Biotyping of *Campylobacter pylori*

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**Summary.** Ninety-one biochemical tests were done on each of 50 strains of *Campylobacter pylori* isolated from the gastric mucosa of patients with gastritis and peptic ulcers in Singapore. The API ZYM system distinguished three biotypes of *C. pylori*. The organism was found to be biochemically different from *C. jejuni* and *C. coli*. We propose a biochemical identification kit that would be of use in identifying and differentiating biotypes of *C. pylori*, and would distinguish them from *C. jejuni* and *C. coli*.

**Introduction**

*Campylobacter pylori* has emerged in the last few years as a bacterium of great interest in view of its close association with gastritis and peptic ulceration in man. Many workers have examined isolates of *C. pylori* in tests for oxidase, catalase, urease, nitrate reduction, hippurate hydrolysis, H2S production and the utilisation of carbohydrates. However, only a few workers have exploited pre-formed enzymes to study the enzymic activities and for rapid identification of *C. pylori*.

In this paper, we present a study in which biochemical tests were used to biotype *C. pylori* and to compare the biochemical characteristics of local isolates with those of six foreign strains of *C. pylori* and reference strains of other campylobacters.

**Materials and methods**

**Bacteria**

Fifty strains of *C. pylori* were isolated from endoscopic gastric biopsies of patients with gastritis and peptic ulcer, at the Singapore General Hospital and the National University Hospital, Singapore. Consent was obtained from the patients before undergoing gastroscopy. Four West German isolates, strains 1238, 1341, 1368 and 1369, were kindly supplied by Dr G. Kasper (Institut für Mikrobiologie, Munich, Federal Republic of Germany). Two Australian isolates, NCTC nos. 11637 and 11638, were kindly supplied by Dr C. S. Goodwin (Royal Perth Hospital, Australia). All the isolates of *C. pylori* were grown on moist chocolate agar (CA; Oxoid Blood Agar Base No. 2 supplemented with 5% lyced horse blood) for 3–4 days, in a humidified incubator at 37°C with CO2 5%.

Two other *Campylobacter* species were used for comparative biochemical studies. *C. jejuni* strain C31, and *C. coli* strains C127 and C128 were kindly supplied by Dr Agnes Labigne (Pasteur Institute, Paris, France). These strains and a local isolate of *C. jejuni*, were grown for 2 days on CA as described for *C. pylori*.

**Biochemical tests**

The 91 biochemical tests performed included catalase, oxidase, and a hippurate hydrolysis test; API 20NE (20 tests), API 50CH (49 tests) and API ZYM (19 tests) were used according to the manufacturer's instructions (API System S.A., La Balme Les Grottes, France).

*API 20NE (non-enteric gram-negative rods).* The API 20NE system is a standardised micromethod containing eight conventional tests and 12 assimilation tests for the identification of gram-negative rods not belonging to the family Enterobacteriaceae. The inoculum for the conventional tests was prepared by making a suspension of the test organism in 2 ml of 0.85% sterile saline supplemented with fetal calf serum (FCS; Gibco Diagnostics) 10% equivalent to 0.5 on the MacFarland scale. The inoculum for the assimilation tests was prepared by making a suspension of the test organism in 2 ml of 0.85% sterile saline supplemented with FCS equivalent to 0.5 on the MacFarland scale. The suspensions were incubated for 24–72 h at 37°C in a humidified CO2 incubator.

*API 50CH (Carbohydrates).* The API 50CH kit 5030 is a micromethod system used to study biochemical and nutritional characteristics by assessing the ability of a test organism to utilise different carbon sources by oxidative or fermentative pathways. The 49 substrates in this system are carbohydrates and derivatives (heterosides, polyalcohols, uronic acids). A suspension of campylobacter colonies was prepared in 1 ml of FCS equivalent to 0.5 on the MacFarland scale. The suspension was added into a vial of API 50CHE medium and mixed well. Alternatively, the cell suspension was
prepared in Brain Heart Infusion Broth (BHI; Gibco Laboratories) supplemented with FCS 10%. The tests for oxidation of carbohydrates were performed on API 50CH strips according to the manufacturer’s instructions. The API 50CH kit was incubated for 3–72 h in a humidified CO₂ incubator at 37°C.

