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DNA Probes for K-Antigen (Capsule) Typing of *Escherichia coli*

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DNA restriction fragments derived from the polysaccharide biosynthesis regions of cloned *Escherichia coli* K1, K5, and K12 capsular antigen genes hybridized only with DNA of strains determined by conventional methods to be of the same K serotype. A probe derived from the common transport region hybridized to all encapsulated *E. coli* strains.

Strains of *Escherichia coli* isolated from human extraintestinal infections frequently possess acidic polysaccharide capsules known as K antigens (15, 19). These are assumed to be virulence factors and are thought to act by protecting the cell against the bactericidal action of complement and phagocytes (15, 19, 24). However, although about 70 different K antigens have been identified in *E. coli*, only a few are frequently found associated with invasive strains (19, 20).

Routine identification of specific K types is conventionally carried out primarily by serological techniques, countercurrent immunoelectrophoresis being the method of choice (13, 18). K1, K5, and K12 are among the most frequently encountered K types associated with isolates from human upper urinary tract infections (16, 19), and the K1 capsule is in addition produced by more than 80% of strains causing neonatal meningitis (21). However, although K12 antiserum is readily available, K1 and K5 are very poor immunogens, probably because they resemble host structures (7, 14), and therefore only low-titer polyclonal antiserum against these antigens is available. Recently, however, high-titer immunoglobulin G monoclonal antibodies were prepared against K1 (8), and immunoglobulin M anti-K5 monoclonal antibodies have also been reported (1).

Bacteriophages that specifically lyse strains possessing K1 or K5 capsules are also used in typing (10, 12). At present it is necessary to use a combination of serological and phage typing methods to obtain unequivocal identification of K1 isolates, since certain isolates may be positive in one or other of the tests but not both (4, 9). In addition, it is possible that other surface structures interfere with capsule determination by phage typing, perhaps by shielding phage attachment sites, or that some K antigens, for example, K5 and K95, act as receptors for the same phage in the absence of a serological cross-reaction. However, preliminary studies in which plasmid DNA carrying the entire gene cluster for K1 expression was used as a probe suggested that screening of clinical isolates by hybridization might overcome such difficulties (9).

In this paper we describe colony hybridization (11) typing with defined K-antigen-specific probes derived from plasmid subclones of cosmids. The genetic determinants for production of the K1, K5, K7, K12, and K92 capsular polysaccharides have recently been cloned and physically mapped (2, 5, 22; I. Roberts, R. Mountford, R. Hodge, K. Jann, and G. Boulnois, J. Bacteriol., in press). The organization of the genes for the synthesis and surface assembly of capsular polysaccharides is the same for all K antigens so far examined. There are three functional regions (Fig. 1); region 1 specifies functions required for translocation of the polysaccharide from the periplasm to the cell surface, region 2 encodes enzymes for sugar biosynthesis and polymerization, and region 3 may be involved in a postpolymerization modification of the polysaccharide. To determine the efficacy of colony hybridization in identifying K antigens, we used DNA probes derived from the antigen-specific regions (region 2) of the cloned K1, K5, and K12 determinants and the common transport region (region 1) from the K1 clone to screen clinical isolates of *E. coli* whose K serotypes had been determined by conventional methods.

Strains used in this study (total number, 128) were isolated in Finland (23, 25) or the Federal Republic of Germany (9) from patients with urinary tract infections, meningitis, septicemia, or other septic infections and from the feces of healthy individuals. An alternative approach of attempting to collect one example or a few examples of every K serotype was rejected in favor of using well-characterized collections of isolates of various clonal groups in which only 11 capsular types (presumably the commonest in human extraintestinal infections) were represented (see Table 1). Serotyping and phage typing were performed by the standard methods used by the State Serum Institute, Copenhagen, Denmark (18). Strains were screened by colony hybridization (11) as described previously (3). Briefly, isolates were inoculated in a regular pattern on nitrocellulose filters placed on the surfaces of nutrient agar plates and were grown overnight at 37°C. Bacterial growth was lysed by exposure to alkali, and released DNA was immobilized on the filters by baking the filters at 80°C for 2 h; cell debris was removed by washing the filters gently with hot water. Probe fragments (Fig. 1) were excised from low-melting-temperature agarose gels and radiolabeled by the random priming method of Feinberg and Vogelstein (6). After hybridization overnight at 65°C (11), the filters were washed extensively in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) as described previously (3).

