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Restriction Endonuclease Analysis of the *ilvGEDA* Operon of Members of the Family *Enterobacteriaceae*

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Four of the genes required for the biosynthesis of isoleucine and valine form the *ilvGEDA* operon in *Escherichia coli* K-12 and *Salmonella typhimurium*. The structural relationship of these genes was examined in eight other members of the family *Enterobacteriaceae* by genomic Southern blot hybridization. These genes are contiguous in all the strains examined, and specific restriction sites appear to be highly conserved, indicating the possible functional importance of the DNA sequences of which they are part.

In *Escherichia coli* K-12 and *Salmonella typhimurium*, the genes for the biosynthesis of the amino acids isoleucine and valine are divided into five transcriptional units (1, 18). Four of the genes are regulated as a single unit, the *ilvGEDA* operon. As an aid to understanding the regulation of the metabolism of isoleucine and valine, the structure and organization of the *ilvGEDA* operon in other bacteria are being analyzed. Traditionally this has necessitated either the establishment of the technology for genetic analysis within an organism or the interspecies transfer of portions of the chromosome. Recently this has been most effectively accomplished by the use of recombinant DNA technology. As an alternative route for the analysis of the *ilvGEDA* genes, cloned portions of the *E. coli* K-12 *ilvGEDA* operon were hybridized with restriction endonuclease digestions of DNA from other members of the family *Enterobacteriaceae*. These studies are similar to those of Riley and co-workers (13–15) and demonstrate that interspecies DNA:DNA hybridization can serve as an effective tool for the detailed analysis of genetic organization.

The strains of enteric bacteria examined in this study are listed in Table 1. Genomic DNA prepared from each organism was digested with each of eight restriction endonucleases (*Ava*I, *Bam*HI, *Cla*I, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, and *Sal*I). *Hin*dIII digestions of DNA from *E. coli* K-12 T31-4-4 (wild-type *ilvGEDA* operon) and CU505 Δ(*ilvGEDAYC-rep*)*2049*, which deletes the entire *ilvGEDA* operon), were used to monitor the fidelity of the hybridization. The restriction fragments generated in these digestions were separated by size by using agarose gel electrophoresis and then transferred to nitrocellulose (19) as described previously (8). The nitrocellulose filters were subsequently hybridized with restriction fragments corresponding to portions of the *ilvGEDA* operon of *E. coli* K-12. The results of these studies are presented in Fig. 1.

Our analysis depended on the specificity of hybridization under the conditions employed in these studies. The conclusion that the hybrids detected were specific for the *ilvGEDA* genes is based on several criteria. First, the hybridization probes were restriction fragments known to code for specific portions of the operon. Second, a *Hin*dIII restriction digest of *E. coli* K-12 wild type and a strain deleted for entire operon (CU505; Δ(*ilvGEDAYC-rep*)*2049* [8]) were included in all hybridizations. Even at the lowest stringency, no nonspecific hybridization could be detected with either wild-type DNA or with the *ilvGEDA* deletion DNA. Third, the total size of the region defined for each strain is nearly identical, and single genomic restriction fragments of some species hybridized to probes that defined the 5′ and 3′ termini of the *E. coli* K-12 operon. Fourth, because *ilvG* encodes one of three isozymes of acetohydroxy acid synthase (*ilvB* and *ilvIH* encode the other two), a unique set of controls is possible. Both wild-type and the Δ(*ilvGEDA-rep*)*2049* DNA were digested with *Eco*RI and hybridized separately with probes specific for *ilvG*, *ilvB*, or *ilvIH* (the *ilvB* and *ilvIH* probes were generously provided, respectively, by G. W. Hatfield and J. M. Calvo). Even under the conditions of lowest stringency used in this study (i.e., 42°C and 25% formamide), no cross-hybridization between these three genes was detected. Thus, under the conditions used in this study, the restriction fragment probes were specific for homologous regions in each of the bacterial strains.

The restriction maps for the strains analyzed in this study may not be complete for the restriction endonucleases used. It is clear that restriction fragments of less than 500 base pairs could go undetected. Our principle conclusions are that the *ilvGEDA* structure is conserved and that there appear to be sites at which specific sequences are conserved. The organization of the genes was found to be identical to that of *E. coli* K-12 and *S. typhimurium* in all the organisms studied. The *ilvGEDA* genes were organized in the same order, G-E-D-A, and were confined to a region of the chromosome similar in size to the corresponding regions in *E. coli* K-12 and *S. typhimurium* LT2. They contradict the earlier report that the *ilv* genes are not clustered in *Yersinia pseudotuberculosis* (12). The conservation of these genes in this diverse sample of members of the family *Enterobacteriaceae* is strong evidence that the *ilvGEDA* organization is ubiquitous within the family, and that the operonic organization observed in *E. coli* K-12 and *S. typhimurium* LT2 is also maintained. This conclusion is also supported by studies of regulatory mutants of the *ilv* genes that were isolated in *Serratia marcescens* (9). In fact, this organization may be conserved in many other bacteria, as these genes have been reported to be clustered in *Bacillus subtilis* and *Pseudomonas aeruginosa* (17).

