

ENZYME BIOTYPES OF *HELICOBACTER PYLORI* ISOLATED FROM PENANG, PENINSULAR MALAYSIA

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Abstract. A total of 52 clinical strains of *Helicobacter pylori* were characterized on the basis of preformed enzyme production with API ZYM kits. Using the biotyping schemes as defined by Reina and Alomar (1989), Kung *et al* (1989) and Matsumoto *et al* (1996), 15.3% (8/52), 13.5% (7/52) and 11.5% (6/52) of the isolates were not biotypable, respectively. Two enzymes, valine arylamidase and cystine arylamidase could be additionally used to differentiate between isolates. Our isolates were either negative or positive for both the enzymes or positive only for cystine arylamidase. We propose the incorporation of these two enzymes into the Matsumoto *et al* (1996) biotyping scheme to biotype strains into additional enzyme biotypes.

INTRODUCTION

Helicobacter pylori was first isolated in Australia by Warren and Marshall (1983) from gastric antrum of patients with active chronic gastritis. The organism is now accepted as an etiologic agent for active chronic gastritis and peptic ulcer disease (Korman and Tytgat, 1995). Infection with *H. pylori* is causally associated with a risk of gastric carcinoma in humans (Blaser *et al*, 1995; Moller *et al*, 1995). The bacteria are also known to be heterogeneous. Studies on enzyme typing of *H. pylori* have been reported in Spain (Reina and Alomar, 1989), Singapore (Kung *et al*, 1989; Hua *et al*, 1998)), India (Sharma *et al*, 1995) and Japan (Matsumoto *et al*, 1996). In the present study, we attempted to biotype fifty-two clinical strains of *H. pylori* isolated in Penang, Peninsular Malaysia on the basis of preformed enzyme production.

MATERIALS AND METHODS

A total of 52 strains of *H. pylori* were isolated from antral biopsies of 34 patients comprising of 14 Chinese, 4 Malays, 14 Indians and 2 Bangladeshi foreign workers undergoing routine gastroduodenoscopy at the Hospital Seberang Jaya, Penang, Malaysia. These patients were confirmed endoscopically with either antral gastritis or peptic ulcer. At endoscopy, informed consent was obtained from all patients. Biopsy tissues for isolation of *H. pylori* were transported in Stuart transport medium (Oxoid, Basingstoke, UK) and macerated in 200 μ l phosphate buffered saline pH 7.2 (Behringwerke AG, Marburg, Germany). One hundred

μ l aliquots were each streaked on two plates of Eugon agar supplemented with 10% (v/v) human blood and incubated in a 10% CO₂-air atmosphere at 37°C for 4 days (Uyub *et al*, 1994). Pure cultures were tested for the presence of preformed urease in a urea test broth (BBL, Cockeysville, MD, USA).

MacFarland standard, API ZYM kit and related reagents were manufactured by BioMerioux, France. To determine the preformed enzyme profile, 96-hour cultures were suspended in 2 ml of 0.85% saline and turbidity adjusted to a scale of 5.0 on the MacFarland standard. Using a micropipette, 85 μ l of each culture were inoculated into each cupule of the API ZYM strip. The strips were incubated in a humidified 10% CO₂-air atmosphere for four hours at 37°C. Two reagents (Zym A and Zym B) were then added and the resulting color intensities developed in each cupule were compared against the API ZYM color chart.

RESULTS

In the first part of the study, 20 patients (7 Chinese, 4 Malays, 8 Indians and a Bangladeshi worker) were enrolled and a strain was isolated from each patient. In another study, 14 patients (7 Chinese, 6 Indians and a Bangladeshi worker) were enrolled and two or three strains were isolated from each, giving a total of 32 strains. Therefore, a total of 52 clinical strains were studied for their preformed enzyme production using the API ZYM kit. All the strains were urease positive.

Table 1 shows the preformed enzyme profile of the strains identified by the various biotyping schemes. The Reina and Alomar (1989) scheme

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Table 1
Preformed enzyme profile of *Helicobacter pylori* according to the various biotyping scheme.

Enzyme	Biotype according to :												
	Reina and Alomar (1989)				Kung <i>et al</i> (1989)			Matsumoto <i>et al</i> (1996)					
	I	II	III	IV	I	II	III	Ia	Ib	IIa	IIb	III	IV
Alkaline phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+
Esterase (C4)	+	-	-	-	-	-	+	+	+	-	-	-	-
Esterase lipase (C8)	+	-	-	-	-	- ^a	+	+	+	-	-	-	-
Leucine arylamidase	+	+	+	-	+	+	+	+	-	+	-	+	-
Acid phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+
Naphthol-AS-β1-phosphohydrolase	+	+	-	-	-	+	+	+	+	+	+	-	-
Total	38.5% (20/52)	46.2% (24/52)	0	0	0	48.1% 25/52	38.5% 20/52	38.5% 20/52	0	46.2% 24/52	3.8% 2/52	0	0

^a some strains were weakly positive.

identified 44 of our 52 strains with 38.5% (20/52) as biotype I and 46.2% (24/52) as biotype II. None of the strains could be biotyped as biotypes III or IV.

Table 2 shows the preformed enzyme profile of the 8 non-biotypable strains according to Reina and Alomar (1989). However, of the eight, the biotyping scheme of Kung *et al* (1989) biotyped an additional strain while that of Matsumoto *et al* (1996) biotyped two strains. Overall, out of 52 strains, 48.1% (25/52) were biotype II, 38.5% (20/52) were biotype III, and 13.4% (7/52) were unidentified according to Kung *et al* (1989).

