JOURNAL OF BACTERIOLOGY, Sept. 1983, p. 1116–1122
0021-9193/83/091116-07$02.00/0

Vol. 155, No. 3

Isolation and Preliminary Characterization of *Escherichia coli* Mutants Deficient in Exonuclease VII

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Received 3 March 1983/Accepted 21 June 1983

Strains of *Escherichia coli* containing reduced levels of exonuclease VII activity due to mutations in the *xseB* gene have been isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Seven mutants of independent origin deficient in exonuclease VII activity were obtained. Four of these contained defects in *xseA*, a locus which has been previously identified, and three others contained mutations in a gene distinct from *xseA*, which we have designated *xseB*. Genetic mapping studies place the *xseB* locus between *proC* and *dnaZ*. Exonuclease VII purified from KLC835 (*xseA*$^+$ *xseB3*) is more heat labile than enzyme purified from the parent strain PA610 (*xse*$^+$), showing that *xseB* is a structural gene for exonuclease VII. The isolation of λ transducing phage carrying *xseA* is also described.

Exonuclease VII isolated from *Escherichia coli* K-12 is a single-stranded DNA-specific nuclease which hydrolyzes DNA from both 3′ and 5′ termini. The enzyme has been shown to retain 80% of its activity in the presence of EDTA, making possible a specific assay for exonuclease VII in crude extracts. This property of the enzyme led to the initial isolation of *xse* strains of *E. coli*, all of which have now been shown to contain mutations at a locus designated *xseA* near the guanine operon (5, 16). In addition, *xseA* has been shown to be a structural gene for the enzyme (5). We have now shown that exonuclease VII is composed of two nonidentical subunits (17). The studies reported here describe the isolation and preliminary characterization of strains containing mutations in a second locus, which we have designated *xseB*, coding for another subunit of exonuclease VII. In addition, the isolation of the *xseA* gene on phage λ is described.

MATERIALS AND METHODS

Bacterial and phage strains. All strains used were derived from *E. coli* K-12. A list of the strains used and their properties is given in Table 1. Phage P1 *vir* was used for generalized transduction (11). Figure 1 is a map of the *E. coli* chromosome showing the positions of relevant markers and the points of origin of the Hfr strains used.

Medium. The medium utilized has been previously described (5). Pyridoxine was added to a final concentration of 5 μg/ml when required.

Preparation of cell extracts. The preparation of crude cellular extracts of cultures grown in 96-well plates has been previously described (5). When exonuclease VII activity was determined quantitatively, extracts were prepared from large cultures by sonic irradiation. Approximately 15 ml of exponentially growing cultures at an absorbance at 590 nm of 1.0 were collected by centrifugation at 10,000 rpm in a Sorvall SS-34 rotor and suspended in 1 ml of 20 mM Tris-hydrochloride buffer (pH 8.0)–10 mM 2-mercaptoethanol–0.1 mM EDTA–10% (wt/vol) glycerol to yield approximately 2.5 mg/ml of protein. The cells were then disrupted by sonic irradiation with 5 min of pulsed irradiation (output setting no. 1, 30% duty cycle), using a Heat Systems model W-375 sonicator.

Mating and transduction procedures. Transductions were carried out using P1 *vir* as described by Miller (11). All bacterial matings were performed as described previously (5, 11).

Isolation of mutants. A mass screening technique that was utilized in the original isolation of *xseA* mutant strains was followed (5). *E. coli* PA610 and KLC786 were mutagenized with 1 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) per ml as described previously (5). In addition, a culture of strain KLC786 was mutagenized with NTG at 5 mg/ml. To follow the efficiency of mutagenesis, the fraction of cells unable to utilize rhamnose as a carbon source and the proportion which developed auxotrophic requirements in addition to requirements of the parental strains were determined. Approximately 4% of the cells became *rha*$^-$, and 50% developed additional auxotrophic requirements after exposure to 1 mg of NTG per ml. Approximately 10% of the cells became *rha*$^-$ after exposure to 5 mg of NTG per ml.

