Chromosomal Insertions of Tn917 in Bacillus subtilis

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Received 20 March 1986/Accepted 9 May 1986

We describe 46 insertions of the Streptococcus faecalis transposon Tn917 into the chromosome of Bacillus subtilis. These insertion mutations were mapped genetically. Some caused auxotrophic requirements, and others were cryptic. These insertions were scattered around the B. subtilis chromosome. The mutant strains were useful in several ways for mapping and cloning B. subtilis genes and were added to the Bacillus Genetic Stock Center collection. Among the auxotrophic markers were a new serine auxotrophy and deletion-insertions that caused auxotrophy in one case for homoserine and threonine, in another case for uracil and either cysteine or methionine, and in a third case for leucine, isoleucine, and valine.

Youngman et al. (13) have described methods for "shot-gunning" the Streptococcus faecalis transposon Tn917 into the Bacillus subtilis chromosome. We used these methods, with some variations, to isolate a group of insertion mutations of various sorts. Some were cryptic; others caused auxotrophic requirements. As a group, these insertion mutations covered most of the B. subtilis chromosome and could be used in several ways for studying the genome of B. subtilis. For example, any insertion mutation can be exchanged for an altered transposon that carries the Escherichia coli plasmid pBR322 within it, and nearby genes can be cloned from such an insertion (10, 11). Alternatively, any Tn917 insertion can be replaced by different altered transposon (12) that contains a promoterless E. coli lacZ gene preceded by a good B. subtilis ribosome binding site; if a B. subtilis chromosomal promoter causes transcription into the transposon in the proper orientation, ß-galactosidase will be expressed. Control of the B. subtilis promoter can then be studied by monitoring ß-galactosidase concentrations.

We collected a number of such Tn917 insertions and (with the help of the Cornell University Biological Sciences 487 classes of 1982, 1983, 1984, and 1985) located them on the chromosome map of B. subtilis; in a few cases we characterized them further. Because we think that these insertion mutations will be useful to people researching the B. subtilis genome, we submitted strains carrying the mutations to the Bacillus Genetic Stock Center (Ohio State University, Columbus), which will supply the strains to persons requesting them. We include here a brief description of the strains.

MATERIALS AND METHODS

Bacterial strains and transposon vector. All bacteria except strain W23 were derivatives of B. subtilis 168. Strain W23 was isolated independently (8). Most of the Tn917 insertions were originally isolated in strain CU3601. This strain was constructed as follows. Strain CU1064 metB5 attSP0 (15) was transduced via phage PBS1 to become a carrier of pTV1, the transposon vector described by Youngman et al. (13). The resulting strain, CU3270, was subjected to the transposition regimen described below, and a glutamate auxotroph carrying gltAB::Tn917 was isolated. This strain, CU3278, had lost pTV1; its insertion was the source of the transposon in strain CU4132. CU3278 was transduced to Met* by a PBS1 phage grown in CU1065 trpC2 attSP9 (15); a transductant that had become Met* and Trp* was chosen and labeled CU3599. Strain CU3599 was transformed to Glt* with DNA extracted from B. subtilis W23. One transformant was labeled CU3600. Its genotype is trpC2 attSP0 gltAB+W23. Strain CU3600 was transduced by the PBS1 phage to become a carrier of pTV1; a transductant was labeled CU3601 trpC2 attSP9 gltAB’T (pTV1).

All of the Tn917 insertions isolated in CU3601 were eventually transferred by transformation or by PBS1 transduction into strain CU1147 trpC2 (SPB c2) (7). The selection was for resistance to the macrolides-lincosamides-streptogramin B (MLS) antibiotics, a trait of the transposon. CU1147 is lysogenic for the heat-inducible mutant of phage SPB. As far as we know, the strains are all isogenic with CU1147 except for the transposon insertion that each carries.

The transposon vector pTV1 (13) is a plasmid that carries the 5.3-kilobase transposon Tn917, a chloramphenicol resistance marker, and a heat-sensitive replication system; pTV1 can replicate well in B. subtilis at 30 or 37°C, but cannot replicate at 50°C. The transposon encodes resistance to the MLS antibiotics. We used 1 μg of erythromycin ml⁻¹ and 25 μg of lincomycin ml⁻¹ as selective conditions for MLS resistance (MLS'). A subinhibitory level of erythromycin (20 ng ml⁻¹) induces transposition (13). Because we found that low concentrations of mitomycin C also induce transposition, we sometimes also added 10 ng of mitomycin C ml⁻¹.

