth of *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 2000;19:718–20.

10 Hazell SL, Harris AG, Trend MA. Evasion of the toxic effects of oxygen. In: Mobley HLT, Mendz GL, Hazell SL, eds. *Helicobacter pylori:* Physiology and Genetics. Washington: ASM Press, 2001;167–75.

11 Kelly DJ, Hughes NJ, Poole RK. Microaerobic physiology: aerobic respiration, anaerobic respiration, and carbon dioxide metabolism. In: Mobley HLT, Mendz GL, Hazell SL, eds. *Helicobacter pylori:* Physiology and Genetics. Washington: ASM Press, 2001;113–24.

12 Mendz GL, Shepley AJ, Hazell SL, Smith MA. Purine metabolism and the microaerophily of *Helicobacter pylori*. *Arch Microbiol* 1997;168:448–56.

13 McGowan CC, Necheva A, Thompson SA, Cover TL, Blaser MJ. Acid-induced expression of an LPS-associated gene in *Helicobacter pylori*. *Mol Microbiol* 1998;30:19–31.

14 Rektorschek M, Weeks D, Sachs G, Melchers K. Influence of pH on metabolism and urease activity of *Helicobacter pylori*. *Gastroenterology* 1998;115:628–41.

15 Salama NR, Otto G, Tompkins L, Falkow S. Vacuolating cytotoxin of *Helicobacter pylori* plays a role during colonization in a mouse model of infection. *Infect Immun* 2001;69:730–6.

16 Gobert AP, McGee DJ, Akhtar M, Mendz GL, Newton JC, Cheng Y, Mobley HL, Wilson KT. *Helicobacter pylori* arginase inhibits nitric oxide production by eukaryotic cells: a strategy for bacterial survival. *Proc Natl Acad Sci USA* 2001;98:13844–9.

17 Goodwin C, Blincow E, Warren J, Waters TE, Sanderson CR, Easton L. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J Clin Pathol* 1985;38:1127–31.

18 Xia HX, Keane CT, O'Morain CA. Culture of *Helicobacter pylori* under aerobic conditions on solid media. *Eur J Clin Microbiol Infect Dis* 1994;13:406–9.

19 Tompkins DS, Dave J, Mapstone N. Adaptation of *Helicobacter pylori* to aerobic growth. *Eur J Clin Microbiol Infect Dis* 1994;13:409–12.

20 Marcelli SW, Huai-Tzu C, Chapman T, Chalk PA, Miles RJ, Poole RK. The respiratory chain of *Helicobacter pylori*: identification of cytochromes and the effects of oxygen on cytochrome and menaquinone. *FEMS Microbiol Lett* 1996;138:59–64.

21 Kelly DJ. The physiology and metabolim of the human gastric pathogen *Helicobacter pylori*. *Adv Microb Physiol* 1998;40:137–89.

22 Olczak AA, Olson JW, Maier RJ. Oxidative-stress resistance mutants of *Helicobacter pylori*. *J Bacteriol* 2002;184:3186–93.

23 Goodwin A, Kersulyte D, Sisson G, Veldhuyzen van Zanten SJ, Berg DE, Hoffman PS. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. *Mol Microbiol* 1998;28:383–93.

24 Kwon DH, Kato M, El-Zaatari FAK, Osato MS, Graham DY. Frame-shift mutations in NAD(P)H flavin oxidoreductase encoding gene (*frxA*) from metronidazole resistant *Helicobacter pylori* ATCC43504 and its involvement in metronidazole resistance. *FEMS Microbiol Lett* 2000;188:197–202.

25 Mendz GL, Mégraud F. Is the molecular basis of metronidazole resistance in microaerophilic organisms understood? *Trends Microbiol* 2002;10:370–5.

26 Donelli G, Matarrese P, Fiorentini C, Dainelli B, Taraborelli T, Campli ED, Bartolomeo SD, Cellini L. The effect of oxygen on the growth and cell morphology of *Helicobacter pylori*. *FEMS Microbiol Lett* 1998;168:9–15.
27 Yamaguchi H, Osaki T, Takahashi M, Taguchi H, Kamiya S. Colony formation by *Helicobacter pylori* after long-term incubation under anaerobic conditions. *FEMS Microbiol Lett* 1999;175:107–11.
28 Kelly DJ, Hughes NJ. The citric acid cycle and fatty acid biosynthesis. In: Mobley HLT, Mendz GL, Hazell SL, eds. *Helicobacter pylori:* Physiology and Genetics. Washington: ASM Press, 2001:135–46.
29 Mendz GL, Hazell SL, Srinivasan S. Fumarate reductase: a target for therapeutic intervention against *Helicobacter pylori*. *Arch Biochem Biophys* 1995;321:153–9.
30 Hughes NJ, Clayton CL, Chalk PA, Kelly DJ. *Helicobacter pylori porCDAB* and *oorDABC* genes encode distinct pyruvate: flavodoxine and 2-oxoglutarate: acceptor oxidoreductases which mediate electron transport to NADP. *J Bacteriol* 1998;180:1119–28.
31 Nagata K, Tsukita S, Tamura T, Sone N. A cb-type cytochrome-c oxidase terminates the respiratory chain in *Helicobacter pylori*. *Microbiology* 1996;142:1757–63.
32 D'Mello R, Hill S, Poole RK. The cytochrome bd quinol oxidase in *Escherichia coli* has an extremely high oxygen affinity and two oxygen-binding haems: implications for regulation of activity *in vivo* by oxygen inhibition. *Microbiology* 1996;142:755–63.
33 Kather B, Stingl K, van der Rest ME, Altendorf K, Molenaar D. Another unusual type of citric acid cycle enzyme in *Helicobacter pylori*: the malate: quinone oxidoreductase. *J Bacteriol* 2000;182:3204–9.
34 Skouloubris S, Labigne A, De Reuse H. The AmiE aliphatic amidase and AmiF formamidase of *Helicobacter pylori*: natural evolution of two enzyme paralogs. *Mol Microbiol* 2001;40:596–609.
35 Miles AA, Misra SS. The estimation of the bactericidal power of blood. *J Hyg* 1938;8:732–49.
36 Mendz GL, Jimenez BM, Hazell SL, Gero AM, O'Sullivan WJ. De novo synthesis of pyrimidine nucleotides by *Helicobacter pylori*. *J Appl Bacteriol* 1994;77:1–8.
37 Burns B, Hazell S, Mendz GL. Acetyl-CoA carboxylase activity in *Helicobacter pylori* and the requirement of increased CO_2 for growth. *Microbiology* 1995;141:3113–8.
38 Cottet S, Corthesy-Theulaz I, Spertini F, Corthesy B. Microaerophilic conditions permit to mimic *in vitro* events occurring during *in vivo Helicobacter pylori* infection and to identify Rho/Ras-associated proteins in cellular signaling. *J Biol Chem* 2002;277:33978–86.
39 Olczak AA, Seyler RWJ, Olson JW, Maier RJ. Association of *Helicobacter pylori* antioxidant activities with host colonization proficiency. *Infect Immun* 2003;71:580–3.
40 Cederbrant G, Kahlmeter G, Ljungh A. Proposed mechanism for metronidazole resistance in *Helicobacter pylori*. *J Antimicrob Chemother* 1992;29:115–20.

