Cloning the gyrA gene of Bacillus subtilis

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ABSTRACT

We have isolated an eight kilobase fragment of Bacillus subtilis DNA by specific integration and excision of a plasmid containing a sequence adjacent to ribosomal operon rrn 0. The genetic locus of the cloned fragment was verified by linkage of the integrated vector to nearby genetic markers using both transduction and transformation. Functional gyrA activity encoded by this fragment complements E. coli gyrA mutants. Recombination between the Bacillus sequences and the E. coli chromosome did not occur. The Bacillus wild type gyrA gene, which confers sensitivity to nalidixic acid, is dominant in E. coli as is the E. coli gene. The cloned DNA precisely defines the physical location of the gyrA mutation on the B. subtilis chromosome. Since an analogous fragment from a nalidixic acid resistant strain has also been isolated, and shown to transform B. subtilis to nalidixic acid resistance, both alleles have been cloned.

INTRODUCTION

Neither the biochemical reactions which take place nor the role that origin sequences play in the initiation of DNA replication or in regulating timing of the cell cycle are adequately understood. At present no gram positive bacterial origin has been isolated or precisely defined. The B. subtilis origin would be of special interest, since it is the gram positive organism about which we have the greatest genetic information. In this species the recD, recF, guaA, gyrA, gyrB, and ts8132 genes are known to lie near the origin (1), but their precise location has been difficult to define. Bacillus origin sequences are thought to be membrane bound and possibly important in regulating initiation of sporulation (2,3), but the DNA responsible for these properties or for autonomous replication has not been functionally identified.

The origin has been located between purA and cysA on the B.
subtilis genetic map using marker frequency analysis from transformation data (4). Subsequent density transfer experiments determined that within this region the ts8132 marker is closest to the origin (5) but the precise location on the physical map is still unknown.

Seiki et al (6) first characterized chromosomal DNA restriction fragments which were preferentially labeled when B. subtilis initiated a synchronous round of replication. They subsequently cloned the BamHI fragment B7 which was believed to become labeled earliest (7). The recombinant plasmid pMS102'B7 contains the Bacillus B7 insert which is a 5.55 kilobase (kb) fragment containing the 5' end of a ribosomal operon (8). We subcloned a non-ribosomal segment from B7 and used the resultant plasmid, pE6', to isolate adjacent DNA fragments. The chromosomal locus of this Bacillus DNA was verified by genetic methods. The newly cloned fragments were tested for the ability to complement chromosomal mutations in the origin region and were found to contain the gyrA gene. A preliminary report of this work was presented at the 83rd Meeting of Am. Soc. for Microbiol.

MATERIALS AND METHODS

Enzymes, chemicals, antibiotics and growth media

Restriction enzymes were obtained from Bethesda Research Laboratories (BRL) and New England Biolabs; T4 DNA ligase was purchased from BRL. All enzymes were used according to the manufacturers instructions. Alpha \(^{32P}\) dCTP was purchased from New England Nuclear Co. Ampicillin, nalidixic acid, novobiocin, and chloramphenicol were purchased from Sigma Biochemical Co. Tryptose Blood Agar Base (for Bacillus) was purchased from Difco Laboratories. L agar (for E. coli) was made according to Miller et al (9). Ampicillin was used at a concentration of 100 \(\mu\)g/ml in solid medium and 30 \(\mu\)g/ml in liquid medium. Chloramphenicol was used at a concentration of 5 \(\mu\)g/ml for initial selection and 20 \(\mu\)g/ml for subsequent testing. Nalidixic acid was present at 50 \(\mu\)g/ml and novobiocin at 2.5 \(\mu\)g/ml.

