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Development of a Scheme for Genotyping *Helicobacter pylori* Based on Allelic Variation in Urease Subunit Genes

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Helicobacter pylori **urease subunit genes in 383 isolates from 10 countries were investigated by PCR-restriction fragment length polymorphism (*Hae*III) analysis. Eighty-two different *ureAB* profiles were documented by reference to known sequences. Variation among 51% of strains was accounted for by 10 predominant patterns, which provided a unique framework for categorizing isolates with geographically diverse origins.**

Helicobacter pylori is the causative agent worldwide of chronic gastritis and is a significant risk factor in the development of peptic ulceration and gastric cancer (5). Intense urease activity is a key pathogenicity factor (12), and in urease, the first genomic region to be sequenced, genes are highly conserved (2, 13, 28). Because DNA restriction digest analysis of isolates from different patients indicates a high level of intragenic diversity, the approach has been applied widely for genotyping (1, 2, 6, 7, 10, 15, 17, 18, 23–27). In view of the importance of urease as a candidate vaccine (14) and as a target for PCR specific-detection assays (15, 19), we have developed a scheme for indexing *ureAB* variation to facilitate identification of clinical isolates.

The 383 isolates of *H. pylori* were collected between 1992 and 1997 from gastric or duodenal biopsy tissue taken during routine endoscopy of patients presenting with symptoms of dyspepsia (10, 11, 18) or from the gastric juice of healthy volunteers (21). Three reference cultures (NCTC 11637, NCTC 11638, and NCTC 12455 strain 26695) were included. Isolates were mostly from United Kingdom ($n = 323$), but strains from nine other countries (Australia [3 isolates], Canada [8 isolates], China [1 isolate], Italy [4 isolates], Nigeria [7 isolates], Peru [2 isolates], South Africa [4 isolates], Turkey [26 isolates] and the United States [5 isolates]) were also included in the study to determine geographical diversity. Strains were cultured on 10% (vol/vol) Columbia blood agar at 37°C in a variable atmosphere incubator (Don Whitley Scientific Ltd., Shipley, United Kingdom) under microaerobic conditions (5% O_2, 5% CO_2, 2% H_2, and 88% N_2) and preserved at −196°C. Genomic DNA was extracted, the *ureAB* region was amplified, and restriction fragment length polymorphism (RFLP) analysis was performed as described previously (11, 18, 23). DNA fragment sizes were estimated from migration distances by using polynomial curve-fitting functions (16). Profiles were recorded in Microsoft Excel as derived fragment sizes (in base pairs) and analyzed as numerical strings according to their positions in the amplicon (Table 1). A novel Visual Basic macro (details are available from the corresponding author) was written to calculate genotype frequencies and to search for matches and partial matches with existing database profiles (9).

The 2.41-kb *ureAB* amplicon was obtained from all 383 isolates of *H. pylori*. A total of 82 *ureAB Hae*III RFLP patterns were defined and designated by a *ureAB* (uAB) profile number (uAB1 to uAB82). Examples of typical profiles are shown

TABLE 1. Details of the 10 most common *H. pylori ureAB* profiles

Profile	Band sizes[a] (bp)	Frequency[b]	Reference strain	Other designations
uAB1	315, 457, 444, 678, 515	25	NCTC 13085	60190, ATCC 49503
uAB2	315, 457, 444, 383, 295, 515	18	NCTC 11637[c]	RPH 13487, CCUG 17874, ATCC 43504
uAB3	315, 457, 1,122, 515	23	NCTC 12455	26695[d]
uAB4	772, 1,059, [63], 515	14	NCTC 12385	
uAB5	772, 827, 232, [63], 515	12	NCTC 13082	
uAB6	772, 827, 295, 515	7	NCTC 13089	
uAB10	315, 457, 827, 295, 515	11	NCTC 11638	RPH 13491, CCUG 17875
uAB11	315, 901, 615, [63], 515	9	NCTC 13090	
uAB19	315, 457, 444, 165, 450, [63], 515	7	NCTC 13088	
uAB32	772, 444, 678, 515	6	NCTC 13094	

[a] Bands are ordered from 5′ to 3′ ends according to predicted locations of *Hae*III sites within known *ureA* and *ureB* gene sequences (3, 13, 28). The 63-bp fragment (in brackets) was not detected in the RFLP analysis.
[b] Based on the numbers of infected patients and excluding multiple isolates from individual patients.
[c] Type strain of species.
[d] Genome sequence has been published (28).

