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I-*Ceu*I Reveals Conservation of the Genome of Independent Strains of *Salmonella typhimurium*

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The enzyme I-*Ceu*I, encoded by a class I mobile intron inserted in the gene for 23S rRNA in *Chlamydomonas eugamatos*, cleaves a specific 19-bp sequence in this gene. This sequence is present only in the seven genes for rRNA in *Salmonella typhimurium* and *Escherichia coli*. Partial digestion with I-*Ceu*I of DNA from 17 wild-type strains of *S. typhimurium* indicates that the chromosome of these strains is strongly conserved, for the digestion products closely resemble those of strain LT2. The lengths and order of chromosomal segments are conserved in 15 of the strains; 2 show some rearrangements. *Xba*I digestion indicated heterogeneity without revealing the genomic structure. Because of conservation of I-*Ceu*I sites in genes for rRNA and conservation of the number and locations of these genes, I-*Ceu*I provides an excellent tool for the rapid examination of the chromosomes of related species of bacteria; differences in the fingerprints indicate the occurrence of chromosomal rearrangements such as insertions or inversions.

The use of type II restriction endonucleases has revolutionized the study of molecular biology; the intron-encoded endonucleases (3) are beginning to have an impact on the field. I-*Ceu*I (5, 7, 17) is a representative of this kind of endonuclease. Many others have also been reported, and some are commercially available, including I-*Ppo*I (18), I-*Sce*I (3), I-*Sce*II (4), I-*Tev*I and I-*Tev*II (1), and I-*Cre*I (6).

I-*Ceu*I is encoded by a class I mobile intron which is inserted into the *rrl* gene for the large-subunit rRNA (23S rRNA) in the chloroplast DNA of *Chlamydomonas eugamatos*. I-*Ceu*I is specific for and cuts in a 19-bp sequence in the *rrl* gene. Because rRNA sequences are strongly conserved, this 19-bp sequence is present in the genomes of many enteric bacteria (12, 13, 16) and in *Rhizobium meliloti* (9), as well as in chloroplasts and mitochondria of eukaryotes. Because I-*Ceu*I cleaves only *rrn* genes and because the number and locations of these genes are highly conserved in enteric bacteria (10), related wild-type strains yield similar I-*Ceu*I fingerprints which give valuable genomic information on DNA insertions or rearrangements; fingerprints with *Xba*I, a type II restriction endonuclease with a 6-bp site, are much more variable, providing little direct genomic information. Some investigators have reported difficulties in working with I-*Ceu*I, and so in this report we present our protocol for its use.

Salmonella typhimurium LT2, which was obtained from Joshua Lederberg (20), was originally isolated by Lilleengen (11). The strains designated SARA2 to SARA18 were obtained from Robert Selander (2) as part of *Salmonella* reference collection A (SARA), a reference set of electrophoretic types established by using multilocus enzyme electrophoresis; strain LT2 is designated SARA2. Luria-Bertani (LB) medium (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, 3.5 ml of 1 M NaOH) was used for cultivation of all strains; solid medium also contained 1.5% agar and 0.2% glucose. Strains were maintained in 15% glycerol at −76°C, and a single colony was isolated prior to use. Endonucleases used were I-*Ceu*I from New England Biolabs and *Xba*I from Boehringer Mannheim. Most other chemicals, including agarose, were from Sigma Chemical Co.

Higher-quality DNA is needed for I-*Ceu*I than for other endonucleases, and so we modified our techniques as follows. Bacterial cells were grown overnight with shaking in LB broth, and then 3-ml volumes were centrifuged in 15-ml plastic tubes, resuspended in 0.5 ml of cell suspension solution (10 mM Tris-HCl [pH 7.2], 20 mM NaCl, 100 mM EDTA), warmed to 70°C in a water bath, and mixed with 0.5 ml of 1.2% agarose (regular, not low melting point) which had been boiled and then held at 70°C. The mixture was then immediately drawn into a tuberculin syringe from which the needle adapter had been cut. When the agarose rod had hardened after a 20-min incubation at room temperature, it was sliced into 1-mm-thick discs which were then put into 3 ml of lysing solution (10 mM Tris-HCl [pH 7.2], 50 mM NaCl, 100 mM EDTA, 0.2% sodium dodecyl sulfate, 0.5% *N*-laurylsarcosine) and gently shaken at 70°C for 90 min. The discs were drained, washed once in wash solution (20 mM Tris-HCl [pH 8.0], 50 mM EDTA), and then treated with 3 ml of proteinase K solution (1 mg of proteinase K per ml, 100 mM EDTA, 0.2% sodium dodecyl sulfate, 1% *N*-laurylsarcosine) at 42°C for 18 h. The discs were washed in wash solution and treated with PMSF solution (1 mM phenylmethylsulfonyl fluoride in wash solution) for 1 h at room temperature with gentle shaking to remove the activity of proteinase K. After two washes in wash solution and one in storage solution (10-times-diluted wash solution), the discs were ready for either digestion with endonucleases or storage (in storage solution at 4 to 8°C). This method regularly yields DNA which digests well with I-*Ceu*I and with other enzymes. There are several changes from the methods reported earlier (14), including elimination of the use of lysozyme, but the most important modification is lysis at 70°C rather than at 37°C; this precludes the use of low-melting-point agarose. Enzyme preparations should be titrated prior to use, since digestion with a high concentration of enzyme or digestion for extended times resulted in smears in the gel.