DNA Isolation and hybridization

Small scale lysates of plasmid-containing E. coli were prepared according to the method of Holmes and Quigley (10);
Table 1: The bacterial strains used in this study.

| Bacterium   | relevant genotype | source         |
|-------------|-------------------|----------------|
| E. coli     |                   |                |
| HB101       | recA              | G. Rapoport    |
| NK5830      | gyrAr             | J. Wang        |
| LL308       | gyrAr             | K. Drlica      |
| DP 50       | gyrAr             | J. Hoch        |
| S90C        | gyrAr             | B. Glickmann   |
| BS24        | gyrAr             |                |
| KNK453      | gyrAts            | K. Drlica      |
| B. subtilis |                   |                |
| 168         | gyrA+ trpC2       | this laboratory|
| Kit 1       | purA cysA         | R. Dedonder    |
| B4          | guaA              | J. Hoch        |
| ts8132      | ts8132            |                |
| 168 N10     | gyrAr             | this laboratory|
| 168 Nov1     | gyrBr             | C. Orrego      |
| BD224       | recE4             | D. Dubnau      |

those from B. subtilis according to the method of Scheer-Abramowitz et al (11). Large scale plasmid preparations were made according to a modified version of the Norgard procedure (12) which used 1% SDS to lyse the cells instead of sodium deoxycholate and Brij-58. Chromosomal DNA was isolated from Bacillus according to Saito et al (13). Electrophoresis in agarose gels was carried out as previously described (14). Restriction fragments were isolated from agarose using DE81 paper according to the method of Dretzen et al (15). DNA was transferred to nitrocellulose following the method of Smith and Sommers (16). The methods for nick translation and hybridization have been described previously (14).

Bacteriological manipulations

Competent E. coli were prepared and transformed according to the method of Dagert and Ehrlich (17). Transformation of competent B. subtilis (18) and B. subtilis protoplasts (19) was as described. Ninety minutes of incubation under non-selective conditions to allow phenotypic expression preceded the selection of transformants for chloramphenicol and 3 hours.
incubation preceded selection for nalidixic acid or novobiocin resistance. PBS1 transduction of \textit{B. subtilis} was carried out according to Hoch et al (20). Lambda phage were prepared using the procedure described in Ferrari et al (21).

**RESULTS**

**Preparation of a plasmid for integrative mapping**

The 5.55 kb \textit{BamH} fragment B7, cloned by Seiki et al (7), contains a portion of a ribosomal operon (8). These sequences could hybridize to and theoretically recombine with any of the 10 ribosomal operons on the \textit{Bacillus} chromosome. In order to obtain a chromosomal fragment which would function both as a unique genetic marker and a hybridization probe that would not react with ribosomal sequences, we subcloned the 2.2 kb \textit{BamH} to \textit{EcoR} fragment, E6', (Fig. 1) from pMS102'B7 which contains the B7 fragment, into the vector pJH101 (22).

**Integrative mapping**

Genetic localization of the E6' fragment on the \textit{Bacillus} chromosome was accomplished using an integrative mapping method of Haldenwang et al (23). We first introduced pE6' into the rec+ \textit{B. subtilis} 168 strain by transformation. As shown in Figure 2, the plasmid integrated into the homologous region in the chromosome. A single fragment from the strain containing the integrated plasmid hybridized with pE6' and this band was larger than in the wild type strain. The new band had a size of 24 kilobase pairs, the predicted length if two copies of pE6' had been integrated in

![Figure 1: Restriction enzyme map of Bacillus origin region.](image)

Distances (in kilobases) between cleavage sites are indicated. Abbreviations: B7, \textit{BamH} fragment cloned by Seiki et al (7); E6, \textit{EcoR} fragment cloned into pnaF and pnaB (see text); E6', \textit{BamH} to \textit{EcoR} fragment subcloned into pE6' and used for mapping and "rolling" (see text); rrnO, ribosomal operon located in origin region (8); R1, \textit{EcoR}; Bam, \textit{BamH}; Sal, SalI.
Figure 2: Hybridization of chromosomal DNA from the wild type and strain with pE6' integrated. DNA samples were electrophoresed thru 0.8% agarose and transferred to nitrocellulose as described in Methods. Plasmid pE6' was labelled with P32 by nick translation for use as probe. Although each of the samples shown was run on the same gel, it was necessary to expose the autorad of individual lanes for different lengths of time to assure clarity of illustration. Lambda/HindIII and lambda/Sall size standards (in kilobases) are indicated at the left. Lanes: 1, 168 chromosomal DNA digested with HindIII; 2, 168 pE6' chromosomal DNA digested with HindIII; 3, undigested 168 pE6' chromosomal DNA; 4, 168 chromosomal DNA digested with EcoRI; 5, plasmid pE6' digested with EcoRI; 6, undigested plasmid pE6'.