Isolation of λ transducing phage for *xseA*. A low-frequency transducing phage lysate for *xseA* was obtained by temperature induction of strain KS504 (15). The lysate was plated on strain LG102 (lysogenic for P2 phage) to isolate phage of the SPI$^-$ (insensitive to phage P2 interference) phenotype. Twenty plaques were picked; from these, λ plate stocks were made (11, 15). The SPI$^-$ phage were then used to infect 2 ml of strain KLC381 grown to an absorbance of 0.5 at 590

TABLE 1. Bacterial strains

Strain	Relevant genotype	Source or construction
Hfr KL16	*thi-1 rel-1* λ^-	CGSC[a] strain 4245
HfrH	*thi-1 rel-1* λ^-	CGSC strain 259
Hfr KL226	*rel-1 tonA22 spoT1* λ^-T2^r	CGSC strain 4311
PA610	F^- *thr-1 leu-6 his-1 argH1 lys-25 lacY1 malA1 xyl-7 ara-13 mtl-2 gal-6 purE43 tonA2 thi-1 rpsL9*	F. Jacob
PC0207	*proA35*	CGSC strain 5498
PC0567	*thi-1 his-68 tyrA2 trp-45 purC50 guaB lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL125 tonA2 supE44 tsx-70* λ^-	DeHaan (12)
χ342	*proC29 metB1 relA1 spoT1* λ^-	CGSC strain 4515
N43	*acrA1*	CGSC strain 5583
AT3143	F^- *pyrF30 ilv-277 met-65 his-53 purE41 proC24 pdxC3 xyl-14 lacY29 rpsL97 cycA1 cyc-2 tonA32 tsx-63* λ^-	CGSC strain 4539
K797	*phoR79*::Tn*10*	CGSC strain 6456
AX727	*dnaZ2016*(Ts)	Walker (6)
LG102	P2 lysogen	Sunshine (9)
CBK103	F^- *thyA cysG*::Tn*5*	Berg (14)
KS504	HfrH Δ(*gal-bio*) λ *cI857* within *guaB*[b]	Shimada et al. (15)
KLC101	*guaA21 thyA trpA33 rha*	Chase
KLC147	*xseA7 thyA* KLC101	Chase
KLC381	λ^s Δ(*xseA-guaB*) KS504	Vales et al. (16)
KLC488	*xseA5 thyA* KLC101	Vales and Chase
KLC786	*malA*$^+$ λ^s PC0567	This work
KLC839	*purE*$^+$ *acrA1* AT3143	P1 transduction: AT3143 × N43
KLC840	*proC*$^+$ *xseB2* KLC839	P1 transduction: KLC839 × KLC815
KLC1011	*xseB*$^+$ KLC840	This work
KLC1020	*dnaZ2016*(Ts) *xseB2* AX727	This work

[a] CGSC, *E. coli* Genetic Stock Center.
[b] Δ, Deletion.

nm in tryptone broth containing 0.2% maltose. The infection was carried out at 37°C for 30 min at a multiplicity of infection of 0.5. Infected cells were collected by centrifugation for 10 min at 9,000 rpm in a Sorvall SE-12 rotor and suspended in 0.2 ml of a solution containing 0.25 mg of lysozyme per ml in 33 mM Tris-hydrochloride buffer (pH 8.0)–0.1 mM EDTA. Cell extracts were prepared and assayed for exonuclease VII activity as previously described (5).

Assay of exonuclease VII in cell extracts. The preparation of cell extracts and the subsequent assay of exonuclease VII have been described previously (5) and were followed in these studies with the following exceptions. Reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.9), 50 mM Tris-hydrochloride buffer (pH 7.9), 8.3 mM EDTA, 10 mM 2-mercaptoethanol, and 0.5 to 1 nmol of single-stranded T7 [^{3}H, 5'-^{32}P]DNA. This DNA substrate was uniformly labeled with tritium and contained 5' termini labeled with [γ-^{32}P]ATP in the reaction catalyzed by polynucleotide kinase. The preparation and use of this substrate have been previously described in detail (3, 4). DNA was denatured by heating. The mass screening of strains for mutants containing defects in exonuclease VII activity involved assaying for the enzyme in crude extracts from cultures of strains grown in 96-well plates. The acid-soluble ^{32}P radioactivity produced was spotted on Norit impregnated paper which was then exposed to X-ray film. This procedure has been described in detail (5).

