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Conservation and Variation of Nucleotide Sequences Within Related Bacterial Genomes: Enterobacteria

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We have assessed the degree of relatedness of several portions of the *Escherichia coli* genome to the corresponding portions of the genomes of representative enteric bacteria, using the Southern transfer and hybridization technique (E. Southern, J. Mol. Biol. 98:503-517, 1975). The degree of relatedness varied among the regions examined. Judging both by the relative amounts of deoxyribonucleic acid in the various enteric genomes that are highly homologous and by the conservation of positions of restriction enzyme cleavage sites in these regions, the enteric genomes have diverged to greater extents in some parts of the genomes than in others. Portions of the genomes (including the *tnaA* and *thyA* genes, the *trp* operon, and one other unassigned segment) appear to have evolved in concert with the genome as a whole. By contrast, the *lacZ* gene and portions of the genome that are homologous to phage λ vary more widely, perhaps reflecting a separate evolutionary origin for these segments of deoxyribonucleic acid.

Contemporary enteric genomes are presumed to have descended from a common ancestor, diverging one from another as the bacterial genomes underwent a succession of changes. Some of these changes were simple base substitutions; others entailed genome rearrangement, such as the inversion of a large section of the genome that included the *trp* operon in either *Escherichia coli* or *Salmonella typhimurium* (9). Additions or deletions of segments of DNA up to 150 kilobases (kb) seem to have played an important role in the evolution of enteric genomes, as was suggested previously to account for some of the differences between the *E. coli* and *S. typhimurium* genetic maps (20). For instance, the absence of an inducible β-galactosidase in *S. typhimurium* and its presence in *E. coli* may be a consequence of the deletion of the *lac* region in *S. typhimurium* or its addition to the *E. coli* genome (20, 23).

Relatedness among specific small portions of enterobacterial genomes is being studied in fine detail by determining nucleotide sequences of equivalent genes in enteric bacteria as is the case, for instance, for portions of the *trp* operon (e.g., 13, 17, 18) and for 5S RNAs (12). Relatedness among enteric genomes as a whole have been studied by DNA-DNA hybridization of total DNAs (4). The overall amount of homology between many enteric genomes has been assessed by determining the extent of hybrid formation between the total DNAs of pairs of bacteria. The degree of homology between the *E. coli* genome and other enteric genomes is not uniform throughout the genomes. By varying the stringency of conditions for formation of hybrids and by determining the thermal stabilities of the hybrids so formed, Brenner and his colleagues have found that many enterobacterial genomes contain a fraction that is highly homologous to *E. coli* DNA, a fraction that contains a higher proportion of mismatched bases, and a fraction that is either poorly homologous or contains no homology and does not form DNA-DNA hybrids under the conditions employed (4, 5).

Short of obtaining the complete nucleotide sequences for homologous genes in a group of enteric genomes, one can gain information on the degree of relatedness of selected portions of these genomes by applying the Southern transfer and hybridization technique (22). To gain more detail about the relatedness of specific portions of enterobacterial genomes, and to learn more about the mechanisms of evolution of bacterial genomes, we focused on the portions of the *E. coli* genome that are carried by five phage DNAs. We studied the extent of homology of these regions with the equivalent portions of other bacterial genomes by measuring the extent of hybridization of the probe DNAs with restriction fragments of various enteric bacterial DNAs that were partially separated by electrophoresis. These studies extend and expand earlier studies that measured the extent of binding to total enterobacterial DNAs of mRNA containing the *lac* genes (4) and mRNA's for various *trp* genes (11, 15).

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MATERIALS AND METHODS

Phages. The λ transducing phages used in these experiments were described previously (1).

Bacteria and preparations of bacterial DNA. The enteric bacterial strains used and their sources are listed in Table 1. DNA was prepared from these strains by a modification of the method used previously (1) since some of the enteric DNAs were difficult to free from material which interfered with digestion by restriction enzymes. The following steps were incorporated into the method previously outlined. (i) Lysed suspensions were treated with a higher concentration of Pronase (2 mg/ml) for 7 to 12 h at 37°C. (ii) In some cases, redissolved DNAs were dialyzed against 0.1 M sodium phosphate-0.1 M EDTA (pH 7.0) (two changes, 12 h each), followed by two changes of 0.01 M sodium phosphate-0.01 M EDTA (pH 7.0) and a final exhaustive dialysis against 10 mM Tris-hydrochloride (pH 7.5)-1 mM EDTA. DNA preparations gave OD_{260}/OD_{280} ratios of 1.8 to 2.0 (OD, optical density).

Restriction enzyme digestion and hybridization procedures. The preparation of ^{32}P-labeled phage DNAs, restriction enzyme digestion of bacterial DNAs, agarose gel electrophoresis, and transfer and hybridization techniques were described (1). The concentrations of enteric bacterial DNA samples were determined by the fluorescence assay of Thomas and Farquhar (24), with the single exception of *Klebsiella aerogenes* DNA, which was estimated by densitometric tracings of ethidium fluorescence photographs (although in this case variation between replicate experiments was as much as 10%).

Determination of relative amounts of hybridized DNA. Autoradiograms were traced on a Joyce-Loebl microdensitometer using a fixed ratio arm and a single wedge. The peaks were cut out and weighed. Peak weights determined from the same autoradiograph of a single filter were corrected for any differential DNA loading and taken as a measure of the ^{32}P-labeled λ or ^{32}P-labeled λ transducing phage DNA that was hybridized.

RESULTS

DNA was isolated from 10 enteric bacteria representing nine genera. *Proteus morganii* and *P. mirabilis* were both included since these species are only distantly related to each other, and in fact have been proposed to belong to two separate genera (8). Agarose gel electrophoresis gave a single band for all of the initial DNA preparations (>30 kb shear fragments), except that of *Shigella dysenteriae* DNA which contained, in addition to the high-molecular-weight shear band, a plasmid of approximately 2 kb pairs (data not shown). Plasmids of 30 and 2 kb molecular weights are known to be present in this strain (19).