this region. Since monomer forms of plasmid molecules are not efficient in stable transformation by competent Bacillus, this doublet may have arisen through a multimer intermediate. If only one copy of pE6' had integrated, a 16 kb Hind III fragment would hybridize with pE6'. No band the size of free plasmid was detected in undigested whole cell DNA hybridized using radioactively labeled pE6' as a probe. PBS1 transducing phage were prepared using as host the strain with the integrated plasmid sequences. Linkage of pE6' to purA and cysA by virtue of the associated chloramphenicol resistance marker from the plasmid verifies that E6' is derived from the origin region of the chromosome (Table 2). This is the first genetic proof that B7, the earliest labeled fragment from which pE6' was derived (and hence the ribosomal operon rrnO), is located in the region where the origin has been placed.
Table 2. Two factor transduction cross for mapping integrated pE6' a

| Donor    | Recipient | Selection (no.) | Recombinant Class | No. |
|----------|-----------|-----------------|-------------------|-----|
| 168 pE6' | Kit 1     | Cm\textsuperscript{r} (100) | 1 1 0 52          |     |
| (111)    | (000)     |                 | 1 0 1 52          |     |
| purA     | (100)     |                 | 1 1 0 59          |     |
| cysA     | (100)     |                 | 1 0 1 43          |     |

a. Possible order: purA pE6' cysA

Since transduction values indicated that pE6' integrated between purA and cysA, we tested its linkage to several other origin-region markers by transformation (Table 3). The tight linkage to the determinant for nalidixic acid resistance

Table 3. Two factor transformation crosses for mapping integrated pE6' a

| Donor    | Recipient | Selection (no.) | Recombinant Class | No. |
|----------|-----------|-----------------|-------------------|-----|
| 168 pE6' | N10       | Cm\textsuperscript{r} (100) | 1 1 61            |     |
| (11)     | (00)      |                 | 1 1 101           |     |
| 168Novr  | (00)      | Cm\textsuperscript{r} (200) | 1 1 87            |     |
| B4       | (00)      | gua\textsuperscript{+} (200) | 1 1 14            |     |
| ts8132   | (00)      | Cm\textsuperscript{r} (200) | 1 1 40            |     |
|          |           | temp\textsuperscript{r} (200) | 1 1 3             |     |

a. Possible order: nov nal pE6' guaA ts8132
Figure 3: Integration of pE6' and excision of pAK1. Only one copy of pE6' is shown integrated for simplicity, although data indicates that two copies exist (see text).

Abbreviations: Cat, chloramphenicol-acetyltransferase from pCl94; ori, plasmid pBR322 replicon; amp, betalactamase gene of pBR322; Sph, SphI; other restriction enzymes as in Fig. 1.

indicates that this gene lies very close to the chromosomal location of the E6' sequence.

Isolation of adjacent chromosomal fragments

The bacterial strain with integrated plasmid pE6' sequences, described above, was used as a source of total chromosomal DNA for subsequent cloning experiments. The DNA was digested to completion with the restriction enzyme SphI, ligated in dilute solution and introduced by transformation into E. coli strain HB101. Recipient E. coli clones resistant to ampicillin were isolated. As shown in Fig 3, the only site for SphI in plasmid pE6', is within the tetracycline gene of pJH101. In theory, all the plasmid molecules larger than pE6' which could be regenerated and recovered using ampicillin selection are those containing the vector linked to Bacillus chromosomal DNA to the next SphI site upstream in the Bacillus DNA. The plasmid pAK1, with an 8 kb insert, was recovered following this strategy (Fig 3). This method, which we have termed "rolling" along the chromosome because it can be used in successive cycles, is an extension of the technique described by Mejean et al (24). The Bacillus insert in the plasmid pAK1 contains appropriate internal
restriction sites predicted from Yoshikawa's early labeling experiments (ref 6). In addition, pAK1 contains the 6.2 kb E6 fragment to which pE6' hybridizes in total Bacillus chromosomal DNA digested with EcoRI (see text and figs 1 and 2). Plasmid pE6' also hybridizes to the E6 fragment in pAK1 (data not shown). Genes in the cloned sequences

