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Biotype-Specific Probe for *Vibrio cholerae* Serogroup O1

RICHARD A. ALM† AND PAUL A. MANNING*

Department of Microbiology and Immunology, The University of Adelaide, G.P.O. Box 498, Adelaide, South Australia 5001, Australia

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The O1 serogroup of *Vibrio cholerae* can be divided into two biotypes, El Tor and Classical. Current tests to distinguish between these biotypes are often difficult to interpret. On the basis of the difference in sequence of the *hlyA* gene in these biotypes, we have developed a simple probe that can easily and reliably differentiate between the two biotypes.

The two biotypes of *Vibrio cholerae* serogroup O1, Classical and El Tor, were originally distinguished on the basis of their abilities to produce a soluble hemolysin which is secreted into the extracellular medium (4). Strains of the Classical biotype are invariably nonhemolytic; however, El Tor strains now appear to be variable in their hemolytic phenotype (3). In fact, El Tor strains can interconvert between being hemolytic and nonhemolytic (5).

The structural gene *hlyA* for the hemolysin has been characterized and sequenced from both biotypes (1, 7). Nucleotide sequence comparison of *hlyA* genes from both Classical strain 569B and El Tor strain O17 revealed the presence of an 11-base-pair deletion in strain 569B that results in a frameshift and generates a stop codon. This produces a truncated protein product of 27 kilodaltons in strain 569B, rendering it nonhemolytic, unlike the wild-type 82-kilodalton hemolysin (Fig. 1). Interestingly, the deleted region has an inverted repeat arrangement that may have been the basis for its generation.

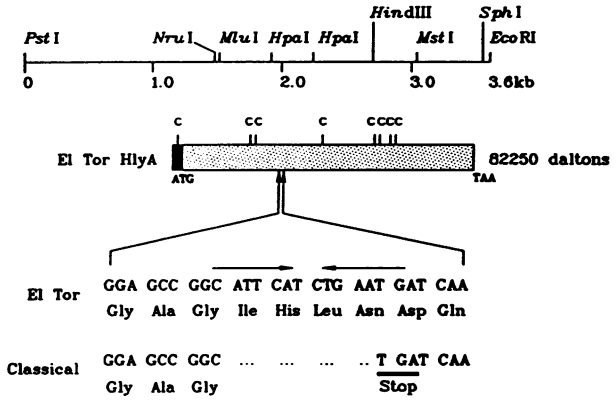

FIG. 1. Map of the region of DNA containing the *hlyA* gene of *V. cholerae* showing the differences between strains O17 (El Tor) and 569B (Classical). The stretch of 11 base pairs deleted in the Classical strain that results in a truncated protein product is indicated. The inverted repeat homology within this region is designated by the head-to-head arrows.

Using an Applied Biosystems 381A DNA synthesizer, we constructed a 19-base-pair synthetic oligodeoxynucleotide that spans this 11-base-pair deletion with 4 bases on either side. This oligodeoxynucleotide (5′-CGGCATTCATCTGA ATGAT-3′) was then radiolabeled using polynucleotide kinase and [α-^{32}P]dATP by the method of Maniatis et al. (6) and was used to probe whole genomic DNA from 118 *Vibrio* strains, including non-O1 environmental isolates and non-*V. cholerae* vibrios. Figure 2 shows a nitrocellulose filter containing genomic DNA of a selection of strains (Table 1) that