Assay of exonuclease VII in cells obtained from matings and transductions. The assays of exonuclease VII activity in transductants and mating recombinants were performed in 96-well plates as described above with the exception that the substrate utilized was single-stranded T7 [^{3}H]DNA. In this case, acid-soluble ^{3}H radioactivity was determined directly by counting samples of supernatant fluid after precipitation with trichloroacetic acid in Hydrofluor in a Packard scintillation spectrometer.

Purification of exonuclease VII. Exonuclease VII was purified from strains PA610 and KLC835 through the DEAE-cellulose step of Chase and Richardson (3) with the addition of a polyethylene glycol-dextran sulfate phase partition step after the streptomycin sulfate precipitation (17). The purified enzyme was assayed by the standard exonuclease VII assay (3).

Protein determination. Protein was determined by the method of Lowry et al. (10).

Chemicals. NTG was purchased from Aldrich Chemical Co. Pyridoxine was obtained from Sigma Chemical Co.

RESULTS

Isolation of *xse* mutant strains. *E. coli* strains containing mutations in a structural gene for exonuclease VII, *xseA*, have already been identified (5). Recently, we determined that exonuclease VII is composed of two nonidentical subunits (17). To determine the chromosomal

TABLE 2. Exonuclease VII activity[a] in extracts

Strain	Exonuclease VII sp act[b] (nmol/mg protein)		43°C/30°C[c]
	30°C	43°C	
PA610 (*xse*$^+$)	2.2	5.6	2.5
KLC815 (*xseB2*)	<0.1	<0.1	
KLC833 (*xseA10*)	0.1	<0.1	
KLC834 (*xseA11*)	<0.1	<0.1	
KLC835 (*xseB3*)	2.3	0.3	0.13
KLC786 (*xse*$^+$)	2.9	6.2	2.1
KLC801 (*xseB1*)	<0.1	<0.1	
KLC836 (*xseA12*)	0.2	1.6	8.0
KLC837 (*xseA13*)	<0.1	<0.1	

[a] Strains KLC815, KLC833, KLC834, and KLC835 were derived from PA610. The remaining strains were derived from KLC786. Strain KLC801 was isolated from a culture of KLC786 mutagenized with 5 mg of NTG per ml. All other mutant strains were obtained after mutagenesis of PA610 or KLC786 with 1 mg of NTG per ml. Strains KLC836 and KLC837 were derived from the same mutagenized culture of KLC786. Similarly, strains KLC815, KLC834, and KLC835 were isolated from the same mutagenized culture of PA610, with KLC833 being derived from an independent culture of the same parent. All extracts were prepared by sonic irradiation from cells grown exponentially at 30°C, and each contained approximately 2.5 mg of protein per ml. The standard exonuclease VII assay (3) was used with a single-stranded uniformly labeled T7 [^{3}H]DNA substrate. Specific activity is given as nanomoles of acid-soluble ^{3}H produced in 30 min at the specified temperature per milligram of protein.

[b] The assay was not sensitive enough for specific activities of <0.1 to be reliably measured.

[c] Ratio of activity at 43°C to that at 30°C during assay.

location of the gene coding for the second subunit, we isolated additional strains deficient in exonuclease VII activity. *E. coli* PA610 and KLC786 were independently mutagenized with NTG, and approximately 2,000 extracts of two independently mutagenized cultures of each strain type, or a total of 8,000 extracts, were assayed for exonuclease VII activity. The mutagenized cells were grown at 30°C on minimal medium to eliminate strains containing auxotrophic mutations in addition to mutations of their parental strain. The assay was performed at 43°C so that strains containing temperature-sensitive enzyme activity could be identified. A total of seven strains having reduced levels of exonuclease VII activity were obtained (Table 2).