There was complete agreement between the results of colony hybridization screening and the standard tests for the determination of capsular antigens (Table 1). Thus, among the strains tested, the biosynthesis genes were serotype

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specific at the level of DNA sequence homology (note that the K5-specific probe did not hybridize with the DNA of K95 strains, which are sensitive to K5-specific phages). The K1-specific probe may hybridize with the DNA of K92 strains (5), but this is unlikely to cause significant practical problems, as K92 strains are rarely encountered. More importantly, our results show that strains of *E. coli* possess genes for the synthesis of only a single K antigen rather than a battery of determinants of which only one is expressed at a time. On the other hand, the transport region is apparently well conserved, the probe hybridizing to the DNA of strains of all K serotypes screened but not to the DNA of any strains that lacked capsules. *E. coli* determinants for K1 production do not hybridize with the DNA of *Neisseria meningitidis* group B, which synthesizes a capsule chemically identical to the K1 polysaccharide (5). However, because our collections of strains comprised only a limited number of K serotypes, we were unable to test the possibility of DNA homology between strains of serotypes for which a strong reciprocal cross-reactivity has been noted (such as K12 and K82 or K92 and the *N. meningitidis* group C polysaccharide). We also probed strains of several gram-negative bacteria, some of which form capsules (*Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Shigella flexneri*, *Salmonella hadar*, *Klebsiella pneumoniae*, *K. aerogenes*, and *Vibrio cholerae*), but found no detectable homology.

Colony hybridization has several advantages over traditional methods of antigen typing. It obviates the need to generate high-titer antisera or phage preparations and is particularly useful for the screening of large numbers of clinical isolates. In some cases it will resolve problems of immunological cross-reactions between antigens or of nonreactions with antisera or phages because of variable environmental conditions affecting K-antigen expression. The common transport probe can be used to detect encapsulated *E. coli* from invasive infections, and the organisms may then be differentiated by using the various antigen-specific probes. In principle, it should be easy to obtain specific probes for all K antigens by screening cosmid libraries constructed from the relevant strains with the transport probe (22).

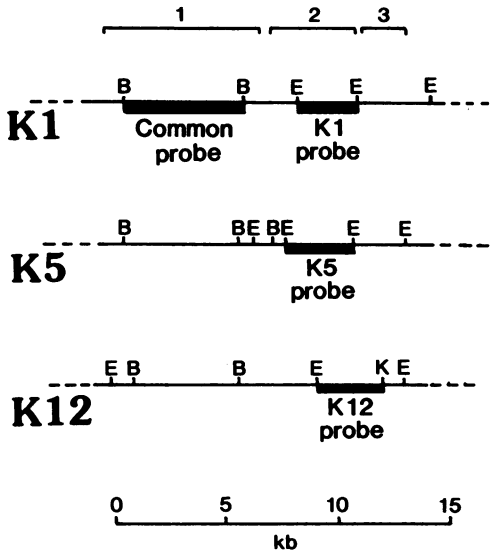

FIG. 1. Partial restriction maps of the K1, K5, and K12 genetic determinants of *E. coli*. The scale shows sizes in kilobases (kb); B, E, and K indicate cleavage sites for *Bam*HI, *Eco*RI, and *Kpn*I, respectively. Brackets 1, 2, and 3 represent the approximate extents of the three functional regions in K1 capsule synthesis and assembly, as described in the text. Thick lines indicate restriction fragments used as probes: the K1-specific probe is a 2.8-kb *Eco*RI fragment of plasmid pKT274, and the K5 probe is a 3.1-kb *Eco*RI fragment of plasmid pGB118; double digestion of plasmid pGB121 with *Eco*RI and *Kpn*I was used to derive the 3.0-kb K12 probe. A 5.5-kb *Bam*HI fragment of the K1 clone pKT274 was used to define common determinants involved in the translocation of capsular components to the cell surface. All plasmids have been described in detail elsewhere (2, 5, 22).

TABLE 1. Colony hybridization screening with capsule-specific probes of *E. coli* isolates of conventionally determined K serotypes

DNA probe[a]	Hybridization with indicated serotype[b]				
	K1 (n = 81)	K5 (n = 11)	K12 (n = 6)	Other[c] (n = 19)	Non-K (n = 11)
K1	+	−	−	−	−
K5	−	+	−	−	−
K12	−	−	+	−	−
Common	+	+	+	+	−

[a] Probes are described in Fig. 1.

[b] +, Hybridization; −, no hybridization. Serotypes were determined by the methods of Ørskov and Ørskov (18).

[c] Includes (n) K2 (8), K13 (4), K14 (1), K52 (1), K53 (1), K93 (1), K95 (2), and K100 (1).

We have evaluated the method described here for the rapid detection of encapsulated *E. coli* in blood or urine samples. If small volumes are available, aliquots of about 10 μl can be spotted onto nitrocellulose filters on nutrient agar plates and incubated overnight at 37°C for processing as described above. Alternatively, it is possible to avoid the growth step if larger samples are available. We were able to detect as few as 10^4 encapsulated bacteria per ml in a total sample of 10 ml collected by suction onto a membrane filter; bacteria were lysed as described above without prior incubation to allow growth and were detected after only 4 h of hybridization at 65°C with the ^{32}P-labeled transport probe. Further evaluation of the system clearly needs to be done in the context of a diagnostic laboratory, but we propose that composite probes containing K1-, K5-, and K12-specific sequences could be similarly used to screen urinary tract isolates. Because of the potential of these serotypes to cause pyelonephritis, patients infected with strains whose DNA hybridized to the probes could be monitored closely, even if at the time of isolation organisms were present in numbers traditionally considered nonsignificant (<10^5 CFU/ml) (17).

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