TABLE 1. *Bacterial strains*

Strain	Source[a]
E. coli W2637 (CGSC 5290)	CGSC
S. dysenteriae SH16	E. Ohtsubo
S. typhimurium LT2	S. Halegoua
C. freundii (ATCC 8090)	ATCC
K. aerogenes T17R1	C. Yanofsky
E. aerogenes (ATCC 13048)	ATCC
S. marcescens	W. Belser
E. amylovora (ATCC 15580)	ATCC
P. morganii	A. C. Wilson
P. mirabilis	S. Halegoua

[a] CGSC, Coli Genetics Stock Center; ATCC, American Type Culture Collection.

The bacterial DNA preparations were digested with the restriction endonucleases *Eco*RI or *Hin*dIII, and the digests were subjected to gel electrophoresis (Fig. 1). Each of the optical patterns of the chromosomal digests differed one from another, indicating that the *Eco*RI and *Hin*dIII restriction target sequences are distributed differently in the genomes of these enteric bacteria. These chromosomal restriction digests were denatured and transferred from the agarose gel to replicate nitrocellulose filters, which were subsequently hybridized to each of five ^{32}P-labeled DNA probes under stringent conditions. For convenience, each of the probe DNAs is referred to by the designation of bacterial gene(s) that are known to be present.

λ probe. λ phage DNA was hybridized to the bacterial DNA digests to enable identification of those sequences that hybridize with vector DNA and also to learn whether or not *E. coli* is unique among enteric bacteria in having chromosomal DNA that is homologous to λ.

Autoradiograms produced by hybridization of both *Eco*RI and *Hin*dIII chromosomal digests with λ probe showed that several enteric bacterial genomes contained segments of DNA that are highly homologous to that of λ (Fig. 2). In addition to *E. coli*, *S. dysenteriae*, *S. typhimurium*, *K. aerogenes*, *Enterobacter aerogenes*, and *P. morganii* contained λ-homologs in the genome. No λ-homologs were seen in the genomes of *Citrobacter freundii*, *Erwinia amylovora*, *Serratia marcescens*, or *P. mirabilis*. The sizes of the restriction fragments in the chromosomal digests that contained λ-homologs were highly variable, showing that no neighboring pairs of the positions of *Hin*dIII or *Eco*RI target sites were conserved in or near the regions of homology to λ DNA in these genomes.

The amount of DNA that was homologous to λ varied in these bacteria. Autoradiograms were traced by densitometry, and areas under the peaks were determined, providing a measure of the amount of λ-homologous DNA that was present in those enteric bacteria that exhibited homology (Table 2). *S. typhimurium* contained about three-fourths the amount of homologous

DNA as was present in the *E. coli* K-12 strain, and almost three times as much as was present in *S. dysenteriae*. The other enteric strains in which λ-homologs were detected had very small amounts compared to *E. coli*.

λ *trp*. When λ *trp* probe was hybridized to enteric chromosome digests, bands were formed that were not present when λ DNA was used as the probe. The new bands served to locate the λ *trp*-homologs among the DNA fragments of the chromosomal digests and are indicated by pointers in Fig. 3. (Some of the λ-homolog bands were absent, presumed to be those parts of the λ genome that were substituted or deleted in the probe.)

Figure 3 shows that all of the enteric genomes contained some regions of close homology to *E. coli trp* DNA. (Although no hybrid band was visible in the *P. mirabilis Eco*RI digest, a faint band could be seen in the *Hin*dIII digest.)

The sizes of the restriction fragments that contained *trp*-homologs varied among the enteric chromosomal DNAs, as would be expected if *Eco*RI and *Hin*dIII target sites occupied different positions in the enteric genomes in and near the *trp* operon. However, a few sites in the *trp* region may have been conserved between some pairs of enteric genomes since in some cases either *Eco*RI or *Hin*dIII fragments that contained *trp*-homologs appeared to be the same

