Species differentiation and identification in the genus of *Helicobacter*

HUA Jie-Song, ZHENG Peng-Yuan and HO Bow

**Subject Headings** *Helicobacter* genus; species; biological features; biochemical tests; identification; differentiation

As early as the nineteenth century, incidental presence of spiral organisms was noted in the stomachs of dogs\(^1\), rats and cats\(^2\). In the early years of this century, spiral organisms were also found in the gastric contents of patients with ulcerative carcinoma\(^1\). During the ensuing 30 years, there were scattered reports of these organisms being found in the stomach of patients with benign peptic ulcers. Doege\(^3\) showed a prevalence of 43% of spiral organisms in a comprehensive autopsy study in 242 human stomach specimens. However, he did not associate the presence of the spiral organism with various gastric diseases.

Controversy existed over the possible role of these spiral organisms in human gastric disease. It was suggested that the bacteria observed in gastric biopsies might represent bacterial contaminants introduced from mouth. This hypothesis gained support with the publication of an extensive histologic study of gastric biopsies from 1000 subjects by Palmer\(^4\). After the publication of the report the interest in gastric bacteria waned.

Interest in the role of gastric bacteria in the pathogenesis of peptic ulcer disease was rekindled when Steer and Colin Jones\(^5\) reported the presence of bacteria deep in the mucus layer of gastric mucosa in patients with gastric ulceration. It was suggested that the bacteria might cause a reduction in gastric mucosal resistance via predisposition to ulceration. Attempts to culture this bacterium yielded growth of *Pseudomonas aeruginosa*. Retrospectively, careful examination of the figures in this publication\(^6\) suggests that the organism seen on the mucosa is a spiral bacterium, a morphological form not associated with *P. aeruginosa*. It is now assumed that the culture of *P. aeruginosa* by these authors represents a contaminant cultured from the endoscope. With the discovery of *Helicobacter pylori* by Warren and Marshall\(^7\), it has been shown that *H. pylori* is associated with gastroduodenal disease\(^8,9\).

The spiral organism was first named Campylobacter pyloridis in 1984\(^10\). However, the rules of Latin grammar changed the name to *Campylobacter pylori*\(^11\). Ribosomal ribonucleic acid sequences showed that the bacterium did not belong to the *Campylobacter* genus\(^12-14\). In 1989, Goodwin in *et al*\(^15\) proposed a new genus called *Helicobacter* on the bases of 5 major taxonomic features: ultrastructure and morphology, cellular fatty acid profiles, menaquinones, growth characteristics and enzyme capabilities. *C. pylori* was, therefore, transferred to the new genus and renamed as *Helicobacter pylori*. The major features\(^15,20\) of *Helicobacter* genus consist of ① Helical, curved or straight unbranched morphology. ② Gram negative. ③ Endospores are not produced. ④ Rapid, darting motility by means of multiple sheathed flagella that are unipolar or bipolar and lateral, with terminal bulbs. ⑤ Optimal growth at 37°C; growth at 30°C but not at 25°C; variable growth at 42°C. ⑥ Microaerophilic, variable growth in air enriched with 100mL/L -CO\(_2\) and anaerobically. ⑦ External glyocalyx produced in broth cultures. ⑧ Susceptible to penicillin, ampicillin, amoxicillin, erythromycin, gentamicin, kanamycin, rifampin and tetracycline. Resistance to nalidixic acid, cephalothin, metronidazole and polymyxin. ⑨ G+C content of chromosomal DNA of 200mol/L–440 mol/L.

It has been a decade since the genus of *Helicobacter* was created. This genus expands rapidly from at first only two species, viz. *H. pylori* and *Helicobacter mustelae*, to 20 species\(^15-35\) and one associated species\(^36\) with a wide variety of sources isolated from either human beings and/or different animals. The characteristic details of the *Helicobacter* genus, which might be useful in the differentiation and identification of different *Helicobacter* species in microbiological laboratory, are listed in Table 1, 2, 3 and 4. The genus of *Helicobacter* will surely continue to enlarge as more data of *Helicobacter* features are available and more animal hosts are investigated. Molecular methods, such as PCR, will provide the most accurate tests in differentiation and identification in future with the publication of the genomic library of *H. pylori*\(^37\).
### Table 1: Locations, key morphological features and growth characteristics of *Helicobacter* species colonizing either humans and/or animals