The bacterial species are organized in Fig. 1 by similarities between the restriction maps. This organization closely follows current estimates of relatedness between members of the family *Enterobacteriaceae* based on DNA:DNA hy-

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TABLE 1. *Enterobacteriaceae* strains

Organism	Strain	Source
Citrobacter freundii	UCIMC 79	V. Williamson[a]
Enterobacter aerogenes	ATCC 15947	ATCC[b]
Escherichia coli B		V. Williamson
Escherichia coli K-12	T31-4-4	This laboratory
Escherichia coli K-12	CU505 Δ(*ilvGEDAYC-rep*)*2049*	David Calhoun
Klebsiella pneumoniae	ATCC 13883	V. Williamson
Salmonella typhimurium	LT2	B. Ames
Serratia marcescens	UCIMC 81	V. Williamson
Shigella sonnei	S3-3	V. Williamson
Proteus vulgaris		C. Yanofsky
Yersinia pseudotuberculosis	ATCC 29833	ATCC

[a] College of Medicine, University of California, Irvine, Calif.
[b] ATCC, American Type Culture Collection.

bridization (6, 7, 17). The restriction maps of *Enterobacter aerogenes* and *Klebsiella pneumoniae* (Fig. 1, lines 5 and 6) appear to be the most similar. Many similarities also occur in the restriction maps of *E. coli* K-12, *E. coli* B, *Shigella sonnei*, and *Citrobacter freundii* (lines 1, 2, 3, and 4). However, there are no continuous patterns between the restriction maps of the 10 species. The restriction map may be conserved between one set of organisms in the 5′ region of the genes, whereas a second set of organisms have conserved restriction maps in a 3′ region. These examples demonstrate that certain regions of the *ilvGEDA* genes show definite similarities to the corresponding regions of other organisms and that these regions of conservation are independent of each other in several cases.

Presumably, restriction sites that are conserved in a majority of the species are located in DNA sequences under strong selection. These sequences may include either active sites of the encoded enzymes or regulatory loci. The *Sal*I restriction site located at map position 4.4 is conserved in 7 of the 10 restriction maps presented in Fig. 1 (*E. coli* K-12, *E. coli* B, *S. sonnei*, *C. freundii*, *K. pneumoniae*, *E. aerogenes*, and *S. typhimurium*). The location of the *Sal*I restriction site has been clearly established in *E. coli* K-12 by determination of the nucleotide sequence (10, 11). Thus, hybridization of the two probes, separated by the *Sal*I site, to distinct restriction fragments defines the structure of the homologous region of each strain. This restriction site is probably within the -10 region of the internal promoter between *ilvG* and *ilvE* that has been described for both *E. coli* K-12 (2) and *S. typhimurium* (2–4). This conclusion is based upon comparison of the sequence of the DNA between *ilvG* and *ilvE* to a promoter consensus sequence (16) and the fact that this site is protected from restriction endonuclease digestion by RNA polymerase (R. P. Lawther and G. W. Hatfield, unpublished data). The apparent conservation of this *Sal*I restriction site predicts the concomitant conservation of the overall nucleotide sequence, suggesting that the putative internal promoter preceding *ilvE* is maintained in many of the enteric bacteria.