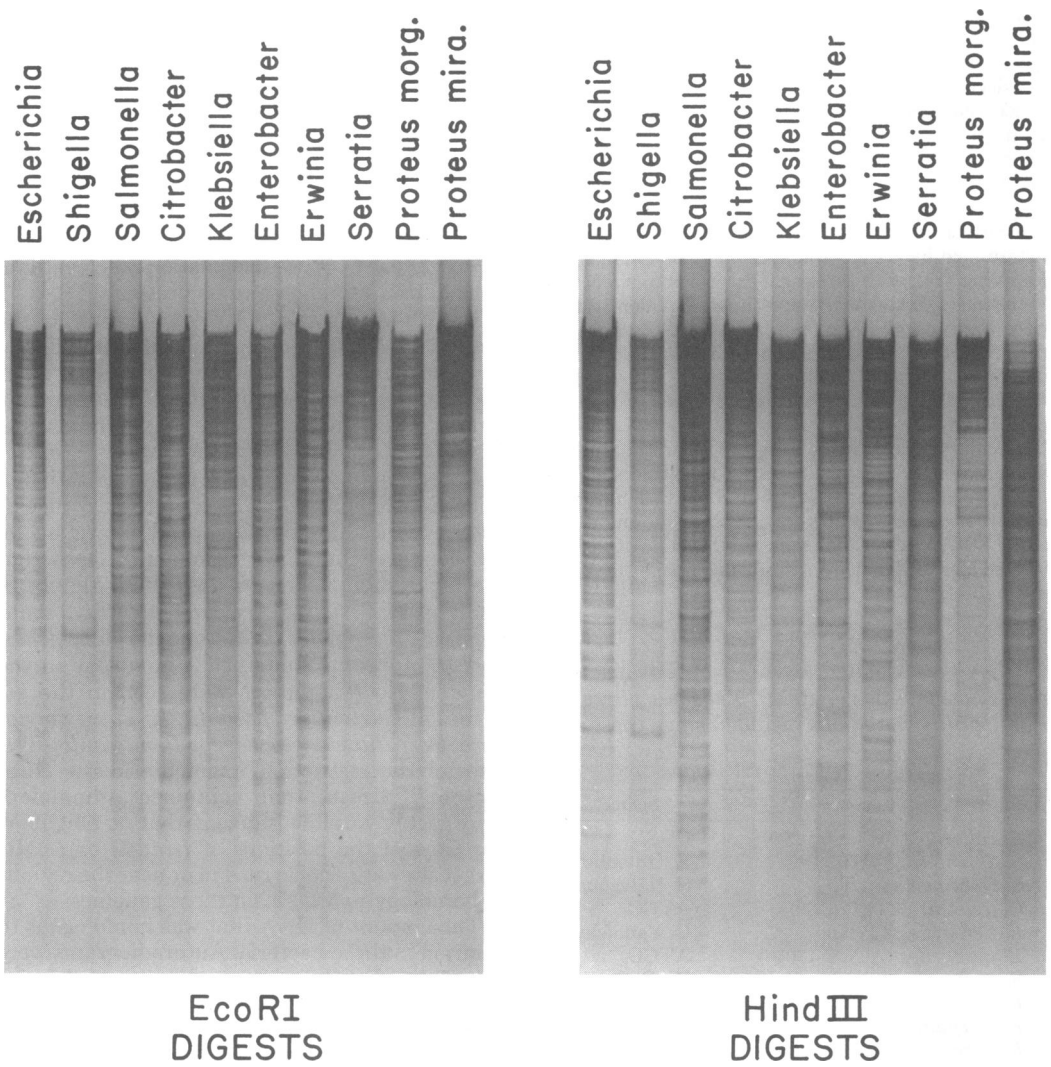

FIG. 1. *Agarose gel electrophoresis of EcoRI and HindIII digests of E. coli DNAs. Digests (about 0.5 μg each) were subjected to electrophoresis through 1% agarose and stained with ethidium bromide and photographed as described in the text.*

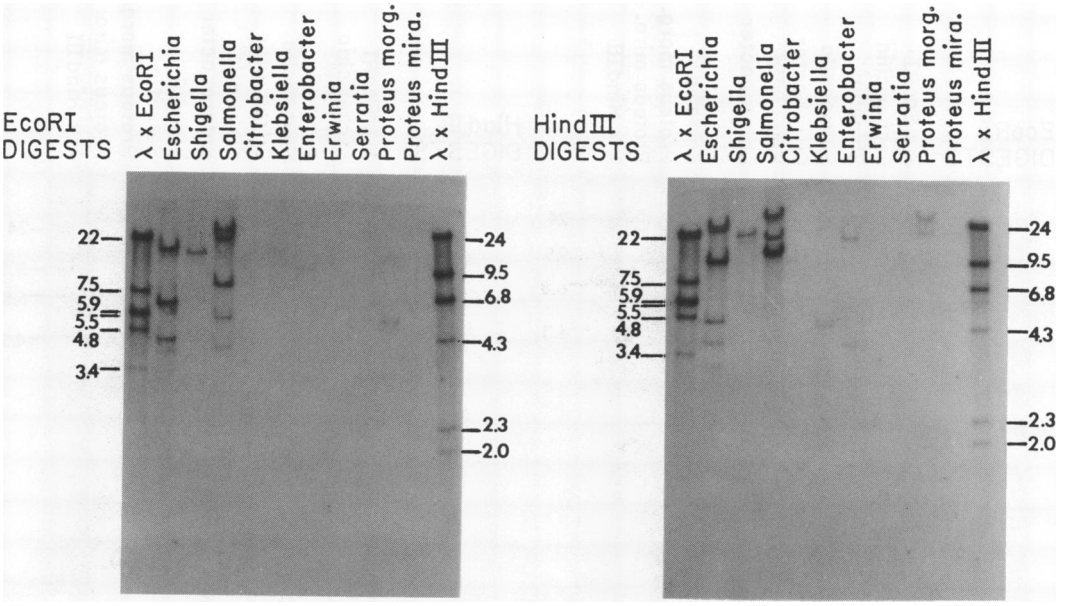

FIG. 2. *Hybridization of ^{32}P-labeled λ DNA to EcoRI and HindIII digests of enteric DNAs. Nitrocellulose filters carrying restriction enzyme digests of enterobacterial DNAs from gels identical to those shown in Fig. 1, with additional outer tracks, of 0.5 ng of EcoRI and HindIII digests of λ DNA, were hybridized with ^{32}P-labeled λ DNA and autoradiographed as described in the text.*

TABLE 2. *Amount of homology to phage λ in enteric genomes*

Strain	Relative homology
E. coli K-12	1.0
S. typhimurium	0.71
S. dysenteriae	0.26
P. morganii	0.10
E. aerogenes	0.07
K. aerogenes	0.01
C. freundii	<0.01
E. amylovora	<0.01
S. marcescens	<0.01
P. mirabilis	<0.01

size in more than one genome.

In two cases, *E. amylovora* and *C. freundii*, the number and sizes of the restriction fragments that contained *trp*-homologs were such that no simple one-to-one correspondence of contiguous chromosomal genes vis-a-vis probe genes could be visualized. Genes homologous to *trp* DNA appear to be organized differently in these two enteric genomes as compared to *E. coli*. To our knowledge, no information is available on the organization of the *trp* operon in either *Erwinia* or *Citrobacter*.

