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Cloning of Extracellular DNase and Construction of a DNase-Negative Strain of *Vibrio cholerae*

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The structural gene *xds* for extracellular DNase of *Vibrio cholerae* was cloned and inactivated by insertion of the transposon Tn*5*. The inactivated gene was introduced into the chromosome of *V. cholerae* by recombination to construct an extracellular DNase-negative strain. Tn*5*-mediated transposon-facilitated recombination was used to establish the position of *xds* between the *pro-1* and *ile-201* markers on the genetic map of *V. cholerae*. The extracellular DNase-negative strain described here should be useful for investigating the role of the *xds*-encoded DNase in the physiology of *V. cholerae* and its plasmids as well as for characterizing other DNases in this organism.

Plasmids are a major agent of genetic exchange in many bacterial species. *Vibrio cholerae*, however, is apparently limited in its ability to accept and maintain plasmids from most incompatibility (*inc*) groups (1, 15, 29, 38, 42). A survey evaluating the incidence of naturally occurring plasmids in clinical and environmental isolates of both O1 and non-O1 *V. cholerae* showed that plasmids are rarely found in the O1 serovar. Only 2 of 111 *V. cholerae* O1 strains contained plasmid DNA, whereas 46 of 187 non-O1 strains carried plasmids. Furthermore, 45 of these 46 plasmid-containing non-O1 strains had small (<5 megadalton [MDa]) cryptic plasmids, and only 1, a clinical isolate from a patient treated with antibiotics, carried an R plasmid (34). When compared with other gram-negative enteric bacteria, the incidence of R plasmids in *V. cholerae* is low (24, 25, 35, 37). This may be explained in part by the limited number of plasmid *inc* groups maintained by *V. cholerae*, but the reasons for the discrepancy between plasmid carriage rates in O1 and non-O1 *V. cholerae* strains remain unknown.

There are a number of barriers to the successful transfer of plasmids between bacteria, including that posed by bacterial DNases. The most notable examples are restriction-modification systems by which the DNA of the recipient bacterium avoids degradation because it contains specific chemical modifications, whereas incoming DNA lacking these modifications is degraded. Surface exclusion (2, 5, 32), growth and mating conditions (11), and the inability to make effective contact between donor and recipient bacteria (16, 20) may also limit plasmid transfer. Assuming that transfer of plasmid DNA to the recipient bacteria occurs successfully, there are additional factors affecting plasmid replication, segregation, and copy number which may result in plasmid instability (18, 21, 23, 26, 39).

In the experiments reported here, we constructed an extracellular DNase-negative recombinant strain of *V. cholerae* with the long-term goal of evaluating the effect of this DNase on plasmid biology and its potential competence for transformation or transfection. Extracellular DNase activity was detected by the formation of zones of DNA hydrolysis around bacterial colonies on DNase test agar. The approach used to construct the extracellular DNase-defective recombinant strain of *V. cholerae* was similar in principle to that used by Koomey et al. (28) to construct deletions of the IgA1 gene in *Neisseria gonorrhoeae* and by Kaper et al. (27) to produce deletions of the cholera toxin gene in *V. cholerae*. The DNase gene was cloned, inactivated by insertion of the transposon Tn*5*, and reintroduced into the *V. cholerae* chromosome by recombination. Because *V. cholerae* does not have a well-defined transformation system, it was necessary to mobilize the insertionally inactivated, cloned extracellular DNase gene into *V. cholerae* by conjugation. Transposon Tn*5* in the inactivated DNase gene was used both for mobilizing the cloned gene back into *V. cholerae* by the F′ plasmid F′$_{ts114}$ *lac*$^+$ Tn*1* Tn*5* (33) and for subsequent mapping of the extracellular DNase locus on the *V. cholerae* chromosome by Tn*5*-mediated transposon-facilitated recombination (Tfr) (33). The method described here avoids the need to construct a special conjugative "helper" plasmid (27), provides for the elimination of the mobilizing plasmid by spontaneous loss, and is generally applicable to construction of other specific *V. cholerae* mutant strains in which the corresponding gene loci can be mapped.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. All *V. cholerae* strains used in this study are members of the O1 serovar. *V. cholerae* 26-3, a biotype eltor, serotype Ogawa strain derived from a clinical isolate from the Philippines collected by R. A. Finkelstein in 1961 (40), was used to prepare chromosomal DNA for cloning. *V. cholerae* GN6300, a biotype eltor, serotype Ogawa strain derived from a clinical isolate collected in Bangladesh (33), was used as the host for construction of the extracellular DNase-negative recombinant strain, *V. cholerae* JN1001. *Escherichia coli* HB101 (10) was used as a recipient in transformation (14) and as the host during mutagenesis with the kanamycin resistance transposon Tn*5* (6). *E. coli* C600 (4) was used as a recipient during transformation and conjugation (31) and as the donor for the mobilization of plasmid pJN50. The *E. coli* K-12 minicell-producing strain was DS410 (19). Plasmid vectors pACYC184 (2) and pBR325 (9) were used for molecular cloning (see Fig. 1 and 3). F′$_{ts114}$ *lac*$^+$ Tn*5* Tn*10* was a derivative of F′$_{ts114}$ *lac*$^+$Tn*10* (13) and was constructed in