Possible conservation of the nucleotide sequence in the

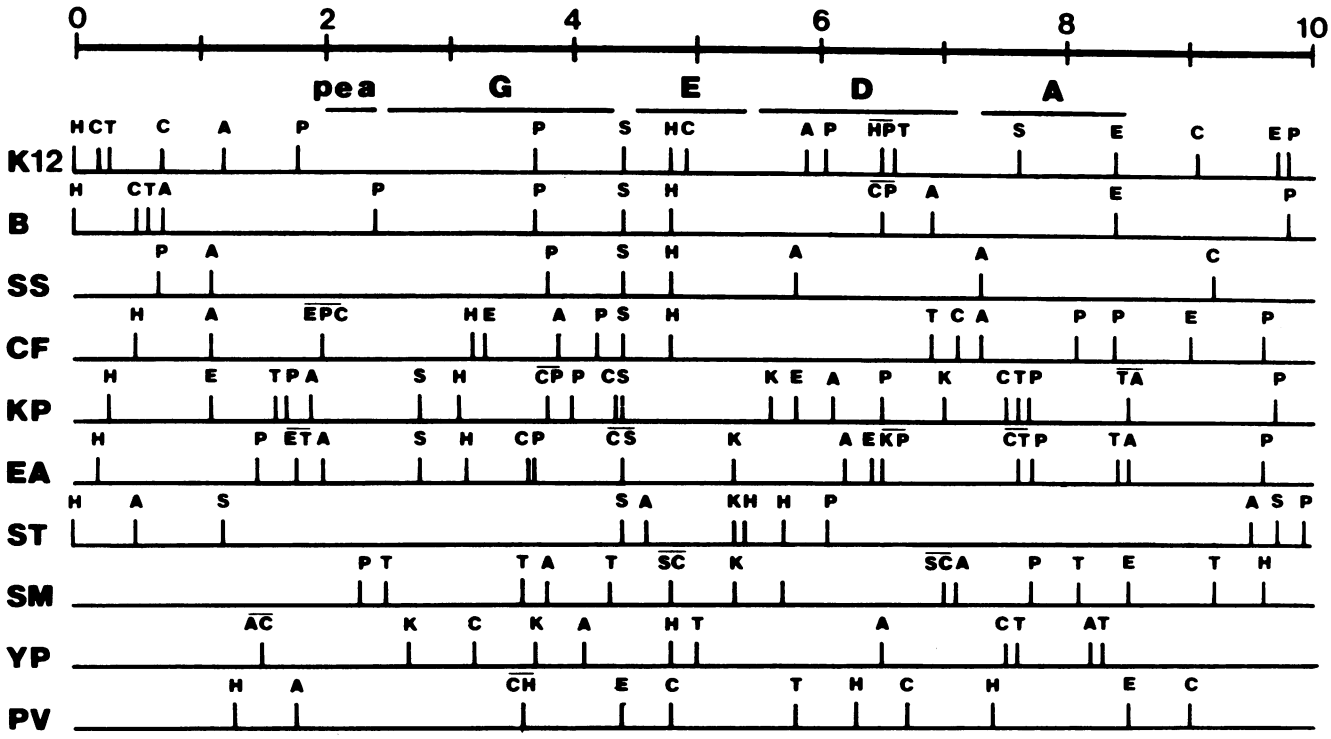

FIG. 1. Comparative restriction map of the *ilvGEDA* genes of 10 members of the family *Enterobacteriaceae*. The upper scale is in kilobase pairs. The strain designations are K-12, *E. coli* K-12; B, *E. coli* B; SS, *S. sonnei*; CF, *C. freundii*; KP, *K. pneumoniae*; EA, *E. aerogenes*; ST, *S. typhimurium*; SM, *S. marcescens*; YP, *Y. pseudotuberculosis*; and PV, *Proteus vulgaris*. Restriction endonuclease sites are indicated by the following letters: A, *Ava*I; K, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; T, *Pst*I; P, *Pvu*II; S, *Sal*I. The restriction maps for *E. coli* K-12 and *S. typhimurium* are based on published data (5, 20) and our unpublished data.

region of the first internal promoter was investigated in organisms which do not maintain the *Sal*I restriction site by using restriction endonucleases which have similar recognition sequences to *Sal*I (recognition sequence, GTCGAC). The restriction enzymes used included *Acc*I (recognition sequence, GTMKAC; M = A or C and K = T or G), *Hin*cII (recognition sequence, GTYRAC; Y = C or T and R = G or A), and *Xho*I (recognition sequence, CTCGAG). These experiments were performed by parallel hybridizations of chromosomal restriction digestions with contiguous *ilvG* and *ilvE* probes. The *Y. pseudotuberculosis* chromosome was found to have an *Xho*I restriction site at this location. This would be the result of a two-nucleotide change in the DNA sequence of this organism, preserving the four-nucleotide core sequence TCGA. This observation further supports our conclusion that the DNA sequence in the region of the internal promoter between *ilvG* and *ilvE* is conserved.

Many of these organisms also conserve a *Pvu*II restriction site between map positions 9.5 and 9.8, 3′ of the *ilvGEDA* genes. The existence of this site in 6 of the 10 organisms studied is strong evidence that the site is conserved. The restriction map of the *ilv* gene cluster of *E. coli* K-12 indicates that the *Pvu*II restriction site at map position 9.8 lies within the beginning of *ilvC* (20). Conservation of this site may indicate clustering of the *ilv* genes as in *E. coli* K-12 and *S. typhimurium*, i.e., the organization (*ilvGEDA*, *ilvY*, *ilvC*) is conserved. The corresponding region of the *S. marcescens* chromosome has a *Hin*dIII restriction site which has the same internal four-nucleotide recognition sequence as that of restriction endonuclease *Pvu*II—AGCT. Of the 10 strains in Fig. 1, 7 have either a *Pvu*II or *Hin*dIII restriction site in the vicinity of map position 9.6. Although we have no direct hybridization data, this would be consistent with retention of the linkage of *ilvC* to *ilvGEDA*.

Analysis by hybridization to chromosomal restriction fragments can be used for the characterization of gene structure. This technique eliminated the need to isolate a specific region of the chromosome in a recombinant plasmid and allowed the simultaneous comparison of the restriction maps from several organisms. An unexpected result from our analysis of these bacterial strains was the detection of incompatibilities in restriction modification systems. It was observed that the restriction endonuclease *Pst*I does not restrict DNA from *S. sonnei*. This type of observation could not be made by more conventional methods of genetic characterization, because the recombinant vectors employed are passed through *E. coli* and would lose the modifications present in the original bacteria.

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