PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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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 nineteen 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[3]. During the ensuing 30 years, there were scattered reports of these organisms being found in the stomach of patients with benign peptic ulcers. Doenges[4] 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[5]. 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[6] 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 predisposal to ulceration. Attempts to culture this bact erium 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, Goodw 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[15,29] 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℃; growth at 30℃ but not at 25℃; variable growth at 42℃. ⑥ Microaerophilic, variable growth in air enriched with 100mL/L -CO_2 and anaerobically. ⑦ External glycocalyx produced in broth cultures. ⑧ Susceptible to penicillin, ampicillin, amoxicillin, erythromycin, gentamicin, kanamycin, rifampin and tetracycline. Resistance to nalidixic acid, cephalothin, metronidazole and polymysin. 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 a nd 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 diff erentiation and identification in future with the publi-cation of the genomic library of *H. pylori*[37].

Department of Microbiology, Faculty of Medicine, National University of Singapore, Lower Kent Ridge Road, Singapore 119260, Republic of Singapore

Correspondence to: Dr. HUA Jie-Song, Department of Microbiology, National University of Singapore, Lower Kent Ridge Road, Singapore 119260, Rep ublic of Singapore
Tel. +65·8743285, Fax. +65·7766872
Email. michuajs@nus.edu.sg
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Table 1 Locations, key morphological features and growth characteristics of *Helicobacter* species colonizing either humans and/or animals

Charastistic	*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	14-20	1-2	1	1
Distribution	Polar	Biopolar	Polar	Biopolar	Polar	Monopolar	Monopolar
Sheath	+	+	+	+	+	-	+
Periplasmic fibers	-	-	-	+	-	-	
Growth at:							
25℃	-	-	-	-	-	-	-
37℃	+	+	+	+	+	+	+
42℃	-	+	-	+	-	+	-
Growth on:							
10g/Lglycine	-		+	-	+		
15g/L NaCl	-		-	-	-		
Tolerance to:							
10g/L bile	-	+	Vary	-	-	+	
Safrain 'O'	-	-	-	-	+	-	
Methyl orange	-	-	+	-	Vary	+	
Growth under:							
Aerobic conditions	-	-	-	-	-	-	-
Microaerobic conditions	+	+	+	+	+	+	Weak+
Anaerobic	Weak+	-	-	+	-	-	+
Susceptibility to:							
Nalidixic acid	R	S	S	R	S	S	S
Cephalothin	S	S	I	S	S	R	R
Cefoperazone	S	S	S	S	S	R	
Metronidazole	S	S		S			

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

Charastistic	*H. acinonyx*	*H. bilis*	*H. bizzozeronii*	*H. cholecystus*	*H. hepaticus*	*H. nuridarum*	*H. mustelae*	*H. nemestrinae*	*H. pametensis*	*H. rodentium*	*H. salomonis*	*H. trogontum*
Host	Cheetah	Mice	Dog	Hamster	Mice	Rat, mice	Ferret	Macaque	Bird, swine	Mice	Dog	Rat
Location	Stomach	Bile,live, intestine	Stomach	Gallbladder	live, intestine	Intestine	Stomach	Stomach	Intestine	Intestine	Stomach	Intestine
Cell size (μm)	0.3×1.5 -2.0	0.5×4.0 -5.0	0.3×5 -10	0.5-0.6× 3.0-5.0	0.2-0.3× 1.5-5.0	0.5×3.5 -5.0	0.5-2.5× 5.0	0.2×2.0 -5.0	0.4-1.5	0.3×1.5 -5.0	0.8-1.2× 5.0-7.0	0.6-0.7× 4.0-6.0
Flagella												
Number	2-5	3-14	10-20	1	2	10-14	4-8	4-8	2	2	10-23	3-7
Distribution	Monopolar	Biopolar	Biopolar	Polar	Biopolar	Biopolar	Peritrich ous	Polar	Biopolar	Biopolar	Biopolar	Biopolar
Sheath	+	+	+	+	+	+	+	+	+	-	+	+
Periplasmic fibers	-	+	-	-	-	+	-	-	-	-	-	+
Growth at:												
25℃	-	-	-	-	-	-	-	-	-	-	-	-
37℃	+	+	+	+	+	+	+	+	+	+	+	+
42℃	-	+	+	+	-	-	+	+	+	+	-	+
Growth on:												
10g/Lglycine	-	+	-	+	+	-	-	-	+	+		-
15g/L NaCl	-	-		-	+	-	-	-	-	+		-
Toleranceto:												
10g/L bile		+	-	+		-	-	-			-	
Safrain 'O'						-	-	-				
Methyl orange						-	-	-				
Growth under:												
Aerobic conditions				-	-	-	-	-	-	-		-
Microaerobic conditions	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic				+	+	-	+	Weak+	Weak+	+		-
Susceptibility to:												
Nalidixic acid	R	R	R	I	R	R	S	R	S	R	R	R
Cephalothin	S	R	S	R	R	R	R	S	S	R	S	R
Cefoperazone			S			S	R	S			S	
Metronidazole	S	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	+		-	+	-		
H_2S production	-		-	-	-		-
Indoxyl acetate hydrolysis	-	+	-	-	+	-	-
Hippurate hydrolysis	-	-	-	-	-	-	+
Nitrate reduction	-	-	+	+	-	+	+
C_4 esterase	+		+		+		+
C_8 esterase lipase	+		+		+		+
Leucine arylamidase	+		-	+	-		+
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 animalsCharacteristic

	H. acinonyx	*H. bilis*	*H. bizzozeronii*	*H. cholecystus*	*H. hepaticus*	*H. muridarum*	*H. mustelae*	*H. nemestrinae*	*H. pametensis*	*H. rodentium*	*H. salomonis*	*H. trogontum*
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+
Urease activity	+	+	+	-	+	+	+	+	-	-	+	+
Oxidase activity	+	+	+	+		+	+	+	+	Weak+	+	+
Alkaline phosphatase activity	+		+	+		+	+	+	+	-	+	-
γ-Glutamyl transpeptidase activity	+		+	-		+	+		-	-	+	+
H2Sproduction		+		-	+	-	-	-	-			-
Indoxyl acetate hydrolysis	-	-	+	-	+	+	+	-	-	-	+	
Hippurate hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	+	+	+	+	-	+	-	+	+	+	+
DNase activity			+					-			+	
G+C content (mol%)	30					35	36	24	38			

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