API ZYM. The API ZYM kit 2520 is a semi-quantitative micromethod that allows the systematic and rapid study of 19 enzymic reactions with very small samples. A bacterial suspension was prepared in 2 ml of 0.85% sterile saline equivalent to 5.0 on the MacFarland scale. The gallery was incubated for 4 h in a humidified CO₂ incubator at 37°C. The colour intensities of the tests after adding reagents were compared against an API ZYM colour chart to determine the number of free nanomoles of product by reference to a scale.

Results

The results of the biochemical tests are summarised in Table I. The isolates of C. pylori, C. jejuni and C. coli studied were all oxidase and catalase positive. All C. pylori isolates were urease positive. C. jejuni and C. coli were urease negative. The C. jejuni strains, but not the C. pylori or C. coli strains, that we tested hydrolysed hippurate.

All the C. pylori isolates, including the two Australian and four West German strains, hydrolysed urea and aesculin and gave negative results in all the other API 20NE tests. The C. jejuni and C. coli strains reduced nitrate, but gave negative results in the other tests in the API 20NE gallery. C. pylori, C. jejuni and C. coli did not assimilate any of the carbohydrates in the API 20NE gallery.

Of the 49 substrates in API 50CH, our local C. pylori isolates metabolised aesculin and 5-keto-gluconate, but the two Australian and the four West German isolates did not. The other two Campylobacter species metabolised the two substrates weakly or not at all. None of the other carbohydrates was utilised by any of the campylobacters in 50CHE medium with or without FCS 10%.

Our API ZYM study showed that all the isolates of C. pylori produced alkaline phosphatase, acid phosphatase and leucine arylamidase, but only some of the isolates produced esterase (C4) or butyrate esterase, esterase lipase (C8) or caprylate esterase or naphthol-AS-β1-phosphohydrolase.

Three biotypes were apparent from the API ZYM results (Table II). Biotype I produced the three basic enzymes: alkaline phosphatase, acid phosphatase and leucine arylamidase. Biotype II produced naphthol-AS-β1-phosphohydrolase in addition to the three basic enzymes. Some isolates in this biotype had very weak esterase lipase activity. Biotype III produced naphthol-AS-β1-phosphohydrolase, esterase (C4) and esterase lipase (C8) in addition to the three basic enzymes.

We found that 60–0% of our local isolates belonged to biotype II and the remainder belonged equally to biotypes I and III (Table II).

The API ZYM study showed that C. jejuni and C. coli differ in their enzymic profiles from C. pylori. All the C. jejuni and C. coli strains tested had esterase lipase (C8), leucine arylamidase, weak naphthol-AS-β1-phosphohydrolase, and acid and alkaline phosphatase activities.

Table I. Biochemical characteristics of C. pylori, C. jejuni and C. coli

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>C. pylori</th>
<th>C. jejuni</th>
<th>C. coli</th>
</tr>
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<tbody>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urea hydrolysis</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metabolism of 5-keto-gluconate</td>
<td>+</td>
<td>-</td>
<td>+*</td>
</tr>
<tr>
<td>Presence of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3. Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. Esterase (C4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5. Esterase lipase (C8)</td>
<td>v</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6. Naphthol-AS-β1-phosphohydrolase</td>
<td>v</td>
<td>+*</td>
<td>+*</td>
</tr>
</tbody>
</table>

* Key: − = negative result; + = positive result; +* = weak positive result; v = variable.
BIOTYPING OF C. PYLORI

Table II. Biotypes of 50 test strains of C. pylori. The three basic enzymes common to all three biotypes are leucine arylamidase, and acid and alkaline phosphatases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Result* given by biotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esterase (C4)</td>
<td>I I I</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>- - +</td>
</tr>
<tr>
<td>Naphthol-AS-β1-</td>
<td>- + +</td>
</tr>
<tr>
<td>phosphohydrolase</td>
<td></td>
</tr>
<tr>
<td>Number of strains</td>
<td>10 30 10</td>
</tr>
</tbody>
</table>

* Key: - = negative result; + = positive result; † = some strains showed weak enzymatic activity.