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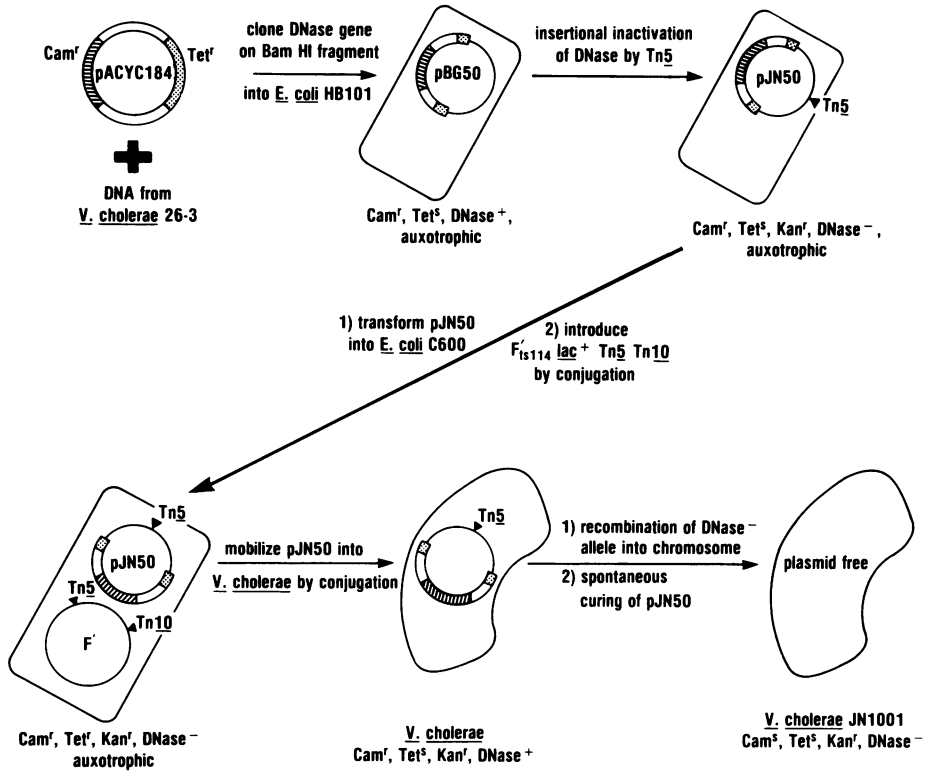

FIG. 1. Schematic diagram of major steps in the construction of the extracellular DNase-negative strain *V. cholerae* JN1001.