Initial mapping studies. To distinguish strains containing *xseA* mutations from strains containing mutations at other loci required for exonuclease VII activity, we made use of the following findings. It had been previously shown that *xseA* is 96% cotransducible with *guaA* and *guaB*, contiguous genes in the guanine operon (12, 13). In addition, previous studies suggested that the locus coding for the second exonuclease VII subunit does not lie in the interval *hisS-upp*, which includes the guanine operon region and hence would not be highly cotransducible with *guaA* or *guaB*. We therefore initially screened the *xse* strains for cotransduction of the *xse* marker with *guaB*. Mutant strains derived from strain KLC786 are *guaB*; hence, *guaB*$^+$ transductants of these strains were selected using P1 phage grown on CBK103 (*xse*$^+$). Transductants were then assayed for exonuclease VII activity. Mutant strains derived from PA610 provided donors for phage P1-mediated transduction of strain KLC381 Δ(*guaB-xseA*) to *guaB*$^+$. Again, transductants were assayed for exonuclease VII activity. The first mutant strain analyzed in this way (KLC815) was shown to be *xseA*$^+$ (data not shown).

Hfr mating experiments were performed to localize the mutation responsible for the defect in exonuclease VII activity in KLC815. Recombinant cells were assayed for exonuclease VII activity, by using the mass screening assay procedure (see above). Approximately 100 recombinants were assayed in each experiment. *E. coli* Hfr KL16 was mated with KLC815, and His$^+$ Str^r recombinants were selected and assayed for enzyme activity. Recombinants isolated after 30 min of mating time did not contain exonuclease VII activity. A second mating experiment with strains HfrH and KLC815 selecting for *purE*$^+$ Str^r recombinants was performed, and after 15 min of mating time exonuclease VII activity was found in recombinant cells. The position of the mutation in strain KLC815 was determined to be in the interval between the origin of transfer of HfrH and that of Hfr KL226 since *purE*$^+$ Str^r recombinants of KLC815 were found to contain exonuclease VII activity after a 5-min mating of KLC815 with Hfr KL226, suggesting that the *xse* mutation was near the origin of transfer of Hfr KL226.

We conclude that a previously unidentified locus required for exonuclease VII activity and clearly distinct from the *xseA* locus lies in this region of the chromosome. We suggest that this locus be designated *xseB*.

Mapping of *xseB* by P1 transduction. Since the mutation in strain KLC815 seemed to lie near the origin of transfer of Hfr KL226, cotransduction frequencies were determined with several markers in this vicinity. The first maker examined was *purE*. Strain KLC815 was transduced to *purE*$^+$ by phage P1 *vir* grown on strain CBK103, but none of the 86 transductants assayed contained exonuclease VII activity. Further transductions were performed using phage

TABLE 3. Transduction of the recipient strain to *xseB*$^-$ or *xseB*$^+$ by phage P1 *vir*

Recipient strain[a]	Donor strain	Selected marker	No. assayed for Xse	No. Xse$^-$ or Xse$^+$	Cotransduction frequency (%)
PC0207	KLC815	*proA*$^+$	88	0 (−)	<0.1
KLC839	KLC815	*proC*$^+$	178	23 (−)	13
KLC1020	KLC1011	*dnaZ*$^+$	52	4 (+)	7.7
KLC840	K797	Tet^r (Tn*10*)	52	9 (+)	17

[a] The recipient strain was routinely grown to approximately 2 × 10^8 cells per ml. In these transductions 2.5 ml of cells and a multiplicity of infection of 0.1 were used.

P1 *vir* grown on strains KLC815, KLC1011, and K797, and selections were for the markers indicated (Table 3). Transductants were assayed for exonuclease VII activity. The results of these studies (Table 3) produced the linkage map shown in Fig. 1. We conclude from these studies that *xseB* lies between *proC* and *dnaZ*.

