Oligonucleotide Probe for Detection of the Enteropathogenic *Escherichia coli* (EPEC) Adherence Factor of Localized Adherent EPEC

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The enteropathogenic *Escherichia coli* adherence factor (EAF) probe detects isolates of enteropathogenic *E. coli* that exhibit localized adherence to HEp-2 cells. A 21-base oligonucleotide probe was constructed on the basis of a sequence from within the 1-kb EAF probe and was shown to have greater sensitivity and specificity than the EAF fragment probe in detecting localized adherent *E. coli*.

Enteropathogenic *Escherichia coli* (EPEC) is a significant cause of acute and chronic infant diarrhea in the developing world. Once a serious cause of "summer diarrhea" and nosocomial outbreaks in hospital nurseries in industrialized countries, diarrhea due to EPEC now occurs less frequently, although outbreaks in nurseries and day care centers are reported occasionally (8, 19).

Historically, EPEC has been defined as *E. coli* strains of certain O:H serotypes that do not produce the heat-labile or heat stable enterotoxins of enterotoxigenic *E. coli* (ETEC) or Sereny test invasiveness of enteroinvasive *E. coli* (EIEC) (8, 19). More recently, the ability of EPEC isolates to adhere in vitro to HEp-2 and HeLa tissue culture cells in a manner described as localized adherence has been used in conjunction with serotyping to identify an EPEC strain (3, 7, 10, 21). This adherence pattern is characterized by clusters of bacteria adhering to localized regions of the tissue culture cell. Localized adherence is a phenotype characteristic of *E. coli* strains of EPEC serotypes, as shown in a large survey of *E. coli* isolates typed for both their O and H antigens in which the localized-adherence phenotype was found in 93% of *E. coli* isolates of EPEC serotypes compared with only 14% of those of non-EPEC serotypes (20).

The ability of EPEC strains to adhere to HEp-2 cells in the localized pattern is associated with a high-molecular-weight plasmid (1) which is necessary for full virulence in volunteers (9). In one such plasmid, pMAR2, from EPEC strain E2348/69, the sequences required for localized adherence have been mapped to within a 35-kb region by transposon mutagenesis and subcloning experiments (2, 17). A 1-kb *Bam*HI-*Sal*I fragment taken from this region of pMAR2 has been cloned and used as a DNA probe to identify EPEC strains which exhibit localized adherence to tissue culture cells. This probe, called the EPEC adherence factor (EAF) probe, has been shown to be 99 to 100% sensitive and specific in predicting localized adherence to HEp-2 or HeLa cells (15, 18).

In this study, we report the development of EAF21, a 21-base oligonucleotide probe which corresponds to a sequence from within the 1-kb EAF probe. Synthetic oligonucleotide probes have several advantages over cloned restriction fragment probes, including more rapid preparation of

high probe concentrations, increased rates of hybridization to target DNA, higher sensitivity due to the presence of only a single strand which is complementary to the target DNA, and absence of potential vector contamination. The 1-kb *Sal*I-*Bam*HI fragment of pMAR2 was previously cloned into pCVD315 (6) to create pJPN16 (J. P. Nataro, unpublished data). The nucleotide sequence of approximately 100 bases of each end of the 1-kb *Sal*I-*Bam*HI EAF fragment was determined by double-stranded sequencing (4) from the *Sal*I and *Bam*HI sites of the EAF fragment by using primers corresponding to the *Hind*III and *Eco*RI sites of pCVD315, respectively. A 21-base oligonucleotide called EAF21, corresponding to the EAF sequence near the *Sal*I site, and a 25-base oligonucleotide called EAF25, corresponding to the EAF sequence near the *Bam*HI site, were synthesized by using a VEGA C300 DNA synthesizer as previously described (22). The nucleotide sequences of EAF21 and EAF25, respectively, are *Sal*I 5' TATGGGGACCATGTAT TATCA 3' and *Bam*HI 5' ACCTGGATCGCCAATGTTCT TGGCG 3'.

Initial experiments with EAF21 and EAF25 revealed good correlation between EAF21 and the EAF fragment probe but not with EAF25, which was therefore not tested further. We then tested 234 *E. coli* isolates of EPEC serogroups, 131 untyped *E. coli* isolates from infants with diarrhea, 38 isolates of ETEC, 57 isolates of enterohemorrhagic *E. coli* (EHEC), 23 isolates of EIEC, and 31 *E. coli* isolates from healthy adults for the ability to hybridize with the EAF fragment and EAF21 oligonucleotide probes. All isolates were obtained from the collections of the Center for Vaccine Development at the University of Maryland, Baltimore.