Plasmid pAK1 was introduced by transformation into competent B. subtilis recipients containing the guaA1 or ts8132 markers. No transformants were obtained after direct selection for these markers. In addition, when plasmid-containing transformants were selected with chloramphenicol, none were gua+ or temperature resistant indicating that neither of the origin region genes, guaA or ts8132, is present in pAK1.

This plasmid was also tested for the nearby gyrA (nal) marker. In E. coli, nalidixic acid resistance is a recessive mutation (25). Similar behavior, although not well characterized, has been reported in B. subtilis (26). Chloramphenicol resistant transformants of nalidixic acid resistant (nal+) B. subtilis strain N10 were selected following exposure to pAK1 DNA. Individual transformants were then tested for conversion to nal sensitivity. Three percent of the chloramphenicol resistant transformants became nalB suggesting that the gyrA gene may be contained on the 8 kb Bacillus chromosomal fragment in pAK1.

Plasmid pAK1 transformants of nal- B. subtilis strain N10 must result from an integration of the plasmid into the chromosome since the vector cannot replicate in Bacillus. Ferrari et al (22) suggested that a single recombination resulting in insertion of a plasmid into the chromosome occurs at a lower frequency [or is less stable] than a double recombination within the chromosomal fragment found on that plasmid. Chloramphenicol resistant N10 transformants were therefore a selected subpopulation of the total but the only class of transformants for which a selection could be devised. This class necessarily represents progeny with duplications of the DNA represented on the plasmid possibly resulting in the presence of wild type and mutant copies of the gyrA gene. Hence, it is not possible to eliminate complementation of gene activity as an explanation for the altered phenotype.
A further indication that pAKl contains a gene with gyrA activity is provided by introducing the plasmid into *E. coli*. When the cloned *E. coli* nal^S^ gene (in the plasmid pSLS447, kindly provided by J. Wang and S. Swanberg) is established in the competent nal^E^ *E. coli* strains LL308 or NK5830, 100% of the amp^E^ transformants become nal^S^. We introduced pAKl by transformation into these same strains. At least 70% of the amp^E^ isolates were nal^S^. When we examined the size of plasmids in mini-lysates of various transformants by electrophoresis in agarose, 75% of those which remained nal^E^ had visibly detectable smaller plasmids. Plasmids from nal^S^ transformants were the same size as the plasmid originally introduced. The frequency of nal conversion is thus considerably higher if only transformants containing full size plasmids are used in the calculations. The high frequency of deletions suggests that these *Bacillus* sequences may be unstable in *E. coli*. The conversion to nal^S^ shows that pAKl contains genetic information encoding gyrA activity and that this wild type *Bacillus* gene is also dominant in *E. coli*. The few nal^E^ transformants with full size pAKl plasmids may have undergone point mutations, or small insertions or deletions not visible by gel analysis.