To assess the thermal stabilities of the homologs that were formed in this and in other similar experiments, the temperature at which probe DNA was dissociated from filter-bound DNA was determined. A single-slot, 12-cm-wide filter of each of three chromosomal digests, *E. coli*, *C. freundii*, and *E. aerogenes*, was prepared and hybridized with λ *trp* probe. Replicate strips were cut from each filter, and these strips were washed at a series of temperatures. Autoradiograms were made and traced by densitometry, and the areas under tracing peaks were determined. The data so obtained contained appreciable scatter, but the melting range was sufficiently narrow that a mid-point temperature of dissociation, T_d, could be estimated from a plot of the thermal elution profile. Representative dissociation profiles are shown in Fig. 4, and T_d values are listed in Table 3. The T_d values were clustered, extremes falling 8°C apart. Each of the hybrid bands that was measured had dissociated within a relatively narrow temperature range, indicating that only well-matched homologs containing less than ~6% mismatch had formed hybrids under the conditions of hybridization that were used in these experiments (16).

The intensities of hybrid bands on autoradiograms reflect the relative amounts of probe DNA that were bound and therefore serve as a rough measure of the total amount of DNA present in a particular chromosomal restriction fragment that was well-matched with the corresponding DNA sequences in the probe preparation. The sum of these measured intensities for all hybrid bands in a bacterial chromosome then serves as an estimate of the sum of the

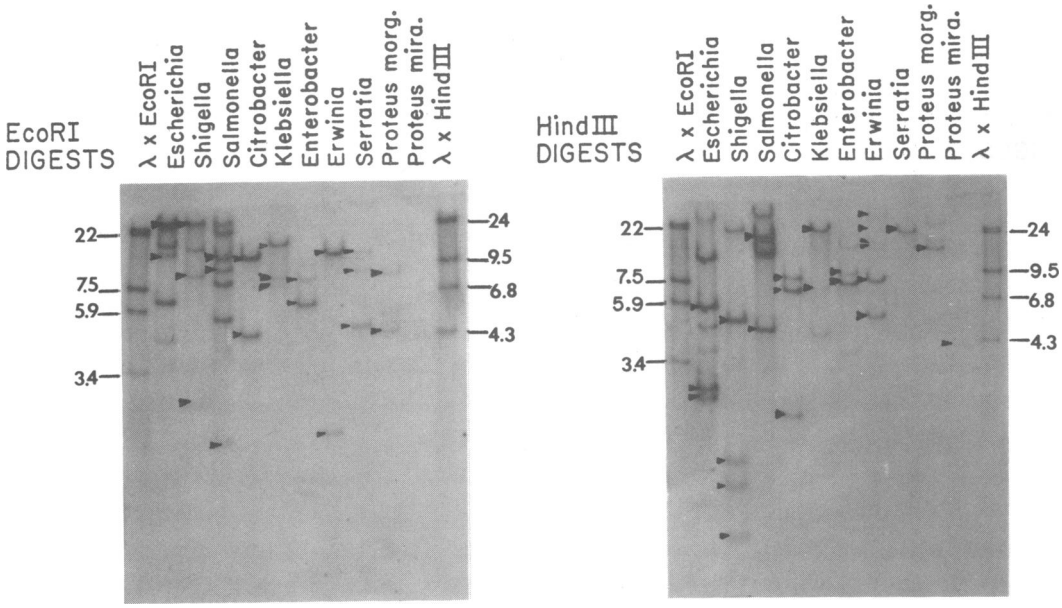

FIG. 3. *Hybridization of ^{32}P-labeled λ trp DNA to EcoRI and HindIII digests of enteric DNAs. See legend to Fig. 2 for details. Pointers show λ trp-homologous bands not present in Fig. 2.*

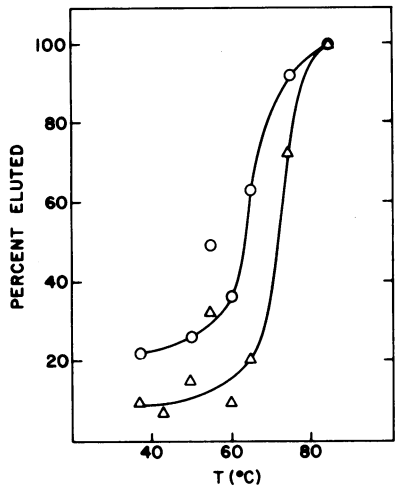

FIG. 4. *Thermal melting of probe DNA from filter-bound restriction fragments. Percent ^{32}P-labeled DNA eluted from hybrids by washes of 50% (vol/vol) formamide-4× SSC at different temperatures was determined for replicate strips as described in the text. Circles are values for the highest-molecular-weight λ-homolog in the E. coli HindIII digest, and the triangles are values for the sum of the E. coli HindIII trp fragments 2 + 3 (see Table 3).*

lengths of the DNA sequences in the chromosomal fragments that were highly homologous to the *E. coli* K-12 probe DNA.

The areas under the peaks of *trp*-homologous hybrid bands were determined for both *Hin*dIII and *Eco*RI digests of each of the enteric bacteria.

TABLE 3. *Temperatures of dissociation of probe DNA from filter-bound restriction fragments*

Homolog	Fragment	T_d(°C)
λ	*E. coli* K-12[a]	
	1[c]	64
	2	64
	3	67
trp	*E. coli* K-12[a]	
	1	69
	2 & 3	72
	C. freundii	64
	E. aerogenes[b]	65

[a] *Hin*dIII digest.
[b] *Eco*RI digest.
[c] Fragments are numbered in order of decreasing molecular weight (see Fig. 2 and 3).

The values were corrected for amounts of chromosomal DNA present on the gel and were averaged between *Eco*RI and *Hin*dIII experiments. The values, expressed relative to *E. coli*-*E. coli* hybrids = 1.0, are presented in Fig. 5.

λ *thy* probe. Experiments similar to those done with λ *trp* probe were carried out using λ *thy* DNA as probe. The λ *thy* probe carries two independent samples of the *E. coli* K-12 genome (1). For convenience, the hybrids formed in experiments using the λ *thy* probe are referred to as *thy*-homologs, even though two separate classes are included in this designation.