our laboratory (33). Meat extract agar adjusted to pH 7.0 (36) and brain heart infusion broth (BHI) (Difco Laboratories, Detroit, Mich.) were used as nutrient media for *V. cholerae*. L agar, L broth, and tryptone broth were used as nutrient media for *E. coli* (31). We used minimal medium V as the solid synthetic medium (33). Antibiotics (Sigma Chemical Co., St. Louis, Mo.) for selection and counterselection were used at the following concentrations: kanamycin (Kan), 25 μg/ml; streptomycin (Str), 75 μg/ml; rifampin (Rif), 30 μg/ml; chloramphenicol (Cam), 25 μg/ml; and tetracycline (Tet), 15 μg/ml. DNase test agar (Difco) was used to screen for the extracellular DNase activity of parental strains and bacteria containing cloned DNA in hybrid plasmids.

Cloning and subcloning. *V. cholerae* 26-3 chromosomal DNA was prepared by phenol extraction and ethanol precipitation of stationary-phase bacteria treated with lysozyme (Sigma), sodium dodecyl sulfate, and proteinase K (Sigma) (7). For primary cloning of the gene for extracellular DNase, 1 μg of *Bam*HI-cleaved *V. cholerae* 26-3 DNA was ligated (T4 ligase; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) to 1 μg of *Bam*HI-cleaved, alkaline phosphatase (Boehringer Mannheim)-treated pACYC184 plasmid DNA (Fig. 1). Endonucleases were purchased from Bethesda Research Laboratories, Gaithersburg, Md., and used as recommended. Sequential digestions with endonucleases requiring different buffers were performed by using the low-salt buffer first. Plasmid DNA was prepared by a mini-lysate procedure scaled down from that of Birnboim and Doly (8). Transformation of *E. coli* strains was done by calcium shock (14).

Tn*5* inactivation of the cloned DNase. *E. coli* HB101 (pBG50) was grown to stationary phase in 2× tryptone broth supplemented with 10 mM $MgSO_4$, 0.2% maltose (wt/vol), and chloramphenicol. A 1:100 dilution of the culture was incubated at 32°C to A_{600} = 0.6, and aliquots were infected with λ*b*221*c*I857Tn*5* (multiplicity of infection, 0.1 to 1.0) (6). After 60 min of incubation at 32°C, the culture was diluted to 1:10 in unsupplemented 1× tryptone broth and incubated for an additional 90 min. Finally, the culture was diluted to 1:10 and grown overnight at 42°C in the presence of kanamycin. Plasmid DNA was isolated from 5 ml of culture, and portions (approximately 100 μg) of DNA were used to transform *E. coli* HB101 with kanamycin resistance as the selection. A Cam^r, Tet^s, Kan^r, DNase$^-$ transformant was isolated, and the insertionally inactivated DNase$^-$ derivative of pBG50 that it contained was designated pJN50 (Fig. 1).

Mobilization of the Tn*5*-inactivated extracellular DNase gene into *V. cholerae* GN6300 and isolation of a DNase-negative recombinant. Plasmid pJN50 was purified from *E. coli* HB101 and introduced into *E. coli* C600 by transformation. Next, F'$_{ts114}$ *lac*$^+$ Tn*5* Tn*10* was introduced into *E. coli* C600(pJN50) by conjugation. The resulting Cam^r, Tet^r, Kan^r, DNase$^-$ *E. coli* C600 was mated with *V. cholerae* GN6300 as follows. Samples (1 ml) of 32°C unshaken, mid-log phase (approximately 2 × 10^8 CFU/ml) *E. coli* and *V. cholerae* cultures were gently mixed and allowed to stand for 30 min at 35°C. The bacteria from the conjugation mixture were collected on a type HA nitrocellulose membrane filter (47-mm diameter; Millipore Corp., Bedford, Mass.). The filter was then placed on meat extract agar prewarmed at 32°C and premoistened by rinsing with BHI followed by incubation for 24 h at 32°C. After incubation, bacteria from the filter were suspended in 5 ml of glucose minimal medium V, and 1-ml samples were plated in glucose minimal medium V soft agar overlays on glucose minimal medium V agar supplemented with chloramphenicol. A Cam^r, Tet^s, Kan^r, DNase$^+$ *V. cholerae*, apparently carrying pJN50 but lacking the F′ plasmid, was isolated. This strain