For pulsed-field gel electrophoresis (PFGE), a Bio-Rad CHEF-DRII apparatus was used. Fragment separation is optimal if a pulse time of about 1 s for each 10 kb of fragment is used; thus, a group of DNA fragments sized from 250 to 350 kb requires a pulse time of around 30 s. Timing of the pulses is

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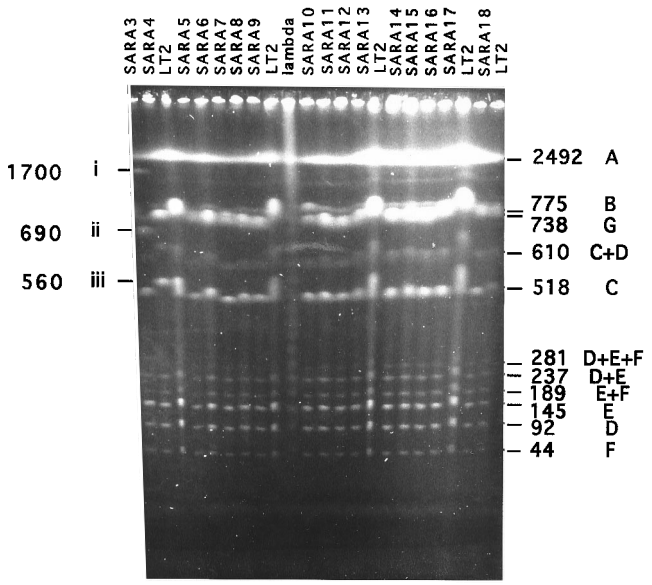

FIG. 1. Separation by PFGE of fragments from I-*Ceu*I digestion of DNA from strains of *S. typhimurium* from the SARA set of strains (2). The following pulse conditions were used with a Bio-Rad CHEF-DRII apparatus at 200 V: pulse times ramped from 50 to 70 s over 17 h, followed by pulse times ramped from 3 to 12 s over 6 h. The lanes marked LT2 represent DNA from strain LT2 (which is SARA2); other lanes show the number of the strain from the SARA set. (The preparation of LT2 DNA was made at a different time and is overloaded in this run; we repeated this test, and LT2 bands looked the same as those for other strains in this gel.) The I-*Ceu*I fragments and their sizes in kilobases are shown on the right. The unusual bands for SARA3 and SARA4 and their sizes are indicated on the left.

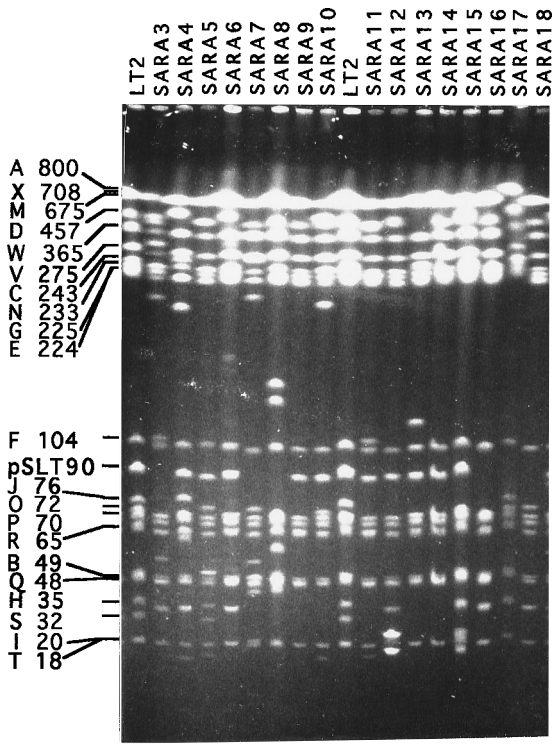

FIG. 2. Separation by PFGE of fragments from *Xba*I digestion of DNA of strains from the SARA set (2). The following pulse conditions were used with a Bio-Rad CHEF-DRII apparatus at 200 V: cycle 1, 3 to 80 s over 12 h at 120°C; cycle 2, 2 to 10 s over 6 h at 150°C; cycle 3, 20 to 25 s over 6 h at 150°C; cycle 4, 60 to 80 s over 4 h at 150°C. Strain LT2 (SARA2) is represented twice; other strains, from SARA3 to SARA18, are represented once. The fragment designations and sizes (in kilobases) are shown on the left and are based on data from strain LT2 reported earlier (12).

also important. Large fragments must be separated from other DNA early in the run; otherwise they are difficult to separate. Therefore, we arrange the pulses in a series of cycles. The first cycle of PFGE is run at pulsing conditions suitable for the whole range of fragment sizes (e.g., ramping from 10 to 120 s over 12 to 20 h), ensuring that all fragments are run out of the wells. The next cycles, if needed, are used to "zoom in" on specific fragment sizes; for example, separation of a crowded group of fragments near 400 kb would be done by ramping from 30 to 50 s for 12 h, and other crowded areas would be separated in the same way.