Determination of *xse* genotype of mutant strains. The *xse* genotype of strains derived from PA610 was determined first by selecting Gua$^+$ transductants of an *xseA-guaB* deletion strain, KLC381, using P1 *vir* phage grown on the respective mutant strains, and screening the recombinants for exonuclease VII activity at 43°C. More than 90% of the Gua$^+$ transductants from the crosses with phage P1 *vir* grown on strains KLC815 and KLC835 were Xse$^+$, showing that these strains were *xseA*$^+$ and suggesting that they were *xseB*$^-$. To confirm this identification, a transduction was performed with strain AT3143 as the recipient, selecting Pro$^+$ recombinants and screening for exonuclease VII activity. Approximately 10% of these transductants were Xse$^-$, confirming that strains KLC815 and KLC835 were *xseB*$^-$. None of the Gua$^+$ transductants of KLC381 derived from the crosses performed with phage P1 *vir* grown on KLC833 and KLC834 were Xse$^+$, suggesting that these strains were *xseA*$^-$. To confirm that KLC833 and KLC834 were *xseA*$^-$, transductions were performed into KLC786 (*guaB*$^-$), selecting Gua$^+$ recombinants and screening for exonuclease VII activity. As expected, more than 90% of these transductants were Xse$^-$.

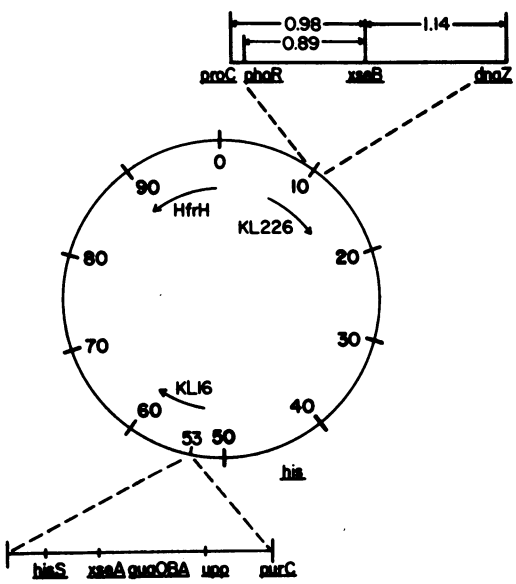

FIG. 1. Genetic map of *E. coli* K-12 (1) indicating the position of pertinent markers referred to in the text and approximate origin and direction of transfer of Hfr strains used in this study. The expanded portion at approximately 10 min shows the relative distances (in minutes) (18) of *xseB* from several markers as calculated from cotransduction frequencies (Table 2).

The *xse* genotype of mutant strains derived from strain KLC786 (*guaB*$^-$) was determined by transducing the respective mutant strains to Gua$^+$, using P1 *vir* phage grown on a *gua*$^+$ *xse*$^+$ strain and then assaying the transductants for exonuclease VII activity. Since more than 90% of the Gua$^+$ transductants of strains KLC836 and KLC837 were *xse*$^+$, both strains contained *xseA* mutations. Strain KLC801 could not be transduced to *xse*$^+$, suggesting that it was *xseB*$^-$. *E. coli* HfrH was mated with KLC801, and Lac$^+$ recombinants were grown and assayed for exonuclease VII activity. After a 15-min mating, exonuclease VII activity was found to appear in recombinant cells, suggesting that KLC801 contained an *xseB* mutation.

Level of exonuclease VII activity in *xse* strains. To quantitate the amount of exonuclease VII activity present, extracts of exponentially growing cultures of the mutant strains were prepared by sonic irradiation. The specific activity of the enzyme in each of these strains was determined at 30 and 43°C and compared with that of their respective parental strain, PA610 or KLC786 (Table 2). Four of the seven strains isolated had little or no detectable exonuclease VII activity at either temperature. Mutant strains KLC833 (*xseA10*) and KLC835 (*xseB3*) contained heat-labile exonuclease VII activity. The seventh strain, KLC836 (*xseA12*), exhibited exonuclease VII activity that was cold sensitive, showing

TABLE 4. Purification of exonuclease VII from wild-type and mutant strains

Fraction	PA610 (*xse*$^+$)		KLC835 (*xseB3*)	
	Sp act[a]	43°C/ 30°C[b]	Sp act[a]	43°C/ 30°C[b]
I. Extract	3.3	2.4	2.5	0.2
II. Streptomycin pellet	15	1.9	17	<0.1
III. Polyethylene-glycol phase	40	1.6	33	<0.1
IV. Precipitate after dialysis	150	1.5	100	<0.1
V. DEAE-cellulose	939	1.8	581	<0.1

[a] The specific activity was determined for the assay at 30°C. The standard exonuclease VII assay was used with a single-stranded T7 [^{3}H]DNA substrate. Under the conditions of the assay, specific activities of <0.1 could not be reliably determined.