was grown in BHI (32°C) supplemented with chloramphenicol, diluted 1:100, and grown in BHI (32°C) supplemented with kanamycin, and it was then subcultured at a 1:100 dilution three times overnight (32°C) in BHI without antibiotics. A 10^{-7} dilution of bacteria from the final subculture was plated on meat extract agar and incubated overnight at 42°C. The pACYC184 replicon is not temperature sensitive, but it was spontaneously lost from *V. cholerae*. Growth at 42°C was used empirically during isolation of the DNase-negative strain to spontaneously eliminate pJN50. A petri plate with approximately 600 colonies was replica plated to meat extract agar containing chloramphenicol or kanamycin. None of the colonies was chloramphenicol resistant, but 45 colonies remained kanamycin resistant. Seven kanamycin resistant colonies were tested, and all were negative for the production of extracellular DNase on DNase test agar. One colony was selected and designated *V. cholerae* JN1001.

Mapping of extracellular DNase gene. The DNase-negative recombinant strain JN1001 was converted into a donor strain for Tfr by introducing the hybrid vibrio sex factor P::Tn*1*, Tn*5*. The conditions of the Tfr matings and the characteristics of the recipient strains are reported elsewhere (33). In this Tfr mating system the Tn*5* transposon provides a homologous region between the sex factor and the chromosome of the donor bacterium; during conjugal mating the bacterial chromosome is mobilized in an oriented fraction from the site of the Tn*5* insertion (33).

Analysis of plasmid-encoded proteins. Plasmids containing selected subclones of the original DNase$^+$ 6.6-kilobase (kb) *Bam*HI fragment from pBG50 were transformed into *E. coli* DS410, and minicells were purified from cultures of each strain. Initially, 500-ml Erlenmeyer flasks containing 200 ml of L broth were incubated overnight at 37°C with shaking with 0.1-ml inocula from cells stored in 30% (vol/vol) glycerol. The cultures were rapidly chilled with crushed ice, centrifuged (GSA rotor; 12,000 rpm, 10 min; Ivan Sorvall, Norwalk, Conn.), suspended in 10 ml of BSG (8.5 g of NaCl, 0.3 g of KH_2PO_4, 0.6 g of Na_2HPO_4, and 0.1 g of gelatin per liter of distilled water), recentrifuged (Sorvall SS34 rotor; 12,000 rpm, 10 min), and resuspended in 2 ml of BSG (21). Separation of the minicells was performed by centrifuging the suspended cells on sucrose gradients in Beckman SW27 nitrocellulose tubes (Sorval HB-4 rotor; 5,000 rpm, 20 min) (3). Minicell bands were collected, pooled if necessary, diluted 1:2 in BSG, centrifuged (Sorvall SS34 rotor; 12,000 rpm, 10 min), suspended in 2 ml of BSG, and rebanded in sucrose gradients. After repurification, the minicells were then diluted to 1:2 in MMM (M9 medium, 0.2% glucose, and methionine assay medium; 12 ml/liter; Difco) (21, 31), pelleted and washed two times in MMM, and diluted with MMM to A_{600} = 1.0. The minicells were incubated for 45 min with shaking at 37°C before the addition of [^{35}S]methionine (15 μl/3 ml; specific activity, 1,058 Ci/mM [50 μCi/ml]) obtained from Amersham Corp., Arlington Heights, Ill. They were then incubated for 1 h with shaking at 37°C, collected by centrifugation, suspended in 100 μl of cracking buffer (21) plus 25 of sample buffer (30), and heated at 100°C for 5 min. Aproximately 10^6 cpm was applied to each sample well of a sodium dodecyl sulfate–12% polyacrylamide gel which was then electrophoresed (18 h at 30 V) by the Weintraub modification of the Laemmli gel system (30, 41). After electrophoresis, the gel was dried and examined by fluorography (17).

