Evidence for the Occurrence of the Same Strain of *Campylobacter pylori* in the Stomach and Dental Plaque

B. SHAMES, S. KRAJDEN, M. FUKSA, C. BABIDA, AND J. L. PENNER*

Department of Microbiology, University of Toronto, Toronto, Ontario, M5G 1L5, and Department of Microbiology, St. Joseph's Health Centre, Toronto, Ontario, M6R 1B5, Canada

Received 26 June 1989/Accepted 17 August 1989

Restriction endonuclease analysis with *HindIII*, *HaeIII*, and *BglII* endonuclease of DNA extracted from each of eight colonies of *Campylobacter pylori* subcultured from a stomach biopsy and from each of eight colonies subcultured from dental plaque of the same patient indicated that at least three strains were present in the dental plaque but only one strain was present in the biopsy. One of the dental strains had restriction patterns indistinguishable from those of the biopsy isolate, providing evidence that both sites were infected with the same strain of *C. pylori*.

In a recent publication describing the examination of human stomach biopsies, saliva, and dental plaque for *Campylobacter pylori*, one patient was found to have the organism both in his stomach and in subgingival plaque (1). Growth from the plaque was of particular interest, since C. pylori has only rarely been isolated from sites other than the stomach. The subject of this study was the examination of *C. pylori* strains from the stomach and plaque to determine if they were epidemiologically linked.

The cultures from the stomach and the dental plaque were subcultured on Columbia blood agar in a microaerophilic atmosphere (5% O₂, 10% CO₂) at 37°C for 72 h. Eight colonies cultured from the stomach and plaque specimens were isolated and resubcultured until three to five plates of each colony type (clone) were available for restriction endonuclease analysis (REA).

The plates were scraped, and the cells (approximately 250 mg) were washed by centrifugation (8,700 × g for 10 min at 4°C) twice in phosphate-buffered saline (pH 7.2) and twice in TE (10 mM Tris hydrochloride, 100 mM sodium EDTA [pH 8.5]). The pellet was suspended in 4 ml of TE, and lysozyme (type II; Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 3.0 mg/ml. The suspension was incubated for 12 min at 37°C and then lysed in sodium dodecyl sulfate (final concentration, 1%). The lysate was incubated for 60 min at 37°C in the presence of RNase A (bovine pancreas; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) at a final concentration of 50 ng/ml. Pronase (final concentration, 0.8 mg/ml; Boehringer Mannheim) and proteinase K (final concentration, 0.5 mg/ml; Boehringer Mannheim) were added, and the lysate was incubated overnight at 37°C. DNA was extracted with an equal volume of phenol-chloroform (1:1, vol/vol) (phenol, recrystallized and ultra pure [IBI]), which was previously equilibrated in 20 mM Tris hydrochloride, pH 7.8 and precipitated overnight in 0.3 M sodium acetate and 2.1 volumes of absolute ethanol at −20°C. The DNA was pelleted by centrifugation at 12,100 × g for 60 min at 4°C. The ethanol was decanted, and the pellet was air dried and redissolved in sterile distilled water. A sample was taken, and the A₂₆₀ and A₂₈₀ were measured in a spectrophotometer (model DB-G; Beckman Instruments, Inc., Fullerton, Calif.) to ascertain purity and for quantitation.

Approximately 20 μg of chromosomal DNA from each *C. pylori* isolate was digested in an excess of enzyme for 4 h at 37°C in *HindIII*, *HaeIII*, and *BglII* (Boehringer Mannheim) as outlined by the manufacturer. The reaction was terminated by the addition of gel loading buffer (40% sucrose, 0.25% bromphenol blue), and DNA fragments were separated by gel electrophoresis in a 0.8% horizontal agarose gel overnight (16 h) at 30 V in one part TAE buffer (40 mM Tris hydrochloride, 20 mM sodium acetate, 2 mM EDTA [pH 7.6]). The gel was stained for 60 min in ethidium bromide (1 μg/ml), destained for 60 min in distilled water, and photographed by filtered (red filter no. 25A [Vivitar] and yellow filter no. Y48-Y2 [Toshiba] combination) UV illumination on P/N type 665 film (Polaroid). Molecular weight markers were obtained from Bethesda Research Laboratories, Gaithersburg, Md.

No evidence of plasmid DNA was found in any of the 16 colonies when undigested DNA was electrophoresed under the conditions described above. DNA from the colonies subcultured from the biopsy specimen gave virtually identical restriction patterns when digested with *HindIII*, indicating that all eight colonies were progeny of the same strain. Restriction patterns obtained with *HaeIII* and *BglII* confirmed the interpretation made from the results with *HindIII*. However, three distinctly different patterns were obtained when DNA preparations from one colony (colony A) yielded one restriction pattern, DNA from a second (colony B) yielded another pattern, and DNA from the remaining six colonies gave a third pattern. Hence it was evident that at least three genetically different strains of *C. pylori* were present in the specimen from the dental plaque. This was confirmed by using the *HaeIII* enzyme, but only two restriction patterns were seen among the DNA preparations digested with *BglII*: one pattern for colony A and another for the seven other colony subcultures.

To compare the isolate from the stomach biopsy with the isolates from the dental plaque, DNA from one colony of the stomach biopsy and DNA from each of three colony types of dental plaque were digested with *HindIII* and electrophoresed in the same gel (Fig. 1A). The isolate from the biopsy specimen (Fig. 1A, lane 1) was found to have a

* Corresponding author.
restriction pattern very similar, if not identical, to that of one colony from the dental plaque (Fig. 1A, lane 2) and quite unlike the patterns of two other dental plaque colonies (Fig. 1A, lanes 3 and 4). It was therefore evident that at least one isolate from the plaque was genetically closely related or identical to the strain from the stomach. This finding was confirmed with the use of the other two endonucleases (Fig. 1B and C).

There are no established systems for serotyping or biotyping strains of *C. pylori*, and investigators interested in the epidemiology of the infections have found that REA is the most satisfactory alternative for this species at present (2, 3). In this study we have confirmed that REA is applicable for this purpose. Moreover, we have shown that it is important to examine more than one colony from the specimen. In our case, the biopsy specimen was subcultured for purity prior to its examination by REA, and thus the possibility that the original specimen may have had more than the one strain that was detected by REA exists. The novel finding from the standpoint of epidemiology, however, is that dental plaque may be a site for *C. pylori* and that it may harbor a strain that occurs in the stomach of the same individual. In the earlier study, it was reported that *C. pylori* was isolated from the stomach for 29 of 71 patients examined and that only one (3.4%) of the 29 patients had the organism present in dental plaque (1). It should be noted that the dental plaque specimens were always taken prior to the gastric biopsy to exclude the possibility of contamination of the tooth surface with *C. pylori* during the withdrawal of the endoscope. Whether dental plaque represents a common or rare ecological niche for this organism has not been established in this limited study, but the finding should encourage the undertaking of systematic investigations of this site and other possible sources to gain further insight into the epidemiology of *C. pylori* infections.

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**LITERATURE CITED**