A more rigorous test for the presence of the gyrA gene was obtained using a recombinant lambda clone, phage 18, which contains the full length 6.2 kb *EcoR*I E6 fragment (Fig 1). Lambda gt WES lambda B chimeric phage were constructed by H. E. Ruley and C. Moran from a fraction of nal^E^ DNA cleaved with *EcoR*I and size fractionated in a gene machine (27). Six kb *EcoR*I *Bacillus* chromosomal fragments were originally chosen for subcloning into this vector because that fraction possessed transforming activity for nal^E^. Phage 18 was identified by hybridization using the plasmid pE6' as a radioactive probe. The analogous *EcoR*I fragment from phage 18 is contained within the *Bacillus* insert in pAKl. They differ in that pAKl was constructed from the wild type nal^S^ *B. subtilis* strain 168 while phage 18 was made with DNA from the nal^E^ *B. subtilis* strain N10. Using DNA purified from phage 18 for transformation, it was possible to select directly for the nal^E^ phenotype in *Bacillus*. The *Bacillus* sequences in phage 18 transform strain 168 to nal^E^ at a
Figure 4: Hybridization of total DNA from transformants of strain 168 containing pnalF. Electrophoresis was as in Fig. 2. The probe was vector pJH101. Lambda/HindIII size markers (in kb) are indicated at the left. Lanes: 1, plasmid pnalF undigested; 2, BD224 containing pC194; 3, 168 pAK1; 4,9,10,11,12,15, 168 transformants containing pnalF which were nalF; 5,6,7,8,13,14, nal8 transformants of 168 containing pnalF; 16, wild type strain 168.

frequency of 9.4 x 10^3 transformants/ug DNA.

The Bacillus DNA from phage 18 was subcloned into the vector, pMK4 (28), a chimeric plasmid capable of replicating autonomously in both E. coli and Bacillus. The new recombinant plasmid was designated pnalF. The analogous EcoRI restriction fragment containing the sensitive allele from plasmid pAK1 was also inserted into pMK4 and designated pnal8. When DNA from pnalF was introduced into rec+ nal8 Bacillus recipients, 84% of the chloramphenicol resistant transformants became nalF. To investigate the state of pnalF in Bacillus transformants, we isolated total DNA from both nalF and nal8 transformants selected on the basis of chloramphenicol resistance. Chromosomal and plasmid DNA were separated by electrophoresis in agarose, the gel
contents transferred to a nitrocellulose filter and the filter hybridized with $^{32}$P labeled vector pJH101 DNA. Figure 4 shows that pnal$^R$ can exist either integrated into the chromosome or as a free plasmid. There does not appear to be a correlation between nal resistance or sensitivity and the state of the plasmid. The high frequency of conversion to nal$^R$ by pnal$^R$ may be due to its ability to both recombine with the chromosome and replicate autonomously. If the nal$^R$ allele is recessive as in E. coli, the wild type nal$^S$ allele must be lost by being recombined out of the chromosome or by exchange thru gene conversion on an autonomous plasmid.

We further tested pnal$^R$ by introducing this plasmid into a gyrA$^{ts}$ mutant of E. coli (29). Ampicillin resistant transformants of this strain, KNK453, were able to grow at 43 C. The cloned E. coli gene on pSLS447 also permitted growth at the non-permissive temperature while the vector plasmid did not. These results indicate that the Bacillus DNA in pnal$^R$ can also complement a temperature sensitive mutation in the E. coli gyrA gene. Plasmid free derivatives of the temperature resistant transformants were identified after growth in the presence of 100 ug/ml of acridine orange. These organisms were no longer resistant to ampicillin and could not grow at the non-permissive temperature. These results prove that complementation and not marker rescue is occurring in this E. coli mutant.

Other properties of the cloned sequences

Because this DNA lies in the Bacillus chromosomal origin region, we tested it for the ability to replicate autonomously in rec$^-$ recipients. The vector portion of pAK1 (plasmid pJH101) cannot replicate in Bacillus (22). Any free plasmid maintenance would therefore require a functional origin in the B. subtilis sequences. When introduced by protoplast transformation, pAK1 did not replicate in the rec$^-$ B. subtilis BD224.

The origin region of the Bacillus chromosome is also thought to be membrane bound (2). Using the assay of Korn et al (30), we were unable to demonstrate that pAK1 possesses membrane binding properties.
Yoshikawa and his colleagues identified B7 as the first BamH1 restriction fragment that is labeled when 3H-thymidine is added to synchronized cultures (6). The exact chromosomal location of B7 had not previously been verified. We subcloned a portion of B7 and genetically mapped it using transduction and transformation to the origin region of the chromosome. If B7 is the first fragment to be replicated, it may contain the origin itself. However, we were not able to demonstrate that this fragment can replicate autonomously, a property exhibited by the cloned E. coli chromosomal origin (31).