The 1-kb EAF probe was labeled with α -³²P by random priming (5), and the EAF21 oligonucleotide probe was end labeled with γ -³²P (12). Colony blots were prepared on Whatman 541 filters (13) and hybridized to the EAF fragment under high stringency (14) or to the EAF21 probe, which was hybridized as follows. Blots were preincubated in hybridization solution containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (pH 7.0), 5× Denhardt solution (10× Denhardt solution is 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 1 mM EDTA (pH 8.0), 0.1% sodium dodecyl sulfate, and 100 μ g of salmon sperm DNA per ml for 1 h at 50°C. The probe was added (2 × 10⁶ cpm per 20 filters), and the blots were incubated at 50°C overnight. On the following day, the blots were washed

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TABLE 1. Hybridization of EAF fragment and EAF21 oligonucleotide probes to *E. coli* isolates of EPEC serogroups, untyped *E. coli* isolates from infants with diarrhea, ETEC, EIEC, EHEC, and normal-flora *E. coli* isolates from healthy adults

<i>E. coli</i> type ^a	Hybridization		No. of strains	No. strains with LA ^b
	EAF	EAF21		
EPEC	+	+	153	ND
	—	—	78	ND
	+	—	1	0
	—	+	1	1
	±	+	1	1
Untyped	+	+	58	ND
	—	—	64	ND
	+	—	4	0
	±	+	1	1
	±	—	4	0
ETEC	—	—	30	ND
	±	—	8	0
EHEC	—	—	56	ND
	±	—	1	0
EIEC	—	—	18	ND
	±	—	5	0
Normal flora	—	—	31	ND

^a Isolates were identified as ETEC, EHEC, or EIEC by DNA probes as previously described (11).

^b LA, Localized adherence; ND, not determined.

three times for 15 min each time at 50°C in 6× SSC–0.1% sodium dodecyl sulfate and then rinsed briefly in 2× SSC at room temperature. After air drying, the blots were exposed to X-ray film and developed.

As shown in Table 1, 153 *E. coli* isolates of EPEC serogroups and 58 untyped *E. coli* isolates from infants with diarrhea hybridized with both the 1-kb EAF fragment and the EAF21 oligonucleotide probe; 78 isolates of EPEC serogroups and 64 untyped isolates did not hybridize with either probe. Thus, for these 353 strains, complete agreement was found between the EAF21 and EAF fragment probes.

Discrepant results, however, were found for 15 strains. These strains were further tested for the ability to demonstrate localized adherence to HEP-2 cells as previously described (16). A greater specificity was demonstrated by the EAF21 oligonucleotide probe than the EAF fragment probe in that one isolate of EPEC serogroup O126 and four untyped isolates hybridized with the EAF fragment probe but did not hybridize with EAF21 nor demonstrate localized adherence to HEP-2 cells. The oligonucleotide probe also demonstrated higher sensitivity than the EAF fragment in that it hybridized to an O55 isolate that exhibited localized adherence to HEP-2 cells, while the EAF fragment probe did not.

It should also be noted that, in general, the oligonucleotide probe produced clearer results than the EAF fragment in that occasional ± “shadows” of hybridization were seen on filters incubated with the EAF fragment (Fig. 1). Two isolates (one untyped and one of EPEC serogroup O128) repeatedly gave a ± result with the EAF fragment but a clearly positive result with EAF21. Both of these isolates demonstrated localized adherence to HEP-2 cells. Four untyped strains also gave a ± result with the EAF fragment

FIG. 1. Autoradiograph of a filter hybridized with the EAF fragment probe as described in the text. In contrast to the strong positive result produced by EPEC strain E2348/69 (position A2) and the negative results produced by ETEC isolates at positions A1, A3, A4, B1, and B3, ± or shadow hybridization occurred between the EAF fragment and two ETEC isolates (positions B2 and B4). These two isolates produced clearly negative results when incubated with the EAF21 oligonucleotide probe (data not shown).

on repeated hybridizations but did not hybridize with EAF21. None of these strains demonstrated localized adherence to HEP-2 cells.

The occurrence of fewer ambiguous results with the oligonucleotide probe was more apparent when ETEC, EHEC, and EIEC isolates were tested. As shown in Table 1, eight ETEC isolates, one EHEC isolate, and five EIEC isolates gave ± shadow results with the EAF fragment probe. None of these isolates hybridized with EAF21, and none demonstrated localized adherence to HEP-2 cells.

In summary, oligonucleotide probe EAF21 was more sensitive and specific than the EAF fragment probe and gave fewer ambiguous results. This probe will be useful in epidemiological studies in which the ability to demonstrate localized adherence to HEP-2 cells is used to identify EPEC isolates.

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