Discussion

We have studied the biochemical characteristics of 50 isolates of C. pylori and compared them with those of C. jejuni and C. coli.

The rapid urease production of C. pylori distinguishes the species from nearly all other campylobacters and this characteristic can be exploited in the rapid diagnosis of C. pylori-associated gastritis. This sets C. pylori apart from C. jejuni and C. coli, as other workers have shown. C. nitrojigzis and some aquatic campylobacters possess weak urease activity. In our study, we did not isolate any urease-negative strains of C. pylori among our 50 isolates, but other workers have encountered such strains.

The C. pylori isolates and the C. coli strains that we studied failed to hydrolyse hippurate, and this is consistent with earlier findings. Failure to hydrolyse hippurate has been used to differentiate C. jejuni from other campylobacters. However, 10 of the isolates of C. pylori tested by Marshall et al. hydrolysed hippurate.

All our strains of C. pylori did not reduce nitrate; this is another biochemical characteristic that separates the other campylobacters from C. pylori. It should be noted that C. fennelliae and the aerotolerant campylobacters also do not reduce nitrate.

In this study, our strains of C. pylori and the other campylobacters metabolised aesculin and 5-keto-gluconate only. This is consistent with other reports that, in the genus Campylobacter, carbohydrates are neither fermented nor oxidised. We found that the Australian and West German strains of C. pylori differed from our local isolates, in that the former could metabolise only aesculin; it appears that there may be slight differences between isolates from different areas in this respect.

The use of API ZYM has enabled us to characterise the strains of C. pylori into three main biotypes which can be differentiated by using a rapid test kit for the six enzymes studied. The Australian and West German isolates of C. pylori are similar to our local isolates which belong to biotype II. Our local isolates metabolised 5-keto-gluconate but the foreign strains did not. The West German isolates also differed slightly from the Australian isolates in that the latter possessed very weak esterase lipase activity. Though the foreign sample size was small, this result might indicate geographical differences in the enzymic characteristics of C. pylori. A similar observation was also made by Feltham et al. who found differences between British and Peruvian isolates. Our isolates of C. pylori were not a homogeneous group, whereas those groups studied by Megraud et al. and McNulty and Dent were homogeneous. We found no correlation between the various clinical diagnoses of the patients and the biotypes of our local isolates which predominantly belonged to biotype II.

Our results show that our isolates of C. pylori are biochemically different from the strains of C. jejuni and C. coli that we have tested, and this agrees with the findings of McNulty and Dent. In enzyme tests, C. jejuni and C. coli have esterase lipase (C8) and weak naphthol-AS-β1-phosphohydrolase activities in addition to the three basic enzymes produced by C. pylori. This characteristic helps to differentiate C. jejuni and C. coli from C. pylori. The differentiation is further supported by genetic analyses indicating that C. pylori belongs to a different ribosomal ribonucleic acid sequence homology group from that of the true campylobacters.

The API ZYM tests for preformed enzymes that we used to identify and differentiate C. pylori are rapid and simple to perform. The results could be read after 4–18 h whereas conventional biochemical tests can take up to 24–72 h. Time saving was also reported with tests that used pre-formed enzyme tablets.

Our findings indicate that a biochemical identification kit for C. pylori might usefully exploit tests for oxidase, catalase, urease, hippurate hydrolysis, nitrate reduction, aesculin hydrolysis, 5-keto-gluconate assimilation, alkaline phosphatase, acid phosphatase, leucine arylamidase, butyrate esterase (C4), caprylate esterase (C8) and naphthol-AS-β1-phosphohydrolase. Such a test kit could also help to differentiate C. pylori from C. jejuni and C. coli, which are also human enteropathogens.
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REFERENCES