We were successful in isolating sequences adjacent to B7 by using a generally useful procedure of specific plasmid integration followed by excision, which we have designated "rolling". A preliminary experiment determined by hybridization that the plasmid pE6' inserted into its homologous fragment in the chromosome (Fig 2). The size of the hybridizing restriction fragment from the strain with the plasmid integrated is the correct predicted size if tandem copies of pE6' had become inserted. Integration of two copies of a plasmid into the chromosome could result from several mechanisms, and might even be enhanced by the requirement of multimer forms of plasmids to stably transform competent Bacillus cells.

Our attempts to demonstrate that the plasmid pAK1, recovered from the rolling procedure and including approximately eight kb of Bacillus DNA, contains the gyrA gene were complicated by the nature of expression of this gene with the nalidixic acid sensitive phenotype. In E. coli it was determined, using F'-derived merodiploids, that the nalR mutation is recessive to the wild type sensitive allele (25). This same type of merodiploid analysis has not been possible with Bacillus until very recently. The dominant nature of the wild type allele is thought to occur because only one sensitive subunit A of gyrase needs to be present on the chromosome to stop DNA replication in the presence of nalidixic acid.

One would expect that all of the nalidixic acid resistant transformation recipients might become nal8 if an entire wild type gyrA gene is present in pAK1 and if the same dominance
relationship of the gyrA gene occurs as in *E. coli*. However, only 3% of nal^R* B. subtilis* strain N10 transformants become nal^S*. This lower-than-expected frequency may result from a different dominance-recessive relationship of gyrA in *Bacillus* than *E. coli*, an incomplete gyrA gene on pAK1, instability of the cloned sequences, or as a result of the specific integration event. We may also be observing a high frequency of gene conversion in favor of the resident nal^R* allele.

Since the recessive nature of the nal^R* mutation was established for the *E. coli* gene, we tested for the presence of gyrA on pAK1 in *E. coli*. In contrast to the situation in *Bacillus*, pAK1 can replicate in *E. coli* and will not integrate into the chromosome. It can be maintained with ampicillin or chloramphenicol selection, but not with nalidixic acid. Would the *Bacillus* gene complement an *E. coli* mutation? Such intergeneric complementation has previously been demonstrated for other *Bacillus* genes (32). Sugino and Bott (26) showed that enzyme activity of the *E. coli* and *Bacillus* gyrase protein in vitro was similar. However, they were not able to demonstrate in vitro complementation of purified *E. coli* subunits with partially dissociated *Bacillus* enzyme.

Not all *E. coli* strains can be used to demonstrate the recessive nature of the nal^R* allele. We used five gyrA mutants and could show conversion of nalidixic acid resistance to sensitivity with only two, mutants LL308 and NK5830. The cloned wild type gyrA allele from *E. coli* (pSLS447) was also positive in only these two strains. It is clear that a specific gyrA allele is important in demonstrating this phenotype.

The *Bacillus* sequences in pAK1 convert a high percentage of transformants of these nal^R* E. coli* strains to nalidixic acid sensitivity. These results demonstrate that this *Bacillus* fragment has the ability to complement gyrA activity in *E. coli*. The wild type *Bacillus* gene exhibits the same dominant nature as the *E. coli* gyrA gene. Approximately 25% of the transformants remained nal^R* but, when plasmids were examined from these isolates, most had undergone deletions large enough to be easily visible indicating experimentally that these *Bacillus* sequences are unstable in *E. coli*. 

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Since phage 18 was constructed with chromosomal DNA from a nalidixic acid resistant *Bacillus* strain, that DNA permitted the direct selection for nalrif. Phage lambda cannot replicate or integrate in *Bacillus* but the *Bacillus* sequences can recombine with the chromosome, converting transformants to nalidixic acid resistance. This result was obtained with phage 18 DNA.