The hybridization patterns of the λ *thy* probe with both *Hin*dIII and *Eco*RI digests are pre-

sented in Fig. 6. Restriction fragments homologous to the *thy* probe DNA were present in all chromosomal digests. A fragment of about 7.5 kb, presumed to contain the *thyA* gene, was present in *Hin*dIII digests of both *E. coli* K-12 and *S. dysenteriae* DNA. Even more striking,

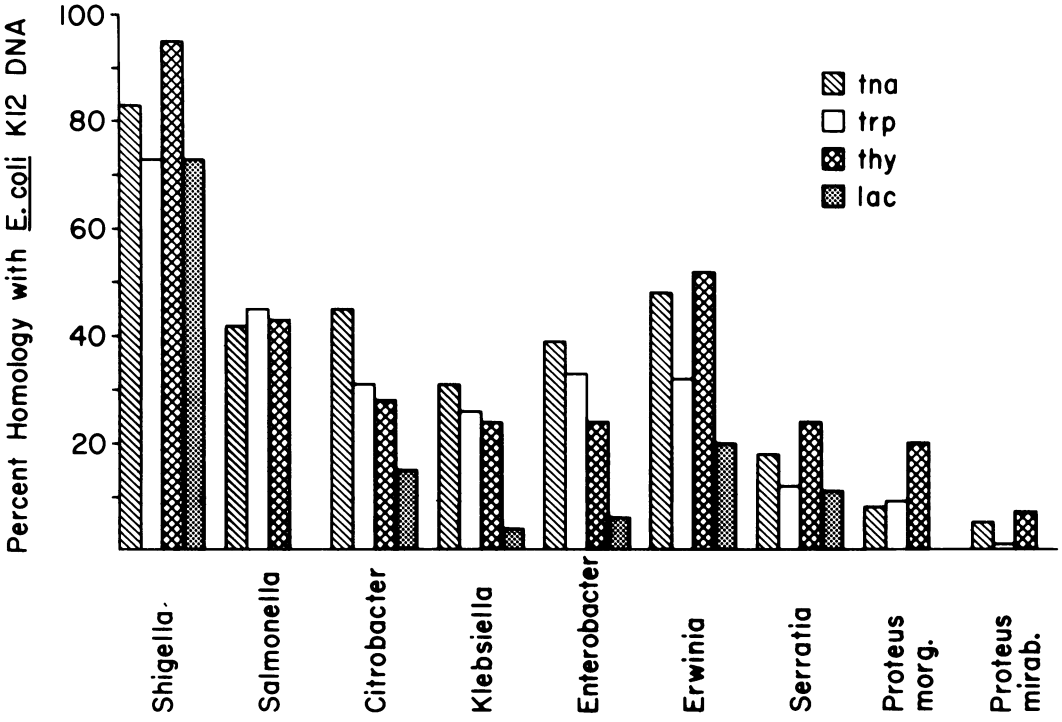

FIG. 5. *Homologies of E. coli tna, trp, thy, and lac probes with EcoRI and HindIII digests of enterobacterial DNAs. The summed relative amounts of hybridization specific to each probe was determined as described in the text. Results from EcoRI and HindIII digests were averaged for each of the probes: λ tna, λ trp, λ thy, and λ lac, and represented with the values obtained for E. coli K-12 taken as 100%.*

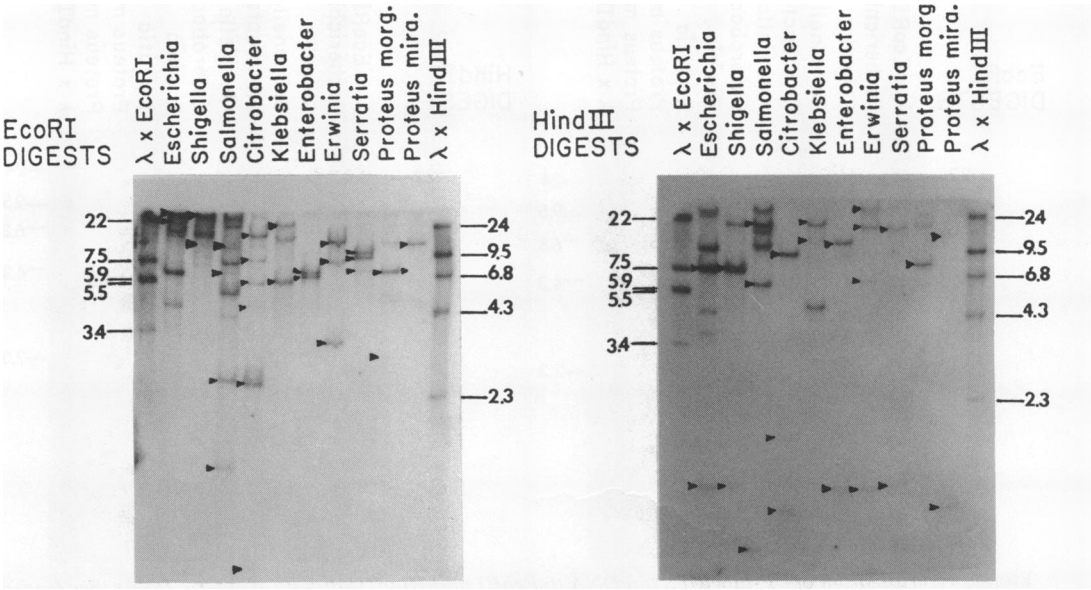

FIG. 6. *Hybridization of ^{32}P-labeled λ thy DNA to EcoRI and HindIII digests of enteric DNAs. See legend to Fig. 2 for details. Pointers show thy-homologous bands.*

the small fragment of about 1.3 kb was present in the *Hin*dIII digests of 5 of the 10 enteric genomes. A fragment of this size was found previously to be conserved in six different *E. coli* strains (1). It seems likely that the unidentified fragment lies in a region which is also highly conserved among the enteric genomes. Other cases of similarity in size of restriction fragments containing *thy*-homologs can be seen by inspection of Fig. 6.