[b] Ratio of activity at 43°C to that at 30°C during assay.

eight times as much activity at 43 than at 30°C.

***xseB* is a structural gene for exonuclease VII.** Table 2 shows that strain KLC835 (*xseB3*) contained approximately wild-type levels of exonuclease VII activity at 30°C but an eightfold reduction in activity at 43°C. This heat-labile activity was associated with exonuclease VII. The enzyme was purified from strains KLC835 and PA610 (Table 4). Throughout the purification, the enzyme from both strains exhibited a constant ratio of activities at 43 and 30°C. For the wild-type enzyme that ratio was about 2:1; for the mutant enzyme, however, exonuclease VII purified from the mutant strain KLC835 remained temperature sensitive throughout purification. Exonuclease VII purified from strains PA610 and KLC835 was assayed at 30 and 43°C as a function of incubation time (Fig. 2). The enzyme purified from strain KLC835 ceased to function approximately 5 min after the reaction mixture was placed at 43°C. Finally, preincubation of purified enzyme for various times at 43°C before its assay for 30 min at 30°C indicated that the half-life of exonuclease VII isolated from strain KLC835 was four- to fivefold less than that of the wild-type enzyme (Fig. 3). All of these results directly associate the temperture-sensitive exonuclease VII activity in strain KLC835 with the enzyme purified from this strain, showing that *xseB* is a structural gene for exonuclease VII.

Isolation of λ d*xseA*. Strain KS504, with prophage λ *c*I857 inserted in the *guaB* gene, was utilized for isolating λ transducing phage carrying *xseA*. Considering the genetic structure required to produce SPI$^-$ λ phage, the fact that the orientation of the *gua* operon relative to *xseA* was known (16), and finally that the orientation of the prophage in this strain had been established by Shimada et al. (15), we surmised that phage exhibiting the SPI$^-$ phenotype derived by temperture induction of strain KS504 would likely include transducing phage for *xseA*. A total of 20 such phage were isolated (see above), and 70% of these were found to restore approximately 50% of the wild-type level of exonuclease