The plasmid pnalrif, constructed from the *Bacillus* sequences in phage 18 inserted into the bifunctional vector pMK4 (28), can also be selected for directly with nalidixic acid. Plasmid pnalrif however, can replicate in *Bacillus*, and can therefore exist either autonomously or integrated in transformants, resulting in a partial diploid for gyrA. Since pnalrif contains a nalrif allele, it should be recessive to the wild type allele in the chromosome if genetic studies with *E. coli* provide an accurate analogy. Why then are 16% of the *Bacillus* transformants nalrif? These results could be explained if only a portion of a gyrA gene is present on pnalrif. The plasmid may also undergo deletions because of instability. A more likely event, though, is that gene conversion has taken place and the sensitive allele is no longer present. Alternatively, the dominance of the sensitive allele of gyrA may not be the same in *Bacillus* as it is in *E. coli*. These testable alternatives are presently under investigation.

We tested pnalrif and pnalrif in an *E. coli* mutant which makes a temperature sensitive form of the gyrA gene product. Our results showed that the strain can grow at the non-permissive temperature when containing either the *E. coli* wild type gene on a plasmid, pnalrif, or pnalrif; again indicating that gyrA activity is encoded by these *Bacillus* sequences. We believe that we have observed complementation rather than marker rescue since the temperature sensitive strain can no longer grow at the non-permissive temperature when cured of the plasmid pnalrif. However, none of the transformants containing pnalrif were resistant to nalidixic acid at the non-permissive temperature. Since this *E. coli* mutation is "leaky", residual expression or activity of the *E. coli* gyrA gene product is probably occurring to make the cells antibiotic sensitive. Although seemingly contradictory with the recent findings of Lother et al (33) that *B. subtilis* gyrA and *E. coli* gyrB subunits do not complement in vitro, these results are
not necessarily inconsistent since the two experimental systems differ.

In constructing pnal\textsuperscript{R} and pnal\textsuperscript{S}, we observed that these plasmids are sometimes unstable in \textit{E. coli}. Soon after the initial transformation, isolates were obtained that had undergone extensive deletions and in the case of pnal\textsuperscript{R}, no longer demonstrated nal transforming activity. This observation again supports the idea that these \textit{Bacillus} sequences are unstable in \textit{E. coli}.

In conclusion, we have shown by genetic mapping procedures that the fragment B7 resides in the origin region of the \textit{Bacillus} chromosome. Plasmid pE6\textsuperscript{R} constructed from a subfragment of B7, was used to isolate additional sequences from the origin region. At least part of these cloned fragments appear to be unstable in \textit{E. coli}. Plasmid pAK1, containing a total of 8 kb of cloned \textit{Bacillus} DNA, complements \textit{E. coli} gyrA mutations. The wild type \textit{Bacillus} sequences are dominant in \textit{E. coli} but this relationship may not be the same in \textit{Bacillus}.

It is possible that a full length gyrA gene may not be present in pAK1, even though this DNA is large enough to encode a functional gyrA protein in both \textit{Bacillus} and \textit{E. coli}. The exact location of this gene on the cloned sequences, as well as the size of any protein(s) being encoded by this DNA are presently being determined.

We expect that a thorough characterization of these genes and their products will permit a better understanding of the recessive nature of the nal\textsuperscript{R} mutation in \textit{Bacillus}, and the effect of DNA gyrase on gene regulation in this organism. The exact nature of the mutation conferring nalidixic acid resistance and a precise identification of the binding site for this antibiotic to the gyrA gene product can be determined since both the sensitive and resistant alleles have been cloned. We can also envision several benefits to recombinant DNA technology which could be derived from the positive selection value for recombinants using cloning vectors containing a functional \textit{Bacillus} gyrA gene (eg, pnal\textsuperscript{S}) if coupled with the appropriate nalidixic acid resistant recipient.
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