The areas under the peaks of *thy*-homologous hybrid bands were determined for both *Eco*RI and *Hin*dIII chromosomal digests to provide a measure of the amount of DNA in the genomes of the enteric bacteria that was well-matched with *E. coli* K-12 DNA. The values were similar to those determined for *trp*-homologs (Fig. 5).

λ *tna* probe. Autoradiograms of hybrids formed with λ *tna* DNA are shown in Fig. 7. All of the enteric chromosomal DNAs contained *tna*-homologs. (Although no band was seen in the *Hin*dIII digest of *P. morganii*, possibly due to technical limitations of the method, one and perhaps a second faint band were present in the *Eco*RI digest.) A few of the fragments containing *tna*-homologs were similar in size in different enteric genomes, indicating that some restriction site positions were conserved, but most fragments differed in size. The areas under the peaks of *tna*-homologous hybrids were determined as before, and the values, similar to those for *thy*- and *trp*-homologs, are presented in Fig. 5.

λ *lac* probe. Autoradiograms of hybrids formed with λ *lac* DNA are shown in Fig. 8. *lac*-homologs were not present in all the enteric chromosomes. No hybrid *lac* bands were seen in either *Hin*dIII or *Eco*RI digests of *S. typhimurium*, *P. morganii*, or *P. mirabilis*. The rest of the enteric genomes contained *lac*-homologs. Fewer homologs were conserved in size in different genomes than was the case for other probes. Thus, less conservation of position of restriction sites was seen in the *lac* region.

The sums of the intensities of the *lac*-homolog hybrid bands in the enteric digests were determined by densitometry. Identification and measurement of intensities of *lac*-homologs were not as straightforward as for the other probe DNAs. For *E. coli* and *S. dysenteriae* *Hin*dIII digests, the presence of a *lac*-homolog near or comigrating with a fragment containing a λ-homolog was only detected by comparing areas of peaks from densitometer tracings of autoradiograms produced by λ probe with those produced by λ *lac* probe, values being normalized to that of a common λ-homolog. For other enteric DNAs, comigration was not observed. The relative amounts of *lac*-homology in the enteric genomes are shown in Fig. 5. In the *S. dysenteriae* and *S. marcescens* genomes, the amount of homology to the *E. coli lacZ* gene was comparable to that found for the *trp*, *thy*, and *tna* homologs. However, the amount of *lac*-homology seems relatively lower in *C. freundii* and *E. amylovora* and

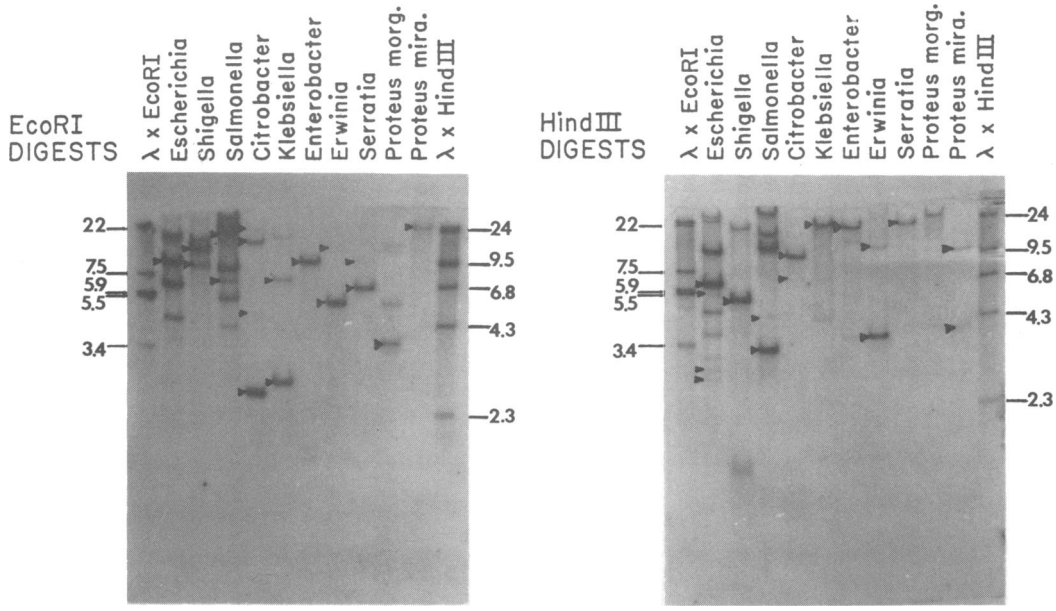

FIG. 7. *Hybridization of ^{32}P-labeled λ tna DNA to EcoRI and HindIII digests of enteric DNAs. See legend to Fig. 2 for details. Pointers show λ tna-homologous bands not present in Fig. 2.*