Quantitative assay of DNase activity. Cell lysates were prepared from 15-ml cultures grown overnight at 37°C in BHI. Cells were harvested by centrifugation (Sorvall SS34 rotor; 8,000 rpm, 10 min), washed once with 25 ml of 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) buffer (pH 7.2)–5 mM 2-mercaptoethanol, suspended in 2 ml of the same wash solution, and lysed by passage through a French pressure cell. The lysate was cleared of cellular debris by centrifugation (Sorvall 50 Ti rotor; 50,000 rpm, 1 h) and frozen. Substrate DNA, polydeoxyadenylate-polydeoxythymidylate (poly[dA-dT]), and [^{3}H]-poly(dA-dT) were purchased from Amersham Corp. (3,835 cpm/μg [0.188 μg/ml]). Reaction mixtures (200 μl) were prepared containing 150 mM HEPES buffer (pH 7.2), 7.5 mM $MgCl_2$, 7.5 mM 2-mercaptoethanol, 5 μg of bovine pancreatic RNase A, 3 μg of poly(dA-dT), and 5 to 25 μl of cell lysate. The mixtures were incubated at 37°C for 30 min; incubation was then terminated by the addition to each sample of 0.5 mg of salmon sperm DNA (Sigma) in a volume of 200 μl, followed by 100 μl of 6% (wt/vol) $HClO_4$. After a 15-min incubation at 0°C the precipitated material was removed by centrifugation (Eppendorf model 5413 microcentrifuge, 15,000 rpm, 3 min; Brinkmann Instruments, Inc., Westbury, N.Y.), 250 μl of each supernatant fraction was mixed with 25 μl of 1 M NH_4OH, and the solubilized ^{3}H was determined in a liquid scintillation counter. The assay was linear with respect to time and the amount of enzyme until approximately 25% of the DNA was degraded to an acid-insoluble form. The concentration of [^{3}H]-poly(dA-dT) used was approximately five times the K_m value of the DNase activity present in the cell lysate.

RESULTS AND DISCUSSION

The procedures used for cloning the extracellular DNase gene of *V. cholerae* and constructing the DNase-negative

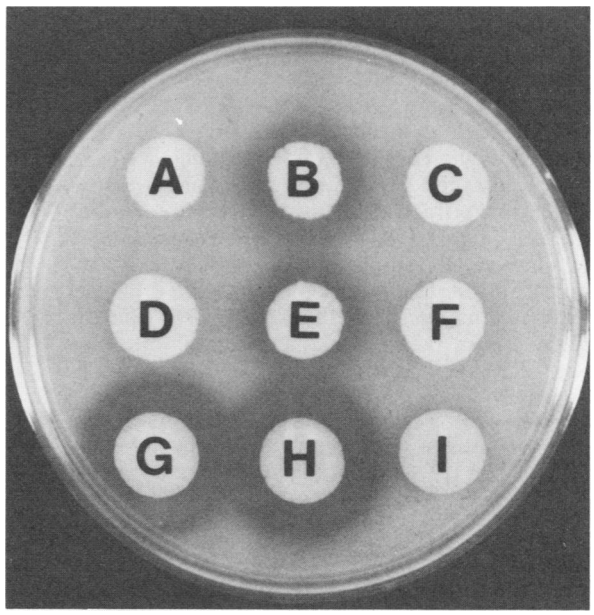

FIG. 2. Results of a qualitative assay of extracellular DNase activity of selected bacterial strains on DNase test agar. The bacterial strains were as follows (wells): A, *E. coli* HB101; B, *E. coli* HB101(pBG50); C, *E. coli* HB101(pJN50); D, *E. coli* C600; E, *E. coli* C600(pBG50); F, *E. coli* C600(pJN50); G, *V. cholerae* GN6300; H, *V. cholerae* GN6300(pJN50); and I, *V. cholerae* JN1001. *E. coli* strains were incubated for 60 h. *V. cholerae* strains were inoculated 24 h later and were incubated for 36 h.