| Characteristic | *H. pylori* | *H. canis* | *H. cinaedi* | *H. felis* | *H. fennelliae* | *H. pullorum* | *H. westmeadii* |
|---------------|-------------|-------------|-------------|-------------|----------------|---------------|-----------------|
| Host          | Human       | Dog, human  | Human       | Cat, dog, human | Human          | Poultry, human | Human           |
| Location      | Stomach     | Intestine   | Blood, rectum | Stomach     | Intestine      | Intestine      | Blood           |
| Cell size (μm) | 0.5×3.0-5.0 | 4.0         | 0.3-0.5×1.5-5.0 | 0.4×5-7.5   | 0.3-0.5×1.5-5.0 | 3×4           | 0.5×1.5-2.0     |
| Flagella      |             |             |             |             |                |               |                 |
| Number        | 4-8         | 2           | 1-2         | 1-2          | 1              | 1             |                 |
| Distribution  | Polar       | Biopolar    | Polar       | Biopolar     | Biopolar       | MONOPOLAR     | MONOPOLAR       |
| Sheath        | +           | +           | +           | +            | +              | +             | +               |
| Periplasmic fibers | -           | -           | -           | -            | -              | -             | -               |
| Growth at:    |             |             |             |             |                |               |                 |
| 25°C          | -           | -           | -           | -            | -              | -             | -               |
| 37°C          | +           | +           | +           | +            | +              | +             | +               |
| 42°C          | -           | +           | +           | +            | +              | +             | +               |
| Growth on:    |             |             |             |             |                |               |                 |
| 10g/L glucose | -           | +           | -           | +            | -              | +             |                 |
| 15g/L NaCl    | -           | -           | -           | -            | -              | -             | -               |
| Tolerance to: | 10g/L bile  | +           | VARY        | +            | VARY           | +             |                 |
| Safrain ‘O’   | -           | -           | -           | +            | -              | +             |                 |
| Methyl orange | -           | -           | +           | -            | -              | -             | -               |
| Growth under: |             |             |             |             |                |               |                 |
| Aerobic       | -           | -           | -           | -            | -              | -             | -               |
| Microaerobic   | +           | +           | +           | +            | +              | +             | Weak +          |
| Anaerobic     | -           | -           | -           | -            | -              | -             | -               |
| Susceptibility to: |           |             |             |             |                |               |                 |
| Nalidixic acid | R           | R           | S           | R            | S              | S             | S               |
| Cephalothin   | S           | S           | S           | S            | R              | R             | R               |
| Ceferazone    | S           | S           | S           | S            | S              | S             | S               |
| Metronizazole | S           | S           | S           | S            | S              | S             | S               |

### Table 2: Locations, key morphological features and growth characteristics of *Helicobacter* species colonizing animals

| Characteristic | *H. pylori* | *H. canis* | *H. cinaedi* | *H. felis* | *H. fennelliae* | *H. pullorum* | *H. westmeadii* |
|---------------|-------------|-------------|-------------|-------------|----------------|---------------|-----------------|
| Host          | Human       | Dog, human  | Human       | Cat, dog, human | Human          | Poultry, human | Human           |
| Location      | Stomach     | Intestine   | Blood, rectum | Stomach     | Intestine      | Intestine      | Blood           |
| Cell size (μm) | 0.3×1.5    | 0.5×4.0    | 0.3×5      | 0.5-0.6     | 0.2-0.3        | 0.5×3.5       |                 |
|                | -2.0        | 10         | 3-10.5     | 5-0         | 15-50          | 5-0           |                 |
| Flagella      |             |             |             |             |                |               |                 |
| Number        | 2-5         | 13-20       | 10-20       | 2-10        | 1-14           | 4-8           |                 |
| Distribution  | Monopolar   | Biopolar    | Biopolar    | Biopolar    | Biopolar       | Biopolar      | Biopolar        |
| Sheath        | +           | +           | +           | +            | +              | +             | +               |
| Periplasmic fibers | -           | -           | -           | -            | -              | -             | -               |
| Growth at:    |             |             |             |             |                |               |                 |
| 25°C          | -           | -           | -           | -            | -              | -             | -               |
| 37°C          | +           | +           | +           | +            | +              | +             | +               |
| 42°C          | -           | +           | +           | +            | +              | +             | +               |
| Growth on:    |             |             |             |             |                |               |                 |
| 10g/L glucose | -           | +           | -           | +            | -              | +             |                 |
| 15g/L NaCl    | -           | -           | -           | -            | -              | -             | -               |
| Tolerance to: | 10g/L bile  | +           | VARY        | +            | VARY           | +             |                 |
| Safrain ‘O’   | -           | -           | -           | +            | -              | +             |                 |
| Methyl orange | -           | -           | +           | -            | -              | -             | -               |
| Growth under: |             |             |             |             |                |               |                 |
| Aerobic       | -           | -           | -           | -            | -              | -             | -               |
| Microaerobic   | +           | +           | +           | +            | +              | +             | +               |
| Anaerobic     | -           | -           | -           | -            | -              | -             | -               |
| Susceptibility to: |           |             |             |             |                |               |                 |
| Nalidixic acid | R           | R           | I           | R            | S              | R             | R               |
| Cephalothin   | S           | S           | S           | S            | R              | R             | R               |
| Ceferazone    | S           | S           | S           | S            | R              | R             | R               |
| Metronizazole | S           | S           | S           | S            | S              | S             | S               |