Selection of transposon insertions. Most of the transposon insertions described below were isolated by the protocol presented here. We grew CU3601 in antibiotic medium no. 3 (Difco Laboratories) supplemented with chloramphenicol (10 μg ml⁻¹) and erythromycin (20 ng ml⁻¹) at 37°C for 18 h with aeration. Transpositions of Tn917 from pTV1 to the bacterial chromosome were selected by plating samples of these cultures on tryptose-blood agar plates (Difco) containing selective concentrations of erythromycin and lincomycin and incubating the samples at 50°C, a temperature that is restrictive for pTV1 replication. The resulting colonies arose from cells in which Tn917 had been transposed from the plasmid to the chromosome. We confirmed this by scoring for the loss of the plasmid marker chloramphenicol resistance. The MLS' clones were then tested for their ability to grow on minimal medium containing tryptophan and (because we were not interested in insertions into the gltA and gltB genes) glutamate. The requirements of the auxotrophic mutations were determined with a grid analogous to that described by Davis et al. (1). The insertions were located on
the chromosome by using phage PBS1 transduction (9), DNA transformation (9), and derivatives of the B. subtilis map kit strains (2).

We were able by a second method to isolate Tn917 insertions linked to a known auxotrophic marker. We produced lysates of the generalized transducing phage PBS1 by growing the phage in mixed cultures of bacteria carrying random Tn917 insertions on their chromosomes. The lysates were prepared as follows. Several cultures of strain CU3601 were grown for 18 h in antibiotic medium no. 3 containing 20 ng of erythromycin ml⁻¹ and 10 ng of mitomycin C ml⁻¹. Each culture was then enriched for transposition events by diluting 1:100 with antibiotic medium no. 3 containing 1 μg of erythromycin ml⁻¹ and 25 μg of lincomycin ml⁻¹ and by incubating the cultures at 50°C overnight with aeration. These cultures were then diluted appropriately with fresh antibiotic medium no. 3, and PBS1 lysates were prepared from them (9). For example, to place transposition insertions near the purA gene of B. subtilis, we used these lysates to transduce a purA16 auxotroph simultaneously to prototrophy and to MLS¹. Essentially all of the transductants then carried cryptic insertions of Tn917 linked to purA.

RESULTS

Observations on transposition events. We produced a strain of B. subtilis, CU3601 rpeC2 attSP⁴, carrying the transposition vector pTV1. When we used 10 mg of erythromycin ml⁻¹ to induce transposition as described above, we found that about 1 cell in 10⁶ could form MLS¹ colonies at 50°C. The frequency of auxotrophs among these colonies was 0.5 to 3% in different experiments. More than 90% of the auxotrophs were Glt⁻; that is, their requirements could be satisfied by either glutamate or aspartate, and the transposon was located in the gltAB region, very close to the B. subtilis terminus of replication. We replaced the gltAB region (which had originated in B. subtilis 168) with the homologous region from strain W23, as described in Materials and Methods. When the newly constructed strain CU3601 was similarly tested, the frequency of Glt⁻ mutants among the auxotrophs was only 25 to 30%. We therefore used CU3601 for the isolation of the insertion mutants, as described here. We found that a large fraction (30 to 50%) of the cryptic insertions were linked (sometimes very closely) to gltAB in strain CU3601.

Several of the insertions of Tn917 were accompanied by deletions of nearby—presumably adjacent—DNA. Two independently isolated insertions into the ilvBC-leuA region (liv-I-1: Tn917 and liv-I-2: Tn917) resulted in apparently identical deletions of 2.7 kilobases of DNA, including the right end of ilvB and most or all of ilvC, as determined both by our failure to recover transformation recombinants with point mutations in the region and by Southern analysis (manuscript in preparation). The urc-83:: Tn917 insertion, which caused auxotrophy for uracil and for either cysteine or methionine, was also accompanied by a deletion. DNA extracted from this strain could not repair point mutations in cysC (with a requirement for cysteine or methionine) or in various pyr genes (with requirements for uracil), all of which were clustered on the B. subtilis chromosome. Similarly, the mth-84:: Tn917 insertion required threonine and either methionine or homoserine. DNA extracted from a strain carrying this marker could not correct a number of point mutations in the thrA gene, nor could it correct a hom mutation which itself was possibly a deletion.