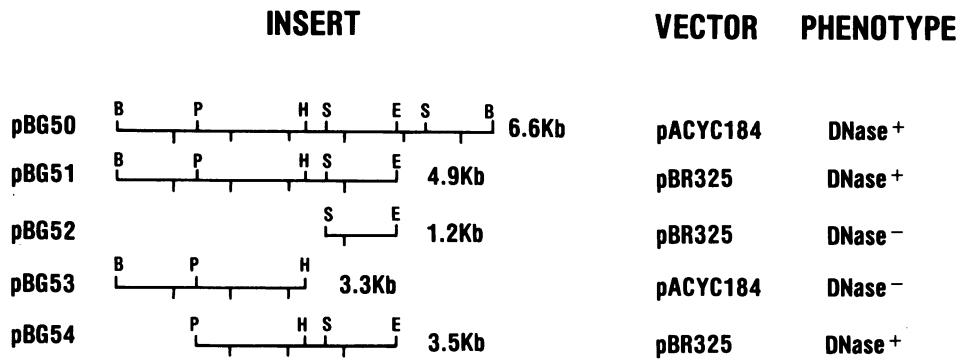

FIG. 3. Physical maps and DNase phenotypes of the *Bam*HI fragment of pBG50, a fragment of chromosomal DNA from *V. cholerae* 26-3 that encodes extracellular DNase, and selected subclones derived from it.

recombinant strain JN1001 are summarized in Fig. 1. We named the structural gene for the extracellular DNase *xds*. The DNase-negative phenotype of *E. coli* HB101 and the DNase-positive phenotype of HB101 with pBG50, the plasmid containing the original clone of *xds*, are shown in Fig. 2A and B. The physical map of the cleavage sites present in the cloned DNA fragment of pBG50 for restriction endonucleases *Bam*HI, *Pst*I, *Hin*dIII, *Sal*I, and *Eco*RI is shown in Fig. 3. The xds^+ allele in pBG50 was inactivated by insertion of the kanamycin transposon Tn*5* to produce plasmid pJN50. The DNase-negative phenotype of *E. coli* HB101(pJN50) is shown in Fig. 2C.

We wanted to use the Tn*5* transposons present in both pJN50 and the F′ plasmid F'_{ts114} lac^+ Tn*5* Tn*10* as sites of homology to promote mobilization of pJN50 by the F′ plasmid during conjugal matings between *E. coli* and *V. cholerae*. Because *E. coli* HB101 is a *recA* mutant, we first purified pJN50 and introduced it by transformation into the $recA^+$ strain *E. coli* C600. The mobilizing F′ plasmid was then introduced into *E. coli* C600(pJN50) by conjugation to construct the donor strain that was subsequently used to introduce pJN50 into *V. cholerae* GN6300 by conjugation (Fig. 1). The DNase phenotypes of *E. coli* strains C600, C600(pBG50), and C600(pJN50) are shown in Fig 2D, E, and F. The F′ plasmid is not stably maintained in *V. cholerae*, and we isolated a transconjugant of *V. cholerae* GN6300 containing only pJN50. Strains GN6300 and GN6300(pJN50) have identical DNase-positive phenotypes (Fig. 2G and H), demonstrating that the chromosomal xds^+ allele of strain GN6300 is dominant over the *xds-201*::Tn*5* allele in pJN50. Replacement of the xds^+ allele on the chromosome by the *xds-201*::Tn*5* allele, through recombination and loss of plasmid pJN50, occurred spontaneously to produce the DNase-negative recombinant strain *V. cholerae* JN1001 (Fig. 1). The phenotype of JN1001 is shown in Fig. 2I.