### Table 3: Key and differential biochemical characteristics of *Helicobacter* species colonizing either humans and/or animals

| Characteristic | *H. pylori* | *H. canis* | *H. cinaedi* | *H. felis* | *H. fennelliae* | *H. pullorum* | *H. westmeadii* |
|---------------|-------------|-------------|-------------|-------------|----------------|---------------|-----------------|
| Catalase activity | +           | +           | +           | +           | +              | +             | +               |
| Urease activity | +           | +           | +           | +           | +              | +             | +               |
| Oxidase activity | +           | +           | +           | +           | +              | +             | +               |
| Alkaline phosphatase activity | +           | +           | +           | +           | +              | +             | +               |
| γ-Glutamyl transpeptidase activity | +           | +           | +           | +           | +              | +             | +               |
| H₂S production | -           | -           | -           | -           | -              | -             | -               |
| Indole acetate hydrolysis | -           | +           | -           | -           | -              | -             | -               |
| Hippurate hydrolysis | -           | -           | -           | -           | -              | -             | -               |
| Nitrate reduction | -           | -           | -           | -           | -              | -             | -               |
| Citrate esterase | +           | +           | +           | +           | +              | +             | +               |
| Citrate esterase lipase | +           | +           | +           | +           | +              | +             | +               |
| Leucine aminopeptidase | +           | +           | +           | +           | +              | +             | +               |
| Acid phosphatase | +           | +           | +           | +           | +              | +             | +               |
| Naphthol-AS-B1-phosphohydrolase | +           | +           | +           | +           | +              | +             | +               |
| DNase activity | +           | +           | +           | +           | +              | +             | +               |
| G+C content (mol%) | 35-37       | 48          | 37-38       | 43          | 37-38          | 34-35         |                 |
Table 4  Key and differential biochemical characteristics of Helicobacter species colonizing animals

|                | H.  | H.  | H.  | H.  | H.  | H.  | H.  | H.  | H.  |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Catalase activity | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| Urease activity | +   | +   | -   | +   | +   | +   | -   | -   | +   |
| Oxidase activity | +   | +   | +   | +   | +   | -   | +   | +   | +   |
| Alkaline phosphatase activity | +   | +   | +   | +   | +   | +   | -   | -   | +   |
| α-Glucosidase activity | +   | +   | +   | +   | +   | -   | +   | +   | +   |
| H2S production | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| Indoxyl acetate hydrolysis | -   | +   | +   | +   | +   | +   | +   | +   | +   |
| Hippurate hydrolysis | -   | -   | -   | -   | +   | -   | +   | +   | +   |
| Nitrate reduction | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| DNase activity | +   | +   | +   | +   | +   | +   | +   | +   | +   |
| G+C content (mol%) | 30  | 35  | 36  | 24  | 38  |     |     |     |     |