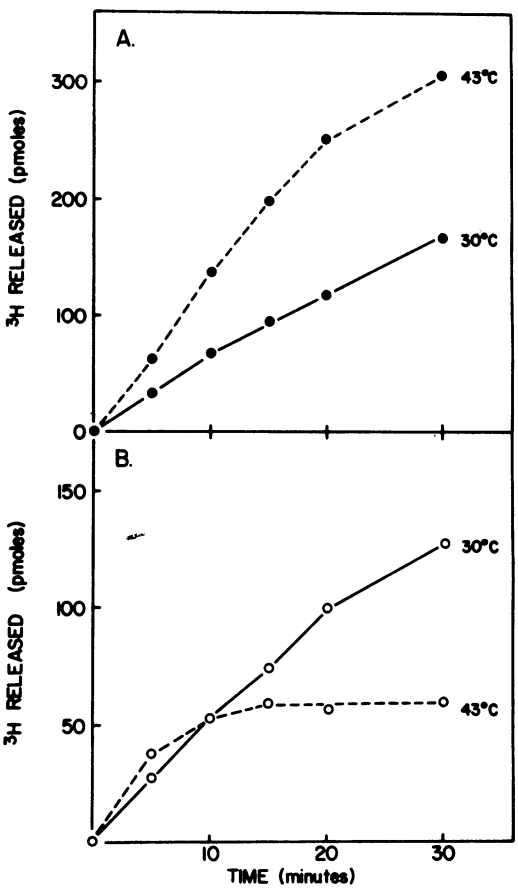

FIG. 2. Time course of exonuclease VII reaction, using exonuclease VII purified from strains PA610 (A) and KLC835 (B). A reaction mixture (1.8 ml) was prepared containing 50 mM potassium phosphate buffer (pH 7.9), 50 mM Tris-hydrochloride buffer (pH 7.9), 8.3 mM EDTA, 10 mM 2-mercaptoethanol, and 12 nmol of single-stranded, sonically irradiated T7 [^{3}H]DNA. The reactions with purified exonuclease VII derived from PA610 and KLC835 (fraction V, Table 4) contained 0.160 and 0.092 U of enzyme, respectively. One unit of exonuclease VII is defined as the amount of enzyme causing the production of 1.0 nmol of acid-soluble ^{3}H in 30 min at 30°C. At the times indicated, 0.15-ml samples were withdrawn, and the trichloroacetic acid-soluble radioactivity was determined.

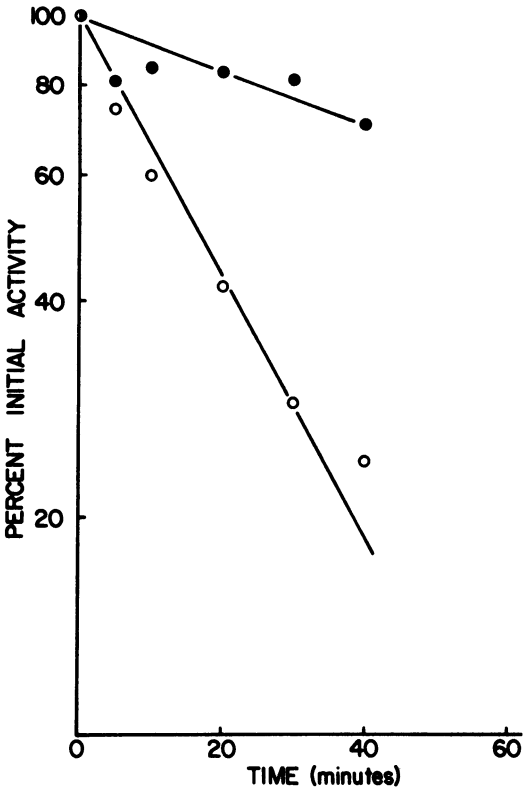

FIG. 3. Heat inactivation of exonuclease VII purified from strains PA610 and KLC835. Exonuclease VII (fraction V, Table 4) purified from strains PA610 (●) and KLC835 (○) was diluted to 7.7 and 8.7 U, respectively, per ml in 50 mM Tris-hydrochloride buffer (pH 7.9)–10 mM 2-mercaptoethanol–0.5 mg of bovine serum albumin per ml and incubated at 43°C. At the times indicated, 0.01-ml samples were assayed in 0.14 ml of the reaction mixture described in the legend to Fig. 2 at 30°C for 30 min. The percentage of initial activity was determined relative to the zero time sample set at 100%.

VII activity upon infection of strain KLC381 Δ(quaB-xseA). Furthermore, lysogenization of strain KLC381 by one of these Δ d*xseA* phage resulted in a strain (KLC428) which produced 80% of the wild-type level of exonuclease VII activity (Table 5). Strains KLC147 (*xseA7*) and KLC488 (*xseA5*) which have no detectable exonuclease VII activity (5) were found to contain intermediate levels of activity relative to the parental strain when lysogenized with λ d*xseA* (Table 5).

DISCUSSION

We previously isolated mutant strains of *E. coli* defective in exonuclease VII activity and showed that these strains are altered at a locus, designated *xseA*, at 53 min on the *E. coli* map (1, 5). Subsequent studies indicated that the enzyme is composed of nonidentical subunits of molecular weights 10,500 and 54,000 and that a locus in addition to *xseA* is required for enzymatic activity. We therefore attempted to isolate additional mutants of *E. coli* defective in exonuclease VII activity. This work has resulted in the identification of a new locus, designated *xseB*, at 10 min on the *E. coli* map.