The presence of transposon Tn*5* in the insertionally inactivated *xds-201* allele of *V. cholerae* JN1001 made it possible to use Tfr to map the *xds* locus. Plasmids pJN2 and pJN8 are derivatives of the vibrio sex factor P. The presence of transposon Tn*1* in each of these plasmids provided a convenient method to select for their presence in strains with chromosomal Tn*5* insertions by resistance to ampicillin. Furthermore, pJN2 and pJN8 contain transposon Tn*5* in opposite orientations. Therefore, they can mobilize the chromosome of JN1001 in a directed manner from the site of the homologous Tn*5* insertion in the *xds* gene, but they do so with opposite orientations. Strains JN1001(pJN2) and JN1001(pJN8) were mated with several different recipient strains of *V. cholerae*, and the progeny were tested to determine which donor markers were inherited at higher frequencies as the result of Tfr (Table 1). The genetic loci are listed across the top of Table 1 in the same order that they occur on the genetic map of *V. cholerae* eltor (33). The results of these experiments demonstrate that the *xds* locus is between *pro-1* and *ile-201* on the genetic map of *V. cholerae*.

To identify more precisely the physical location of the *xds* gene on plasmid pBG50, several additional plasmids were constructed that contained subclones of the original *Bam*HI fragment and their DNase phenotypes were examined (Fig. 3). Plasmids pBG51 and pBG54 had DNase-positive phenotypes, demonstrating that the 3.5-kb *Pst*I-*Eco*RI insert in pBG54 contains the information necessary for functional extracellular DNase activity. Plasmids pBG52 and pBG53 had DNase-negative phenotypes. Therefore, neither the 1.9-kb *Pst*I-*Hin*dIII segment nor the 1.2-kb *Sal*I-*Eco*RI segment of the 3.5-kb *Pst*I-*Eco*RI fragment is sufficient for the synthesis of active DNase. To obtain information about the product of the cloned *xds* gene, we analyzed the synthesis of polypeptides encoded by plasmids pBR325, pBG51, pBG52,

TABLE 1. Mapping of the *xds* locus of *V. cholerae* by Tn*5*-mediated Tfr[a]

Donor strain	Recipient strains and genotypes							
	RJ44 *ilv*	RJ44 *arg*	GN6437 *ile*	RV88 *pro*	RV88 *leu*	GN6437 *ura*	RJ44 *his*	RJ57 *met*
JN1001 *xds-201*::Tn*5*(pJN8)	+	+	+	−	−	−	−	−
JN1001 *xds-201*::Tn*5*(pJN2)	−	−	−	+	+	+	+	+

[a] For each mating, the selected recombinants expressed the Rif^r or Str^r phenotype of the recipient strain and the wild-type donor allele corresponding to the specified auxotrophic marker of the recipient. Symbols: +, the selected donor allele was inherited at an increased frequency characteristic of Tfr; −, the selected donor allele was inherited at a low frequency. Previous studies provide examples of absolute numbers of recombinants observed in Tn*5*-mediated Tfr matings and an analysis of sites of insertion of transposons in Tfr donor strains (22, 33). The site of insertion of transposon Tn*5* in the chromosome of the donor strain JN1001 is the *xds* gene that encodes extracellular DNase. The specific alleles present in the recipient strains were *ilv-5*, *arg-7*, *ile-201*, *pro-1*, *leu-1*, *ura-201*, *his-2*, and *met-4*.

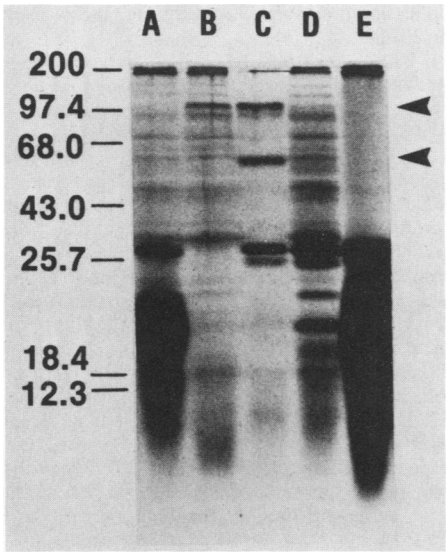

FIG. 4. Autoradiograph of the [^{35}S]methionine-labeled polypeptides synthesized in minicells of *E. coli* DS410 containing the following plasmids (lanes): A, pBR325; B, pBG54; C, pBG51; D, pBG52; and E, pBR328. Numbers on the left indicate the molecular masses (in kilodaltons) of selected polypeptide standards. Arrows point to the plasmid-coded 100- and 60-kDa polypeptides.