The biotyping scheme of Matsumoto *et al* (1996) is a modification of Reina and Alomar (1989) in that biotype I was sub-divided into biotype Ia and Ib and biotype II into biotype IIa and IIb, leaving biotype III and IV undivided. Out of 52 strains, 38.5% (20/52) were biotype Ia, 46.2% (24/52) were biotype IIa, 3.8% (2/52) were biotype IIb and 11.5% (6/52) strains unidentified.

Following the Matsumoto *et al* (1996) scheme, we found no association between biotypes and endoscopic findings or between biotypes and ethnic groups but 3 out of 14 patients were found to be infected with mixed biotypes. Two patients were infected with a mixture of biotype IIa and IIb, another patient had biotype Ia and a non-biotypable strain.

DISCUSSION

In this study, the API ZYM kit was used to

Table 2
API ZYM enzyme profile of eight non-biotypable *Helicobacter pylori* strains according to Reina and Alomar (1989).

Enzyme	Non-biotypable strain			
Alkaline phosphatase	+	+	+	+
Esterase (C4)	+	-	+	-
Esterase lipase (C8)	+	+	-	-
Leucine arylamidase	+	+	+	-
Acid phosphatase	+	+	+	+
Naphthol-AS-β1-phosphohydrolase	-	+	+	+
Total	1	1 ^a	4	2 ^b

1^a identified as biotype II (Kung *et al*, 1989).

2^b identified as biotype IIb (Matsumoto *et al*, 1996).

determine the preformed enzyme profiles of 52 clinical strains of *H. pylori*. The finding that majority of our strains (86.6%) were biotypes I or II is in agreement with that of Reina and Alomar (1989) who reported that 96% of their strains as belonging to both the biotypes. But we were unable to detect biotypes III and IV while they reported the remaining 4% of their strains were equally distributed between these two biotypes.

In Singapore, Kung *et al* (1989) reported that out of 50 strains, 60% were biotype II while the remaining strains were equally either biotype I or III. In a later study, Hua *et al* (1998) found that 80% of their 69 strains were biotype II and the remaining 20% were biotype III. It appears that

Table 3
Source and preformed enzyme profile of 52 strains of *Helicobacter pylori*.

Enzymes	Biotype														
	Iaa	Iab	Iac	Ib	Ic	Id	Iea	Ieb	Iec	IIaa	IIab	IIac	IIfb	III	IV
Alkaline phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esterase (C4)	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-
Esterase lipase (C8)	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Leucine arylamidase	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-
Valine arylamidase	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-
Cystine arylamidase	+	+	-	-	-	-	+	+	-	+	+	-	-	-	-
Acid phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Naphthol-AS-β1-phosphohydrolase	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
Total number of strains	4	3	13	0	1	1	2	1	1	8	2	14	2	0	0
Source of strains															
Chinese	0	1	5	0	1	0	1	1	0	2	0	2	2	0	0
Malay	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Indian	2	1	3	0	0	1	1	0	0	3	1	0	0	0	0
Bangladeshi	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0

our findings are in agreement with both the Singaporean studies in that biotype II was most commonly isolated.

Matsumoto *et al* (1996) found that 83.5% (170/196) of their Japanese strains were not producing leucine arylamidase, which otherwise could have been identified as biotype I or II according to the Reina and Alomar (1989) scheme. They proposed six biotypes designated as Ia, Ib, IIa, IIfb, III and IV to accommodate their non-biotypable strains. Accordingly, most of their strains were biotype Ib (37%) and biotype IIfb (49.5%) while the remaining 13.5% were distributed among the other biotypes. In contrast, most of our strains were biotype Ia (36.5%) and biotype IIa (48.1%) and only 3.8% biotype IIfb, while the remaining 11.5% unidentified. Overall, there appears to be a difference in the frequency of occurrence of various biotypes between Malaysia and Japan.

Out of the six strains unidentified, one each did not produce Naphthol-AS-β1-phosphohydrolase or esterase, while the other 4 strains did not produce esterase lipase, otherwise they could have been biotyped as biotype Ia according to the identification scheme of Matsumoto *et al* (1996). We found that our strains were either positive or negative for valine arylamidase and cystine arylamidase, or positive only for cystine arylamidase, which could be additionally used to further differentiate the strains. We propose the incorporation of these two

enzymes into the Matsumoto *et al* (1996) biotyping scheme. Biotype Ia was sub-divided into Iaa, Iab and Iac while IIa into IIaa, IIab and IIac based on positivity or negativity for valine arylamidase and cystine arylamidase, or positivity for only cystine arylamidase, and biotype Ic, Id, Iea, Ieb and Iec were additional enzyme biotypes (Table 3).

With the proposed system, all of our 52 strains were biotypable but we were not able to associate biotypes and endoscopic findings. The number of patients detected with mixed infection was five using our proposed system in contrast to three using that of Matsumoto *et al* (1996) scheme. Also, we noticed that biotypes Iaa, Id and IIab were isolated from the Indian ethnic group only, while biotypes Ic, Ieb and IIfb were isolated from the Chinese, and biotype Iec from the Bangladeshi foreign worker. The more common biotypes such as Iac, IIaa and IIac were commonly isolated from the Indian and Chinese patients (Table 3). However, further studies are required before any association between biotype and ethnic groups or other factors could be well established.

In conclusion, we found some strains which were not biotypable according to the existing biotyping schemes. We propose the incorporation of two enzymes, valine arylamidase and cystine arylamidase into the Matsumoto *et al* (1996) biotyping scheme to biotype all of our strains into additional enzyme biotypes.

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