The strategy used to map *xseB* was based on the analysis of deletion mutants in the region of the chromosome near *xseA* as well as on studies of λ transducing phage carrying genes from the *gua* region. The inability to obtain an overproduction of exonuclease VII activity after infection with a λ transducing phage carrying *guaB* through *hisS* (Fig. 1) suggested that the phage did not carry both structural genes. Seven newly isolated strains of *E. coli* defective in exonuclease VII activity were characterized as either *xseA*$^-$ or *xseA*$^+$. Four of the seven strains were *xseA*$^-$, but mapping studies of the three remaining strains indicated that they possessed mutant alleles of a previously unidentified locus which we have designated *xseB*. We have determined that *xseB* is a structural gene for exonuclease VII, based on studies of a strain containing temperature-sensitive enzyme activity.

The genotype and specific activity of exonuclease VII in the mutant strains isolated suggest that all seven strains are of independent origin. It is interesting to note that none of the nine *xse* strains originally isolated was *xseB*. Since the substrate utilized in the isolation of those Xse$^-$ strains was duplex DNA with 5′ single-stranded termini produced by limited exonuclease III treatment (3), the question arose as to whether this substrate precluded the isolation of *xseB* strains. The substrate utilized in this work was completely single stranded. However, all three of the *xseB* strains isolated were found to be deficient in the hydrolysis of the former sub-

TABLE 5. Exonuclease VII in strains lysogenic for λ d*xseA*

Strain	Relevant genotype	Exonuclease VII sp act[a] (nmol/mg of protein)
KLC101	Wild-type	7.2
KLC433	λ d*xseA*$^+$ lysogen of KLC101	7.7
KLC381	*xseA*	<0.1
KLC428	λ d*xseA*$^+$ lysogen of KLC381	5.6
KLC147	*xseA7*	<0.1
KLC423	λ d*xseA*$^+$ lysogen of KLC147	0.95
KLC488	*xseA5*	<0.1
KLC492	λ d*xseA*$^+$ lysogen of KLC488	2.9

[a] Under the conditions of the assay, specific activities of <0.1 could not be reliably determined. The assay was performed for 30 min at 37°C.

strate (B. A. Rabin and J. W. Chase, unpublished data). We conclude that the lack of *xseB* strains among the *xse* strains initially isolated was not due to the substrate utilized.

We have described the isolation of λ transducing phage carrying $xseA^+$ which restores exonuclease VII activity upon infection or lysogenization of an *xseA* strain. Phage λ d*xseA* lysogens of *xseA7* or *xseA5* mutant strains exhibited levels of exonuclease VII activity intermediate to that of the parental strain. These results suggest that mutant and wild-type subunits produced by the $xseA^-$ and $xseA^+$ genes competed for the subunit product of the *xseB* locus. The isolation of *xseA* on phage λ will make possible the cloning of the gene into a multicopy plasmid vector. In addition, the *xseB* gene is currently being cloned in our laboratory. Exonuclease VII has proven to be useful in analysis of genes whose transcription results in spliced mRNA molecules (2) and for the convenient isolation from plasmid DNA vectors of insert DNAs joined at polydeoxyadenylic acid-polydeoxythymidylic acid splice points (7). The properties of the enzyme have proven to be useful in the structural analyses of DNA molecules, which in turn has recently led to an observation suggesting that the substrate specificity of exonuclease VII may be more complex than was previously thought (8). The cloning of the exonuclease VII structural genes will permit the construction of strains able to produce increased quantities of exonuclease VII. This will facilitate further in vitro studies of the enzyme as well as improve its availability for use as an enzymatic reagent.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grants GM11301 and GM23451 from the National Institute of General Medical Sciences and CA13330 from the National Cancer Institute. L.D.V. and B.A.R. are Predoctoral Fellows of the National Institute of General Medical Sciences, grant GM07491. J.W.C. is an established investigator of the American Heart Association, grant 78-129.

We are grateful to Robert Bender for many helpful discussions.

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