and pBG54 in *E. coli* minicells (Fig. 4). The larger DNase-positive clone pBG51 encoded two polypeptides of approximately 100 and 60 kDa that were not encoded by the pBG325 cloning vector (Fig. 4, lanes C and A). The smaller DNase-positive clone pBG54 encoded the 100-kDa polypeptide but not the 60-kDa polypeptide (Fig. 4, lane B), and the DNase-negative clone pBG52 encoded neither of these polypeptides (Fig. 4, lane D). These observations provide strong evidence that the DNase is the 100-kDa polypeptide.

Assays of total DNase activity in cell lysates from *E. coli* and *V. cholerae* strains containing cloned *xds*$^+$ and *xds-201*::Tn*5* alleles are shown in Table 2. Preliminary experiments established that at least 90% of the total DNase activity in cultures of *E. coli* or *V. cholerae* remained cell-associated in the mid-log phase cells used in our experiments. The results with *V. cholerae* strains GN6300 and JN1001 demonstrated that insertional inactivation of the chromosomal *xds* gene eliminated approximately 80% of the DNase activity. The DNase activity in *E. coli* C600 was much lower and was only 7% as great as that in *V. cholerae* GN6300. When plasmid pBG50, containing the *xds*$^+$ allele from *V. cholerae*, was introduced into *E. coli* C600, the DNase activity increased approximately threefold. Therefore, the product of the cloned *xds* gene was the most active DNase in *E. coli* C600(pBG50). The introduction of plasmid pJN50, containing the cloned *xds-201*::Tn*5* allele, into *E. coli* C600 resulted in no increase in DNase activity above the basal level found in *E. coli* C600. Within the quantitative limits of these experiments, it appears that insertion of Tn*5* into the *xds* gene causes the complete inactivation of the DNase that it encodes. The residual DNase activity in *V. cholerae* JN1001, therefore, is most likely due to the presence of additional DNases encoded by genes other than *xds*.

TABLE 2. DNase activity in cell lysates from selected strains of *V. cholerae* and *E. coli*

Strain	*xds* allele	DNase activity (μg/min per mg of protein)
V. cholerae GN6300	*xds*$^+$	30.1
V. cholerae JN1001	*xds-201*::Tn*5*	6.4
E. coli C600(pBG50)	*xds*$^+$	6.6
E. coli C600(pJN50)	*xds-201*::Tn*5*	1.8
E. coli C600	Not present	2.0

Further experiments will be required to define the precise limits of the DNA sequence corresponding to the *xds* gene. The amount of DNA required to code for a 100-kDa polypeptide is approximately 2.5 kb. This represents a large part of the coding capacity of the 3.5-kb *Pst*I-*Eco*RI insert in the smallest DNase-positive clone, pBG54, and exceeds the coding capacity of the 1.9-kb *Pst*I-*Hin*dIII segment of the insert in DNase-negative clone pBG53. The orientation of transcription of the *xds* gene has not yet been defined. Because *V. cholerae* JN1001 is viable and its growth is not obviously different from that of the ancestral strain GN6300, it appears that the DNase encoded by the *xds* gene is not essential. The role(s) of this DNase in the physiology of *V. cholerae* has not yet been established. Our experiments defined the structural gene for the extracellular DNase, identified a single 100-kDa polypeptide associated with this enzyme, and established the position of the *xds* locus on the genetic map of *V. cholerae*. The DNase-negative recombinant strain JN1001 should be a valuable tool to analyze the role of the *xds*-encoded DNase in the physiology of *V. cholerae* and its plasmids and should facilitate future studies of other DNases in this organism.

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