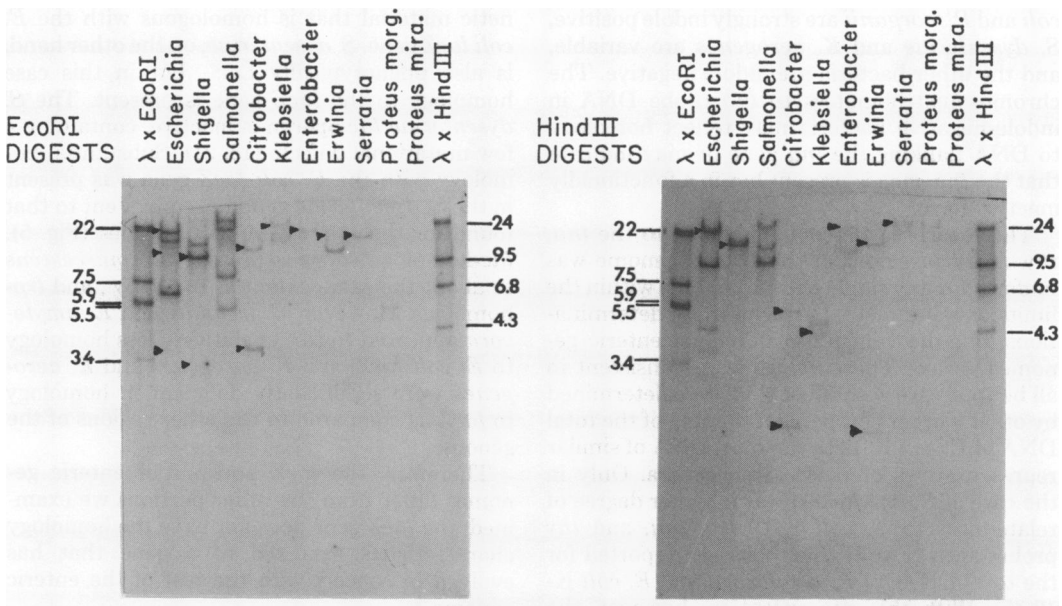

FIG. 8. *Hybridization of ^{32}P-labeled λ lac DNA to EcoRI and HindIII digests of enteric DNAs. See legend to Fig. 2 for details. Pointers show λ lac-homologous bands not present in Fig. 2.*

is substantially lower for *K. aerogenes* and *E. aerogenes*.

DISCUSSION

We have used five probe DNAs to compare selected small portions of the *E. coli* K-12 genome with the equivalent portions of the genomes of nine other enteric bacteria. As one criterion for the degree of relatedness of the selected portions of the *E. coli* genome with the genomes of other enteric bacteria, the total amount of DNA in each of the enteric genomes that is highly homologous to the *E. coli* DNA was estimated, using the Southern transfer and hybridization procedure (22) and densitometric tracing of autoradiograms.

The method provides a rough measure of the sum total of segments of highly homologous DNA that is present in the restriction fragment giving rise to a particular hybrid peak. Runs of nucleotide sequences in chromosomal restriction fragments that are highly homologous to corresponding sequences in probe DNA molecules would be expected to act as nucleation centers and to affect binding of probe DNA under stringent conditions. These well-matched segments are expected to be interspersed with poorly homologous segments in the hybrid molecules. Where nucleotide sequences are known for homologous regions of two or more enteric bacteria (3, 13, 17, 18), uniformity of base sequence divergence has not been found. Rather, regions of highly conserved sequences are interspersed with other regions with variable degrees of homology. For instance, the *lpp* region of *E. coli* and *S. marcescens* genomes can be divided into regions of 95% homology, 82%, and two regions of about 56% homology each (17a). The leader-promoter-operator region of *trpE* in the *E. coli* and *S. typhimurium* genomes has a region of high homology (positions −24 to +8), in which only one base in 32 differs, followed by a region of relatively low homology (positions +9 to +50), in which 15/42 bases differ (3, 13). Similar patterns of interspersion of conserved and variable regions have been found in the *trpA* genes of *E. coli* and *S. typhimurium* (18) and in part of the *trpE* region in *S. marcescens* as compared with *E. coli* and *S. typhimurium* (17). The amount of probe DNA bound to a partially homologous chromosomal restriction fragment under stringent conditions will be a function of the number and length of regions within it that are highly homologous to the probe. The function is undoubtedly a complex one, depending on the length and distribution of the highly homologous segments. Nevertheless, the amount of probe DNA bound gives a rough measure of degree of relatedness of particular portions of enteric genomes to the corresponding portion of the *E. coli* genome.

The data presented in Fig. 5 shows that homologs to the *tna*, *thy*, and *trp* probes were present in each of the bacterial strains examined. Among the enteric organisms used in these studies, *E.*

coli and *P. morganii* are strongly indole positive, *S. dysenteriae* and *K. aerogenes* are variable, and the other bacteria are indole negative. The chromosomal homologs to *tna* probe DNA in indole-negative bacteria may reflect homology to DNA flanking the *tna* gene or may indicate that the *tna* gene is present but in a functionally inactive form.

The measured percent homology to the *tna*, *thy*, and *trp* regions of the *E. coli* genome was uniform for any single enteric genome within the limits of experimental error of these determinations, but the values for individual enteric genomes varied. These values were consistent in all but one case with those that were determined by other workers for percent binding of the total DNA of *E. coli* K-12 to the total DNA of similar representatives of these same genera. Only in the case of *E. amylovora* was a higher degree of relatedness to *E. coli* K-12 *trp*, *tna*, and *thy* probes seen (Fig. 5) than has been reported for the total DNAs of *E. amylovora* and *E. coli* K-12 (7). With this one exception, however, the degree of relatedness of the *trp*-, *tna*-, and *thy*-homologs that was found in these experiments was similar to the degree of relatedness of the total DNAs when they were hybridized at 60°C under the conditions of Brenner and colleagues (4–8, 10). Although the relative values for percent homology agree in the two types of experiments, no particular significance should be attached to the numerical coincidence since the methods employed, although similar in principle, differed in detail, and because the strains used in the experiments as representative of different enteric genera were not always the same in the two sets of studies. Nevertheless, it seems clear that the amount of change that has taken place in the *tna*-, *thy*-, and *trp*-homologs in each enteric bacterium relative to *E. coli* K-12 is similar to the amount of change that has taken place within each genome taken as a whole.