We did not detect a precise excision of Tn917 from a chromosomal location. We plated more than 10⁶ cells of each of more than 40 auxotrophic insertion strains to search for revertants, but we found none that had lost the transposon. Most of these auxotrophs did not have detectable deletions.

On the other hand, a few Tn917-generated auxotrophic insertions could revert to prototrophy. The mth-82:: Tn917 insertion caused uracil auxotrophy, but prototrophic revertants were recovered at a frequency of 10⁻⁵ to 10⁻⁶. The revertants retained Tn917 or at least its MLS¹ marker. We suspect that the transposon lies in a regulatory region of the pyr operon and that mutations or small deletions in the region can suppress the Pyr⁻ phenotype. Similarly, the mth-83:: Tn917 insertion resulted in auxotrophy for threonine and either homoserine or methionine. The insertion was very closely linked to thrA and hom mutations. Mutations could be isolated from strains carrying this insertion that were either Met⁺ or Thr⁺ or else Met⁻ and Thr⁻; all retained the transposon. Again, we suspect that the insertion lies in a regulatory region, possibly between genes encoding homoserine synthetase and homoserine dehydrogenase.

Auxotrophic insertion mutations. Most of the auxotrophic insertion mutations (Table 1) corresponded to the phenotypes described in the most recent edition of the B. subtilis chromosome map (6). A few additional insertion mutations are described below, proceeding clockwise around the chromosome.

| Mutation designation | Map position | Strain designation | Cornell | BSGC² |
|-----------------------|--------------|-------------------|--------|-------|
| cym-84:: Tn917        | 11⁷          | CU4120             | 1A600  |
| purB83:: Tn917        | 54⁷          | CU4121             | 1A601  |
| ath-33:: Tn917        | 55⁷          | CU4122             | 1A602  |
| thiA84:: Tn917        | 70⁷          | CU4123             | 1A603  |
| metD83:: Tn917        | 96⁷          | CU4124             | 1A604  |
| argG85:: Tn917        | 98⁷          | CU4125             | 1A605  |
| argC82:: Tn917        | 100⁷         | CU4126             | 1A606  |
| metC85:: Tn917        | 115⁷         | CU4127             | 1A607  |
| arg-342-82:: Tn917    | 117⁷         | CU4128             | 1A608  |
| pyr-82:: Tn917        | 137²         | CU4129             | 1A609  |
| pyr-83:: Tn917        | 137²         | CU4130             | 1A610  |
| urc-83:: Tn917⁸       | 137²         | CU4131             | 1A611  |
| gltAB81:: Tn917       | 180⁷         | CU4132             | 1A612  |
| arOBC84:: Tn917       | 206³         | CU4133             | 1A613  |
| serA84:: Tn917        | 208³         | CU4134             | 1A614  |
| lysA82:: Tn917        | 210³         | CU4135             | 1A615  |
| nic-82:: Tn917        | 245³         | CU4136             | 1A616  |
| pheA82:: Tn917        | 246³         | CU4137             | 1A617  |
| leuB84:: Tn917        | 250³         | CU4138             | 1A618  |
| liv-I-82:: Tn917³      | 251³         | CU4139             | 1A619  |
| liv-3-83:: Tn917³      | 251³         | CU4140             | 1A620  |
| serC82:: Tn917        | 257³         | CU4141             | 1A621  |
| argA85:: Tn917        | 260³         | CU4142             | 1A622  |
| alaA82:: Tn917        | 281³         | CU4143             | 1A623  |
| mth-83:: Tn917⁴       | 290³         | CU4144             | 1A624  |
| mth-84:: Tn917⁴       | 290³         | CU4145             | 1A625  |
| hisA82:: Tn917        | 305³         | CU4146             | 1A626  |

¹ Auxotrophic insertion mutation designations and map positions are as described by Piggot and Hoch (6). All strains carry the trpC2 marker and are lysogenic for SPB c2.

² BGSC, Bacillus Genetic Stock Center.

³ Requires uracil and either cysteine or methionine (see the text).

⁴ Requires leucine, isoleucine, and valine (see the text).