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TABLE 2. Signature bands for rapid identification of the main *H. pylori ureAB* profiles

Derived band size (bp)	Band presence in profile									
	uAB1	uAB2	uAB10	uAB19	uAB3	uAB11	uAB4	uAB5	uAB6	uAB32
315	+[a]	+	+	+	+	+				
457	+	+	+	+	+					
772							+	+	+	+
444	+	+		+						+
827			+					+	+	
295		+	+						+	
678	+									+
Total no. of bands in profile	5	6	5	6	4	4	3	4	4	4

[a] +, band was present in profile. Data do not include universally conserved and unique single bands.

elsewhere (18). About 18% of the strains had unique uAB profiles, but the 10 most common profiles (Table 1) accounted for about 51% of strains. Profile descriptions were based on 17 fragments of ≤1,578 bp. The 515-bp fragment was conserved, but a scheme using seven diagnostic signature fragments for the rapid identification of the main profiles was devised (Table 2). The precise size and order of fragments in each profile were determined by using the known locations of *Hae*III sites in linear maps of three previously reported *ureAB* sequences (3, 13, 28). NCTC 12455 (strain 26695) had profile uAB3, which was in accord with the *Hae*III digest fragments predicted from the genome sequence (28). The estimated locations of the genetic events involving *Hae*III sites are listed in Table 3. Pattern uAB1 was selected as the core pattern, as it was the most frequent and gave rise to the other common patterns by a series of single or double mutations. The 315-bp region at the 5′ end of *ureA* and the 515-bp region at the 3′ end of *ureB* were highly conserved, whereas most variation occurred in the middle region of *ureB*. An analysis of strains representing the 10 common types indicated that *ureAB* profiling was more discriminatory than *ureC* (*Hha*I) and *ureC* (*Mbo*I) profiling but marginally less discriminatory than combined *ureC* (*Hha*I-*Mbo*I) profiling (25). Although *ureC* (revised designation, *glmM*) is used for genotyping, its main role is now recognized to be related to cell wall synthesis and not to urease activity (4).

Our results confirm and extend the findings of previous studies showing considerable diversity in the *ureAB* region of *H. pylori* isolated from different individuals (2, 18). All strains produced functionally highly active urease, and mutations within the subunit genes were synonymous for the three sequenced strains. The effect of such variations on urease antigenicity is unknown but may be of practical significance when considering the effectiveness of urease-based vaccines in diverse and heterogeneous human populations.

A novel finding was that about half the strains were represented by just 10 *ureAB* RFLP profiles, which indicated a higher degree of conservation than expected. Furthermore, *H. pylori* with the same urease profile infected unrelated individuals, and in some cases, isolates were from widely separated parts of the world. Although it is generally believed that a large and very genetically diverse population of *H. pylori* circulates in the community, certain genomic markers, such as *cagA* and *vacAs1*, are widely distributed and partially conserved (8, 22), and it would appear that this is also true for certain *ureAB* polymorphisms. Further evidence of this conservation was apparent from published data on strains isolated in the United States showing profiles closely matching uAB3 and uAB5 (6). The availability of *H. pylori* urease gene sequences enabled precise sizes to be assigned to each RFLP profile and an exact genomic location to be identified. This novel development of RFLP analysis has considerable potential for studying allelic variation in other regions of the *H. pylori* genome, such as the highly polymorphic *vacA* locus (22).

In conclusion, we found that *Hae*III RFLP analysis was an easy-to-perform, reproducible, and discriminatory means of documenting diversity within the *H. pylori ureAB* operon. Furthermore, differences were independent of variation in other loci associated with pathogenicity, such as *cagA*, *vacA*, 48-kDa *HtrA*, and 26-kDa antigen genes, although analysis of such loci with multiple restriction enzymes could be applied to further discriminate strains when necessary (2, 20). The scheme was developed mainly on U.K. isolates of *H. pylori*, but it appears to be equally applicable to isolates from other parts of the world and thus provides a practical general purpose genotyping framework to facilitate interlaboratory comparability.

TABLE 3. Analysis of genetic events within the *ureAB* operon of *H. pylori*

Change in uAB profile	Genetic event[a]	Location of event (bp)	
		Fragment(s)	Position (operon)
1→2	+	678	1599 (*ureB*)
1→19	+	678	1381 (*ureB*)
	+	678	1831 (*ureB*)
1→3	−	444, 678	1216 (*ureB*)
1→11	+	678	1831 (*ureB*)
	−	457, 444	772 (*ureA*)
2→10	−	315, 457	1216 (*ureB*)
10→6	−	772, 827	315 (*ureA*)
6→5	+	295	1831 (*ureB*)
5→4	−	827, 232	1599 (*ureB*)
4→32	−	63, 615	1831 (*ureB*)
	+	1059	1216 (*ureB*)
32→1	+	772	315 (*ureA*)

[a] Restriction site gained (+) or lost (−).

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