NOTES

Biotype Determinations for Monitoring of EK1 Host Strains Used in Recombinant DNA Research

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The biotypes of eight *Escherichia coli* K-12 strains were determined. Eight different biotypes were observed. Based upon the API biotype computer file, the appearance frequency for these K-12 biotypes ranged from 1/127 (infrequent) to 1/18,046 (rare).

On 7 February 1977, the Cambridge City Council passed the Ordinance for the Use of Recombinant DNA Molecule Technology in the City of Cambridge. This ordinance, in addition to placing the National Institutes of Health guidelines into law, defined the responsibilities of institutions in the city of Cambridge which desired to engage or were engaged in DNA research activity.

In section II-7, part 5, it states that "as part of the institution's health monitoring responsibilities it shall in good faith make every attempt, subject to the limitation of available technology, to monitor the survival and escape of the host organism or any component thereof in the laboratory worker. This should include whatever means is available to monitor the intestinal flora of the laboratory worker."

How does one approach personnel or environmental monitoring of recombinant hosts? Although methods exist for the detection of airborne (3, 4, 8) and laboratory surface contamination (6, 9, 12, 13), as well as for screening clinical specimens (feces, throat, nasopharynx, etc.) for bacteria (2), the methods for determining whether or not the isolated bacterium is a recombinant host are complex and require extensive support from molecular geneticists.

There are at least two approaches for monitoring environmental and personnel exposure to recombinant DNA material. One approach would require all investigators be responsible for their own monitoring (personnel, laboratory surface, air, etc.). The recent review by Bachmann (1) provides the informational basis that would be necessary to formulate this laboratory strategy. For example, a laboratory that used *Escherichia coli* K-12 strains C600 or Ymel or CR 63m individually or in combination would or

should have the expertise and material readily available for verifying these strain designations (see Table 3).

A second approach would be to have a service group or department responsible for this monitoring. Here, the genetic approach would still be possible, provided that a restricted number of host strains was in use. However, when the number of host strains is large (i.e., EK1 equals any K-12 strain), it would be difficult to have the media required to distinguish the various strains available on a routine basis. This difficulty would be in the form of media preparation time, storage, etc. Other approaches could be developed which combine these two in varying degrees.

The first approach could work if all investigators were inclined to perform this task. The second approach could succeed provided there was a direct method for identifying recombinant hosts.

The responsibility for monitoring at the Massachusetts Institute of Technology was assigned to the Environmental Medical Service (Biohazard Assessment Office), and we decided to explore the latter approach.

The recent availability of commercial diagnostic kits to the bacteriology laboratory has simplified the complete differentiation and species identification of several genera of gram-negative bacteria commonly found in cultures of clinical specimens (5, 7, 11, 14). The API 20E (Analytab Products, Inc., Plainville, N.Y.) system for the identification of *Enterobacteriaceae* is used routinely in many clinical laboratories and provides a reasonably standardized method for biotyping bacterial isolates.

The biotype is determined by an organism's response to 21 biochemical tests (Table 1, col-

umn 1). Based upon the test results, API has developed a binary numbering system. Tests are grouped into seven sets of three; the first test in a set has a value equal to one, if positive; the second has a value of two, if positive; and third test has a value of four, if positive; all negative results have a value of zero (see Table 1 for an example). Since the API 20E was introduced, over 100,000 bacterial isolates (including 44,000 *E. coli* strains) have been differentiated. The biotype, the genus-species designation, and the frequency of appearance of the biotype have been stored in a computer file. There are at present about 587 biotypes and frequency indexes of *E. coli* in the file. This systems approach has been verified by testing API 20E biotype results against a computer diagnostic model (10).

Because this extensive *E. coli* data base was available, we wanted to determine if the API 20E system could distinguish between clinical *E. coli* strains and *E. coli* K-12 which might represent novel or rare biotypes.

Eight *E. coli* K-12 strains were obtained from D. Botstein (Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.). These strains were grown on Luria agar

TABLE 1. Biotype determination for *E. coli* K-12 594^a

Test	Result	Score
ONPG	—	
Arginine dihydrolase	—	4
Lysine decarboxylase	+	
Ornithine decarboxylase	—	
Citrate	—	0
H ₂ S	—	
Urea	—	
Tryptophan deaminase	—	4
Indole	+	
Voges-Proskauer reaction	—	
Gelatinase	—	4
Glucose	+	
Mannitol	+	
Inositol	—	5
Sorbitol	+	
Rhamnose	+	
Saccharose	—	1
Melibiose	—	
Amygdalin	—	
Arabinose	+	2
Oxidase	—	

^a The API 20E kit was used in accordance with the manufacturer's specifications. ONPG, *o*-Nitrophenol- β -D-galactopyranoside hydrolysis.