⁵ Requires threonine and either homoserine or methionine (see the text).
TABLE 2. Cryptic insertion mutations

| Mutation designationa | Map positionb | % Linkagec | Strain designation  |
|-----------------------|--------------|------------|---------------------|
| zfa-84::Tn917         | 0°           | 96% to gnuA 75% to purA | CU4147 1A627 |
| zbi-82::Tn917         | 65°          | 31% to thiA* 38% to tre* | CU4148 1A628 |
| zca-82::Tn917         | 74°          | 54% to gfbK' 62% to gfbB' | CU4149 1A629 |
| zce-83::Tn917         | 87°          | 85% to gnb 73% to metD | CU4150 1A630 |
| zdd-85::Tn917         | 121°         | 62% to metC 12% to pyr | CU4151 1A631 |
| zde-85::Tn917         | 126°         | 36% to metC 34% to pyr | CU4152 1A632 |
| zdi-82::Tn917         | 140°         | 97% to cysC | CU4153 1A633 |
| zel-82::Tn917         | 177°         | 7% to citK 40% to gbiB | CU4154 1A634 |
| zet-82::Tn917         | 179°         | 98% to gbiB 38% to citK | CU4155 1A635 |
| zfi-85::Tn917         | 183°         | 34% to gbiB 80% to citK | CU4156 1A636 |
| zff-82::Tn917         | 200°         | 95% to ilvA 47% to hisH | CU4157 1A637 |
| zfg-83::Tn917         | 216°         | 34% to aroD 75% to lys | CU4158 1A638 |
| zgi-83::Tn917         | 245°         | 74% to leuB 82% to pheA | CU4159 1A639 |
| zhb-83::Tn917         | 257°         | 56% to sdh 61% to argA | CU4160 1A640 |
| zhc-85::Tn917         | 260°         | 93% to argA  27% to aroG' | CU4161 1A641 |
| zhi-83::Tn917         | 285°         | 85% to ald 79% to thrA | CU4162 1A642 |
| zib-82::Tn917         | 294°         | 18% to hisA 83% to cysB | CU4163 1A643 |
| zil-83::Tn917         | 317°         | 29% to hisA 58% to cysA | CU4164 1A644 |
| zif-85::Tn917         | 342°         | 20% to sacA 64% to purA | CU4165 1A645 |
| (zfd-81::Tn917)       | 192°         | In SPB 2 c2 proophase | CU2111 1A646 |

a Cryptic insertion mutation designations are explained in the text. All strains carry the trpC2 marker. All strains except CU2111 are lysogenic for SPB c2.

b Map positions are estimated from Piggot and Hoch (6).

c Cotransduction by PBS1 phage except where noted. Selection was for MLS'.

d BGSC, Bacillus Genetic Stock Center.

e Cotransformation with DNA. Selection was for MLS'.

insertion mutations that we isolated) caused auxotrophy for serine or glycine, although growth was improved if both were added. Serine was toxic for *B. subtilis* at concentrations above about 4 μg ml⁻¹ because the cells starved for threonine (unpublished observations). Therefore, we fed such cells glycine (200 μg ml⁻¹), serine (40 μg ml⁻¹), and threonine (40 μg ml⁻¹). We are not certain that this class of insertion mutations differs from the class labeled glyA on the *B. subtilis* chromosome map.

The *liv-3:*Tn917 insertion mutation lies between the *ilvB* and the *ilvC* regions, as determined by transformation mapping. Strains carrying *liv-3:*Tn917 were prototrophic at 30 and 37°C but were Liv⁻ at 50°C (manuscript in preparation).

The *serC* insertion mutation caused auxotrophy for serine, which we used at 4 μg ml⁻¹. The *serC* gene is located clockwise to the *sdh* genes and was 98% cotransduced with the latter. Better growth was achieved with 40 μg each of serine and threonine ml⁻¹.

**Cryptic insertions.** Nineteen strains carrying cryptic insertions of Tn917 in the *B. subtilis* chromosome are listed in Table 2. Their genetic map positions were indicated by the method of Hong and Ames (5) as amended by Davis et al. (1). The insertion is designated by three letters, the first of which is z. The second and third letters indicate the approximate map position of an insertion in one-tenth and one-hundredth segments, respectively, of the total genetic map, starting at the origin of replication (which equals 0 or 100) and proceeding clockwise. The second letter corresponds to the one-hundredth map segments, which are lettered consecutively in a clockwise fashion; i.e., the region from position 0 to position 100 equals a, the region from position 10 to position 20 equals b, and so on. The third letter corresponds to the one-hundredth map segments within any one-tenth segment. Thus, a map position just clockwise of the origin of replication is designated zaa; a position just counterclockwise of the origin of replication is designated zbj. The map positions were determined by PBS1 mapping and, in a few cases, by transformation with nearby markers. The equivalent position on the Piggot-Hoch 360° map (6) is also given.