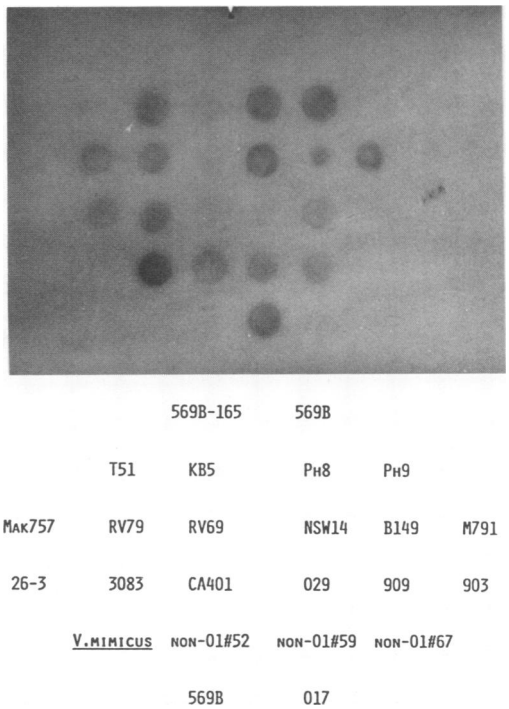

FIG. 2. Autoradiograph of a nitrocellulose filter that shows, after lysis with 0.5 M NaOH and baking at 80°C for 2 h, a sample of strains that were probed with the labeled oligodeoxynucleotide that spans the 11-base-pair deletion. Hybridization was performed at 42°C overnight and was followed by two 30-min stringency washes at 42°C in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (6). The strains, together with their biotypes and hemolytic status, are listed in Table 1.

* Corresponding author.

† Present address: Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2.

TABLE 1. Phenotype of strains shown in Fig. 2

Strain	Biotype	Hemolysin production	Source
569B-165	Classical	No	K. Bhaskaran
569B	Classical	No	I. Huq
T51	El Tor	No	K. Bhaskaran
KB5	Classical	No	K. Bhaskaran
Ph8	El Tor	Yes	K. Bhaskaran
Ph9	El Tor	Yes	K. Bhaskaran
MAK757	El Tor	No	B. C. Deb
RV79	El Tor	No	C. Parker
RV69	Classical	No	C. Parker
NSW14	El Tor	Yes	P. Desmarchelier
B149	El Tor	Yes	P. Desmarchelier
M791	El Tor	Yes	J. Mekalanos
26-3	El Tor	Yes	R. Finkelstein
3083	El Tor	Yes	R. Finkelstein
CA401	Classical	No	R. Finkelstein
029	Classical	No	J. E. Ogg
909	El Tor	Yes	J. E. Ogg
903	Classical	No	J. E. Ogg
V. mimicus		Yes	B. C. Deb
Non-O1 52		Yes	P. Desmarchelier
Non-O1 59		Yes	P. Desmarchelier
Non-O1 67		Yes	P. Desmarchelier
O17	El Tor	Yes	W. F. Verwey

were probed with this oligodeoxynucleotide. The results show that all El Tor isolates regardless of their hemolytic phenotype show homology to this probe, whereas the Classical isolates do not. This indicates that the genetic basis for the nonhemolytic phenotype in Classical and El Tor strains is clearly different.

The *V. cholerae* serogroup O1 El Tor hemolysin has been shown to be immunologically related to the hemolysin produced by non-serogroup O1 *V. cholerae* isolates (8). Furthermore, the genes that encode these proteins display very strong DNA homology (2). The probe binds to all the non-O1 isolates tested. Among other vibrios, *Vibrio mimicus* displays strong homology to the probe (Table 2).

These data imply that all Classical strains tested have the same deletion mutation in the *hlyA* gene and that this 19-base-pair oligodeoxynucleotide probe is more effective for use in distinguishing between the two biotypes of *V. cholerae* serogroup O1 than the other commonly used methods, which are less reliable and often difficult to interpret.

TABLE 2. Summary of *Vibrio* strains tested

Strain	Biotype	Hemolytic status	No. reacting/ total no. tested
V. cholerae O1	El Tor[a]	Yes	45/45
V. cholerae O1	El Tor[b]	No	9/9
V. cholerae O1	Classical[c]	No	0/32
V. cholerae non-O1		Yes	27/27
V. mimicus		Yes	2/2
V. fluvialis		Yes	0/1
V. parahaemolyticus		Yes	0/2

[a] Includes seven nontoxigenic environmental isolates from Australia and Brazil and seven field isolates from Afghanistan and the Philippines.
[b] Includes six isolates from the current pandemic. Four strains were isolated in Calcutta, India, and two were isolated in Bangladesh.
[c] Includes two isolates from the current pandemic in Bangladesh.

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