REFERENCES

1 Bizzozero O, Ueber die schlauformigen Drusen des magendarmassakens und die beziehungen ihres epithels zu dem oberfachenepithel der schleimhaut. Arch F Mikr Anat, 1893;23:82-152
2 Salmon H, Ueber das spirellum des saugteriagemens und sein verhalten zu den beziehungen. Zentralbl Bakteriol Microbiol Hyg, 1896;19:433-442
3 Krieni W, Ueber das auftreten von spirochaten verschiedener form im mageninnielt bei carcinoma ventriculi. Dtsch Med Wochenst,1906;32:872-876
4 Doenges JL, Spirochetes in the gastric glands of macacus rhesus and humans without definite history of related disease. Proc Soc ExpMed Biol,1938;38:536-538
5 Palmer ED, Investigation of gastric mucosa spirochetes of the human. Gastroenterology,1954;27:218-220
6 Steer HW, Colin-Jones DG, Mucosal changes in gastric ulceration and their reponse to carboksonate sodium. Gut,1975;16:590-597
7 Warren JR, Marshall BJ, Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet,1983;1:1273-1275
8 Goodwin CS, Armstrong JA, Marshall BJ, Campylobacter pyloridis, gastritis, and peptic ulceration. J Clin Pathol,1986;39:353-365
9 Wee A, Kang JY, Toh M,Helicobacter pylori and gastric cancer: correlation with gastritis, intestinal metaplasia and tumor histology, Gut,1992;33:1029-1032
10 Marshall BJ, Royce H, Annear DL, Goodwin CS, Pearman JW, Warren JR, Original isolation of Campylobacter pyloridis from human gastric mucosa. Biotmed Letts,1984;25:83-88
11 Marshall BJ, Goodwin CS, Revised nomenclature of Campylobacter pyloridis. Int J Syst Bacteriol,1987;37:68
12 Lau PP, de Brunner V, Belegzellen. Arch F Mikr Anat, 1896;19:433-442
13 Fennell CL, Totten PA, Quinnc TC, Patton DL, Holmes KK, Stamm WE, Characterization of Campylobacter-like organisms isolated from homosexual men. J Infect Dis,1989;159:55-66
14 McNulty CA, Dent JC, Curry A, Ufis JS, Ford GA, Gear MW, New spiral bacterium in gastric mucosa. J Clin Pathol,1989;42:585-591
15 Hazelli SL, Isolation of “Helicobacter heilmannii” from human tissue. Eur J Clin Microbiol Infect Dis,1996;15:4-9
16 Fox JG, Dewhirst FE, Tully JG, Paster BJ, Ya L, Taylor NS, Helicobacter hepticus sp. nov. a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. J Clin Microbiol,1994;32:1238-1245
17 Lee A, Phillips MW, O’Rourke JL, Paster BJ, Dewhirst FE, Fraser GJ. Helicobacter mustaridum sp. nov. a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. Int J Syst Bacteriol,1992;42:27-36
18 Fox JG, Taylor NS, Edmunds P, Brenner DJ, Campylobacter pylori subsp. Mustelae subsp. nov. isolated from the gastric mucosa of ferrets (Mustela putorius furo), and an emended description of Campylobacter pylori. Int J Syst Bacteriol,1988;38:367-370
19 Brendon MA, Goodwin CS, Sly SL, Chilvers T, Schoenknecht FD, Helicobacter nemestinea sp. nov. a spiral bacterium found in the stomach of a pigtailed macaque. Int J Syst Bacteriol,1991;41:148-153
20 Dewhirst FE, Seymour C, Fraser GJ, Paster BJ, Fox JG, Phylogeny of Helicobacter isolates from birds and swine feces and description of Helicobacter pamelatensis sp. nov. Int J Syst Bacteriol,1994;44:553-560
21 Stanley J, Linton D, Burns AP, Dewhirst FE, On SL, Porter A, Helicobacter pullorum sp. nov. genotyp and phenotype of a new species isolated from poult and from human patients with gastroenteritis. Microbiology,1994;140(Pt2):3441-3449
22 Shan Z, Fox JG, Dewhirst FE, Paster BJ, Foltz CJ, Yan L, Helicobacter rodentium sp. nov. a uresa negative Helicobacter species isolated from laboratory mice. Int J Syst Bacteriol,1997;47:627-634
23 Jalava K, Kaatinnen M, Urtainen M, Happonen I, Hanninen M, Helicobacter salomonis sp. nov. a canine gastric Helicobacter sp. related to Helicobacter felis and Helicobacter bizzozorneri. Int J Syst Bacteriol,1997;47:975-982
24 Mendes EN, Queiroz DM, Dewhirst FE, Paster BJ, Moura SB, Fox JG, Helicobacter trogontum sp. nov. isolated from the rat intestine. Int J Syst Bacteriol,1996;46:916-921
25 Trivett Moore NL, Rawlinson WD, Yuen M, Gilbert GL, Helicobacter westmeadi sp. nov., a new species isolated from blood cultures of two AIDS patients. J Clin Microbiol,1997;35:1144-1150
26 Schauber DH, Ghor N, Falkow S, Isolation and characterization of “Flexispira raptini” from laboratory mice. J Clin Microbiol,1993;31:2709-2714
27 Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD. The complete genome sequence of the gastric pathogen: Helicobacter pylori. Nature,1997;388:539-547