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# Perspectives on methodology for *in vitro* culture of *Helicobacter pylori*

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## Abstract

Over the past 25 years, a variety of methods have been developed for culture of *Helicobacter pylori in vitro*. *H. pylori* is a capnophilic and microaerophilic organism that is typically cultured using complex culture media. Analysis of *H. pylori* growth in chemically defined media has provided insight into the nutritional requirements, physiology, and metabolic capacities of this organism.

### Keywords

*Helicobacter pylori*; defined medium; capnophilic; microaerophilic; nutritional requirements; nutrient acquisition

Spiral-shaped bacteria were visualized in histologic sections of human gastric specimens throughout most of the twentieth century, but these organisms remained uncharacterized until 1984, when gastric bacteria (known now as *Helicobacter pylori*) were successfully cultured *in vitro* for the first time by Marshall and Warren (1). These investigators isolated *H. pylori* by placing minced human gastric tissue on non-selective media and culturing at 37°C under microaerobic conditions for 4 days, using methods similar to those used for isolation of Campylobacter species. Over the past 25 years, there have been various improvements in the methods for culture of *H. pylori* from human gastric specimens, but the general approach remains similar to that which was used initially in 1984. General principles include the use of a rich culture medium containing blood or serum, a microaerobic and hypercarbic atmosphere, high humidity, temperature of 37°C, and incubation periods ranging from 4 to 10 days (2). *H. pylori* can be cultured from gastric specimens using non-selective media, but antibiotics are often added to allow selective growth of *H. pylori*. For example, combinations of vancomycin, polymyxin, trimethoprim, bacitracin, nalidixic acid, and amphotericin are commonly included in *H. pylori*-selective media.

After recovery of *H. pylori* from human gastric biopsy specimens, the bacteria can be propagated *in vitro* using a variety of approaches. Commonly used media include Brucella agar, Columbia agar, brain heart infusion agar, or trypicase soy agar as the base, supplemented with sheep blood or horse blood (5–10%) (2). Growth of *H. pylori* on serum-free medium can be accomplished by substituting β-cyclodextrin in place of blood products (3). In addition, egg yolk emulsion medium has been described as a blood-free medium for growth of *H. pylori* (4).

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*H. pylori* is a capnophilic organism that requires an atmosphere enriched in $CO_2$ (typically 5 to 10%) for growth (5, 6). The requirement for high $CO_2$ concentrations is probably related to multiple factors. For example, production of pyruvate through $CO_2$ fixation may provide a route for carbon assimilation (7), and $CO_2$ may have a role in pH homeostasis since *H. pylori* carbonic anhydrase catalyzes interconversion of $CO_2$ and $HCO_3^-$ (8). *H. pylori* is an oxygen-sensitive microaerophile (5), and consequently, microaerobic conditions are used when initially culturing *H. pylori* from gastric biopsy specimens. The sensitivity of *H. pylori* to oxygen is attributed to oxygen-dependent inactivation of essential bacterial enzymes (6). When present in high cell densities, laboratory-adapted strains of *H. pylori* can grow in a range of atmospheric oxygen tensions ranging from microaerobic (<5% oxygen) to fully aerobic (21% oxygen) (5). Several characteristics of *H. pylori*, including hemolysin production, metronidazole resistance, ferredoxin oxidoreductase activity, and ability of the bacteria to induce alterations in epithelial cells, are reported to differ depending on whether the bacteria are grown in microaerobic or aerobic conditions (5, 9–11).

When growth of *H. pylori* in liquid culture is required, a commonly used medium is Brucella broth supplemented with fetal bovine serum (5–10%). As an alternative to fetal bovine serum, β-cyclodextrin, charcoal, or starch may be used (12). The exact mechanisms by which serum, β-cyclodextrin, charcoal, or starch promote growth of *H. pylori* have not been investigated in detail. One possibility is that serum may contain growth-stimulating factors. β-cyclodextrin, charcoal, and starch may bind fatty acids or other toxic metabolites produced by the bacteria (13, 14). Growth of *H. pylori* in liquid culture on a large scale has been successfully accomplished by use of a fermentor (15, 16).

For some experiments, it is desirable to culture *H. pylori* using a defined medium instead of the complex media described above. In general, defined media for culture of *H. pylori* consist of various salts, a purine, vitamins, amino acids, and trace metals (17–20). To enhance bacterial growth, the defined media can be supplemented with either bovine serum albumin or a mixture of β-cyclodextrin and cholesterol (17, 19, 20). Initial formulations of defined media for growth of *H. pylori* had a composition similar to that found in commercially available RPMI tissue culture medium (17), whereas the composition of more recent formulations is similar to that found in F12 medium (20). The use of defined media for culture of *H. pylori* has allowed important insights into the nutritional requirements, physiology, and metabolism of this organism (17–23), and also has facilitated analysis of the *H. pylori* extracellular proteome (24, 25).

Nearly all *H. pylori* strains require arginine, histidine, leucine, methionine, phenylalanine, and valine for growth (17–20). Interestingly, the amino acids that are essential for *H. pylori* growth are similar to the amino acids that are essential for humans (26). There is variability among strains in a requirement for alanine, serine, cysteine, proline, and isoleucine (17–20). Other factors required for *H. pylori* growth include pyruvate, thiamine, and hypoxanthine (20, 21), as well as several metals, including iron, zinc, and magnesium (21). These nutritional requirements correlate with absence of the corresponding biosynthetic genes in the *H. pylori* genome (27).

Although current culture methods provide valuable insights into the biology of *H. pylori*, it is important to recognize the limitations of these systems in replicating conditions present in the human stomach. Numerous bacterial proteins are required for *H. pylori* growth *in vivo* but are non-essential *in vitro* (28). For example, urease (a nickel-containing enzyme) is required for *H. pylori* colonization of the stomach (29, 30) but is not required for *H. pylori* growth *in vitro*. Correspondingly, nickel is probably required for *H. pylori* growth *in vivo*, but does not seem to be required for *H. pylori* growth *in vitro* (21). Mechanisms of nutrient acquisition also may differ during bacterial growth *in vivo* compared to *in vitro.* For

example, *H. pylori* utilizes free iron when cultured *in vitro* (21) but utilizes hemoglobin, transferrin, and lactoferrin as iron sources *in vivo* (31). These examples illustrate that there are likely to be important differences in nutritional requirements, nutrient acquisition and metabolism of *H. pylori in vivo* compared to *in vitro*.

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