The gels are 155 mm wide and 227 mm long, with 25 wells. The gel thickness is about 7 mm, and the agarose concentration is 0.6%.

We find little variation in the lengths of chromosome segments between *rrn* genes in independent wild-type strains of *S. typhimurium*. DNA from 17 strains from the SARA set, representing several different electrophoretic types, was digested with I-*Ceu*I, separated by PFGE, and stained with ethidium bromide (Fig. 1). As reported previously (12, 13), strain LT2 has seven bands due to I-*Ceu*I fragments designated I-*Ceu*I-A to -G, resulting from digestion of I-*Ceu*I sites in the seven *rrn* genes. There are fainter bands due to partial digestion with I-*Ceu*I, and these are the sum of two or more adjacent I-*Ceu*I fragments. Thus, the I-*Ceu*I bands of 44, 92, and 145 kb represent fragments F, D, and E, while the bands of 189, 237, and 280 kb are postulated to be I-*Ceu*I-EF, -DE, and -DEF, respectively. This was confirmed with some strains by dissection of a gel fragment containing the band, redigestion with I-*Ceu*I, and reelectrophoresis, as described earlier (13) (data not shown). These data confirm the order of the fragments in strain LT2 to be I-*Ceu*I-DEF; together with a band composed of I-*Ceu*I-CD fragments, the order of fragments is shown to be I-*Ceu*I-CDEF. The strains SARA3 to SARA18 are independent wild-type strains of *S. typhimurium* whose genome has not been previously studied. The sizes of I-*Ceu*I fragments and of bands resulting from partial digestion are indistinguishable in 15 of the 17 strains, including LT2, indicating that the genome structure of these 15 strains is of the LT2 type in the following respects: all have seven *rrn* genes, all have chromosome segments between *rrn* genes of very similar lengths, and all have the I-*Ceu*I fragments in the order I-*Ceu*I-CDEF. Two of the 17 strains show some differences. In SARA4, the I-*Ceu*I-C fragment is slightly larger than it is in the other strains. In SARA3, the sizes of both I-*Ceu*I-B and I-*Ceu*-G are greatly altered. But overall, the lengths of chromosome segments between *rrn* genes, and the order of these segments in the chromosome, are strongly conserved in wild-type strains of *S. typhimurium.*

Digestion of DNA of LT2 by *Xba*I (Fig. 2) revealed the fragment sizes reported earlier (12). Fragment sizes of other wild-type strains of *S. typhimurium* show some similarity to those of LT2 and to each other, but there are many differences and no obvious separation into classes. Strains SARA2 to SARA5, of electrophoretic type Tm1, do not resemble each other more than they resemble other electrophoretic types.

In using I-*Ceu*I, it is important to titrate its activity prior to the first use, for high concentrations of the extract and long treatment times may cause DNA degradation, and to have very high-quality genomic DNA for digestion. A series of long pulse times must be used early, to separate out large DNA fragments. After this, pulse conditions designed to separate a spe-

cific group of fragments can be used, with the general rule that for optimal separation of a group of fragments, 1 s of pulse time is needed per 10 kb of fragment.

The fingerprint resulting from digestion with *Xba*I shows considerable variation among different strains but does not allow conclusions about genome arrangements to be drawn directly from the data. It is also not possible to determine genome sizes accurately, because some of the single bands may represent more than one fragment. Though data obtained with type II restriction endonucleases such as *Xba*I, *Bln*I, *Not*I, and others cannot be used for genomic comparisons in the same direct way as can data obtained with I-*Ceu*I, the data from these enzymes are still valuable for construction of genomic cleavage maps.

Comparisons of the structure of the genomes of *Escherichia coli* K-12 and *S. typhimurium* LT2 (10, 19) indicate a high degree of conservation. Partial digestion with I-*Ceu*I shows that wild-type strains of *S. typhimurium* are similarly conserved (Fig. 1). However, the genomes of *Salmonella typhi* Ty2 (16), *Salmonella paratyphi* A (15), and *S. paratyphi* C (8) contain genomic rearrangements postulated to be due to homologous recombination in the *rrn* genes. I-*Ceu*I can be used to examine members of the genus *Salmonella*, and other genera of bacteria, for rearrangements of these types.

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