The *lacZ*-homologous portions of the enteric genomes differ from the *tna*-, *thy*-, and *trp*-homologous portions in the amount of such DNA that is present in the enteric genomes. As measured by the intensities of hybrid bands on autoradiograms, the *lac*-homologs do not show the same degree of relatedness as do the *tna*-, *thy*-, and *trp*-homologs (Fig. 8). The *lac* region is variable among enteric bacteria. In some cases the Lac$^-$ phenotype results from absence of *lac* DNA; in other cases the Lac$^-$ phenotype results from inactivating point mutations in the region. Little or no homologous material was present in the genomes of the Lac$^-$ enterobacteria *S. typhimurium*, *P. morganii*, and *P. mirabilis*, indicating that these three chromosomes lack genetic material that is homologous with the *E. coli lacZ* gene. *S. dysenteriae*, on the other hand, is also phenotypically Lac$^-$, but in this case homology to the *lacZ* gene is present. The *S. dysenteriae lac* operon is inactive, containing a few mutational defects (15, 21). Substantial homology with the *E. coli lacZ* gene was present in the *S. dysenteriae* genome, equivalent to that found for the *tna*, *thy*, and *trp* probes (Fig. 5). *lac*-homologs were also present in *S. marcescens* to about the same extent as *tna*-, *thy*-, and *trp*-homologs. However, *C. freundii* and *E. amylovora* appeared to carry relatively less homology to *E. coli lacZ*, and *K. aerogenes* and *E. aerogenes* were significantly deficient in homology to *lacZ* as compared to the other regions of the genome.

Therefore, the *lacZ* portions of enteric genomes differ from the other portions we examined; the *lacZ* gene does not have the homology characteristics expected of a gene that has evolved in concert with the rest of the enteric genome.

Another measure of the relatedness of specific portions of the enteric genomes is given by the degree of conservation of the positions of restriction enzyme target sites. Conservation of homolog-fragment size reflects, in the absence of compensating changes, retention of the nucleotide sequence of the flanking restriction sites and retention of the numbers of intervening base pairs. The positions of some of the restriction sites appear to remain unchanged in some portions of the enteric genomes. The greatest degree of conservation was seen for two *Hin*dIII sites flanking a 1.3-kb chromosomal fragment that is homologous to DNA carried by the λ *thy* probe. A 1.3-kb homolog was present in five of the enteric genomes, and bands that appear to be size variants of this small fragment were present in the other five enteric bacteria. The function of this highly conserved segment of DNA is unknown. Other instances of conservation of fragment size were seen in the *tna*, *thy*, and *trp* regions (Fig. 3, 6, 7); however, fewer examples of conservation of fragment size were seen in the *lac* region of those enteric genomes that have homology to *lacZ* (Fig. 8).

A disparity in the relatedness of different portions of enteric genomes could be understood if not all enteric genes have evolved in concert with one another. Some portions of the genome could have been present at the outset in a common ancestral genome and could have changed during evolution to the same extent on the average as the genomes as a whole, whereas other portions of the contemporary genome could have been acquired more recently and could have undergone changes beginning from a dif-

ferent evolutionary starting point. In the case of the *lacZ* gene of *E. coli*, it has been noted by others that the *lac* region of the *E. coli* genome might have been acquired by insertion, an insertion event that did not occur in the *S. typhimurium* genome (23). A comparative analysis of the genetic maps of these two bacteria suggests that major additions or deletions have occurred in many positions in these genomes, including the *lac* region (20). Such additions and deletions to bacterial genomes could have been a major source of evolutionary divergence, introducing changes over and above those caused by base substitutions and small internal rearrangements.

The disparity that was observed in these experiments in the extent of relatedness of the *E. coli lacZ* gene compared to other portions of the genome suggests that more than one acquisition event may have occurred among the enteric bacteria. The relatively low amount of homology of any part of the *K. aerogenes* and *E. aerogenes* genomes to the *E. coli lacZ* gene may reflect independent acquisition by these genomes of a gene specifying a β-galactosidase, a gene that could have diverged from a common ancestral *Z*-type gene at an earlier time or, perhaps more likely, could have arisen from different sources altogether.

Finally, the λ-homologs in the genomes of the enteric bacteria were found to vary over an even wider range than the *lacZ* gene (Table 2), as might be expected for genetic material that is not believed to be functional and is not essential to the life of the cell. No λ-homologs were visualized in the genomes of *C. freundii*, *E. amylovora*, *S. marcescens*, or *P. mirabilis*. Almost three times as much λ-homologous DNA was found in *S. typhimurium* DNA than in *S. dysenteriae* DNA, even though in other respects the genomes of *E. coli* and *S. dysenteriae* are more highly related than are the genomes of *E. coli* and *S. typhimurium*. Perhaps even more surprising, λ-homologs were found in bacterial genomes as distantly related to *E. coli* as *P. morganii*. The chromosomal λ-homologs might be vestiges of interactions at an earlier time of lambdoid phages with the enteric genomes or with other enteric temperate phages that share some features in common with λ, as has been shown for the *Salmonella* phage P22 by Botstein and Herskowitz (2).

Judging by the uniqueness of the sizes of each of the restriction fragments that contain λ-homologs, the positions of restriction site sequences have changed to a greater extent in and near λ-homologous portions of the DNA than in or near the *tna*, *thy*, and *trp* portions. Therefore, by both criteria used here, i.e., the total amount of homology present in a genome and the conservation of positions of restriction sites, the λ-homologs that are present in the enteric genomes are more variable than are the other portions of the genome that were examined in these experiments. An ancestral genome may have contained λ-homologs, and these may have diverged at a faster rate than other parts of the genome, or, perhaps more likely, the acquisition of λ-homologs may have been more recent, i.e., since the divergence of distinct groups of enteric bacteria from a common ancestor. Some of the enteric bacteria may never have incorporated the phage-like sequences into their genomes; others may have acquired and lost fragments of this genetic material through genetic recombination between one or more phages as well as the bacterial genome.

In summary, the disparity in the amount of relatedness of the portions of the genomes that have been studied can be viewed as reflecting two kinds of processes that were important in the evolution of bacterial genomes: cumulative changes in nucleotide sequences through base substitution and the acquisition of segments of DNA by lateral transmission.

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