(10 g of tryptone, 5 g of yeast extract, 10 g of sodium chloride, 15 g of agar, and distilled water to yield final volume of 1 liter), and isolated colonies were used to inoculate the API 20E system as per the manufacturer's recommendations (Table 2). In addition, 22 clinical *E. coli* isolates were obtained from M. Dul (University of Massachusetts Medical Center, Worcester, Mass.), and M. J. Benfari (Massachusetts General Hospital, Boston, Mass.). Both the biotypes and the expected frequency of appearance of each biotype are presented in Tables 2 and 3 for the 22 clinical *E. coli* isolates and the 8 *E. coli* K-12 strains.

Although only one of the clinical isolates demonstrated an infrequently observed biotype (Table 2, strain 20), six of the eight K-12 strains had biotypes that would be considered as infrequent. Four of these six could be regarded as relatively rare biotypes.

If we compare the K-12 genotypes (Table 3) with the API biotypes, we find only three points of comparison. The K-12 strains designated E15 and XAC are *o*-nitrophenyl- β -D-galactopyranoside negative in the API 20E, and both have a defect in the *lac* region (Table 3). Strain Ymel does not grow on melibiose minimal medium and is also melibiose negative in the API 20E. Essentially then, the API 20E biotyping repre-

TABLE 2. Clinical *E. coli* strains

Strain	Biotype	Frequency estimate
1 ^a	5144552	1/7
2	5144552	1/7
3	5144572	1/9
4	5144572	1/9
5	5144512	1/13
6	5044542	1/42
7	5144550 ^b	1/65
8	5144550	1/65
9	5144550	1/65
10	5144552	1/67
11	5144530	1/90
12	5144502	1/98
13	5144112	1/104
14	5144152	1/112
15	5144510 ^b	1/132
16	5144510	1/132
17	5144530	1/184
18	5144530	1/184
19	5044562	1/208
20	5144520	1/1,342
21 ^c	5144552	1/7
22	1144572	1/44

^a Strains 1 through 20 were supplied by M. Dul (University of Massachusetts Medical Center).

^b Strains 7 and 9 were isolated from separate culture sites of one individual. Strains 15 and 16 are identical.

^c Strains 21 and 22 were supplied by M. Benfiore (Massachusetts General Hospital).

TABLE 3. *E. coli* K-12 strains^a

Strain	Genotype	Biotype	Frequency
594	<i>gal-1 gal-2 strA</i>	4044512	1/127
E15	<i>relA1 tonA22 T₂^R</i> <i>phoA8 PO2A</i>	4044552	1/240
C600	<i>thr- leu-6 thi-1 supE44</i> <i>lacY1 (stable) tonA21</i> λ -	5044510	1/412
Ymel	<i>mel-1 supE57 tyrT58</i>	5044110	1/1,209
p3478	<i>thyA36 polA1</i>	5044000	1/6,229
HB101	<i>thr leu pro</i> (r-, m-, phe- notypic markers) <i>recA</i>	5044400	1/8,403
CR63	F <i>supD60</i>	5004152	1/12,278
XAC	Δ { <i>pro-lac</i> } <i>rpoB nalA</i> <i>argE</i> (am)	4004500	1/18,046

^a The information for 594 and XAC was provided by D. Ross, Department of Biology, Massachusetts Institute of Technology. The information for the remaining strains was taken from Bachmann (1).

sents a separate method for characterizing the K-12 strains. We have tested only eight K-12 strains and have not found identical biotypes for different genotypes. We are presently assembling a larger collection of K-12 strains and intend to examine this more closely.

It must be emphasized that these frequency estimates are based on the number of times a given biotype has been observed. This does not mean that *E. coli* K-12 strain 594 has been seen with a frequency of 1/127, but that the biotype profile was observed with the given frequency.

HB101 and C600 are frequently used recombinant *E. coli* K-12 host strains. The appearance frequencies of 1/8,403 and 1/412 (respectively) are sufficiently rare that it should be possible to distinguish these biotypes from other coliform isolates.

These preliminary results suggest that the API 20E system may be useful in identifying *E. coli* K-12 strains and may therefore facilitate the development of an environmental and personnel monitoring program for recombinant DNA research.

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