None of the cryptic insertions isolated in the two experiments carried Tn917 in SPB because strain CU3601, in which these mutations were originally isolated, lacks the SPB proophase. We therefore included strain CU2111, received from P. J. Youngman (his strain PY37), which is lysogenic for a derivative of SPB c2 into which Tn917 has been inserted (Table 2). When CU2111 is induced by heat shock or by mitomycin C, it releases the SPB c2 phage carrying Tn917; the phage particles are capable of forming plaques and of lysogenizing sensitive or lysogenic *B. subtilis* cells, which they convert to MLS' (14). The transposon is located within the *psrI* N fragment of SPB c2, a fragment that lies near the counterclockwise end of the prophage near map position 192° on the *B. subtilis* genetic map. The transposon also lies in the *SacI* J fragment and in the *Salt* B fragment (3; P. S. Fink, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1982).

Figure 1 shows the 46 Tn917 chromosomal insertions and gives the chromosome locations of genes to which the cryptic insertions were linked.

**DISCUSSION**

The 47 strains included in this study carry Tn917 insertions in most regions of the *B. subtilis* chromosome. Two major gaps remain. One lies between 11 and 54° on the 360° Piggot-Hoch map (6); the other lies between 140 and 177° of the same map. Each of these gaps corresponds to between 10
and 12% of the *B. subtilis* map. We did not search specifically for Tn917 insertions in these regions of the chromosome by the PBS1 linkage method. We have no reason to think that such a search would fail, although there may be clusters of essential genes that would make the search difficult. The region between 182 and 193° is covered by a transposon inserted into the SPβ prophage.

The strains are useful for cloning nearby genes and for studying the regulation of genes carrying the transposon. For these reasons we deposited the strains in the *Bacillus* Genetic Stock Center, which will supply cultures upon request.

Some of the strains carry transposons that have adjacent deletions associated with them. These include strains CU4131, CU4139, and CU4145. Other strains may have undetected deletions associated with them. We never detected a precise excision of Tn917 which would allow us to return an auxotrophic insertion to the prototrophic state.

We have evidence that Tn917 is polar in one orientation (manuscript in preparation), the orientation in which chromosomal transcription enters the right end of the transposon (12). In the other orientation, readthrough transcription can occur at low temperatures (30 to 37°C), but a rather weak transcription terminator prevents most readthrough transcription at 50°C. The phenotype of CU4140 (liv-3::Tn917), which is prototrophic at 37°C (although its growth is stimulated by branched-chain amino acids) and Liv^- at 50°C (requiring leucine, isoleucine, and valine), is an example of the evidence leading to this conclusion.

Some of the auxotropic strains included in this study gave rise to prototrophic mutations that retained the transposon in its original insertion site. We did not examine these strains at length, but they may be useful in investigations of the pathways involved. They include strain CU4129 (pyr-82::Tn917) and strain CU4144 (mth-83::Tn917). Because strain CU3601 carries DNA from *B. subtilis* W23 in its gltAB region, three of the strains in this collection (CU4154, CU4155, and CU4156) may contain DNA of W23 origin.

The Mth (requiring threonine and either homoserine or methionine) and Urc (requiring uracil and either cysteine or methionine) phenotypes of *B. subtilis* described here have not been reported before. The serC mutation is unique. In our experience, spontaneous Liv mutations do not occur in *B. subtilis*, and the ath (adenine and thiamine), cym (cysteine or methionine, located near cysA), and ala (L-alanine) auxotrophic mutations are quite rare among mutations induced either by N-methyl-N'-nitro-N-nitrosoguanidine or by 6-chloro-9-[3-(2-chlorethyl)-amino]propyl]-2-methoxyacridine (ICR-191).

ACKNOWLEDGMENTS

We are grateful to Philip J. Youngman for his generous donations of plasmids (including pTV1) and bacterial strains (including

FIG. 1. Chromosome map of *B. subtilis* insertions of Tn917. Insertions are indicated outside the circle. This map is patterned after the genetic linkage map of Piggott and Hoch (6).
CU211 [PY37]) and for his advice. We thank the 1982, 1983, 1984, and 1985 classes of Biological Sciences 487, Cornell University, Ithaca, N.Y., for carrying out preliminary mapping of many of the insertions described here. We also thank Janice Odebralski and Kevin Mangan for help in the original isolation of the insertion mutants.

This research was supported in part by Public Health Service grant GM33152 from the National Institute of General Medical Sciences.

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