Transposons are mobile genetic elements that can move from one site to another in the genome with the aid of a recombinase called a transposase. They have been used as tools for genetic analysis in many biological systems, and engineered transposons have been developed that incorporate a variety of useful features. Numerous transposon delivery systems have been developed for Escherichia coli and other gram-negative bacteria, and several are commercially available. However, in many cases these incorporate selectable markers that are not conducive to their use in gram-positive bacteria.

Transposons have played a major role in genetic analysis in Bacillus subtilis, which serves as a model gram-positive bacterium. The first to be developed was Tn917, a member of the Tn3 family of replicative insertion elements. Tn917 was adapted for in vivo transposition in this organism. Tn917, a transposon isolated from Bacillus subtilis, was shown to be fully functional in B. subtilis (50). Despite widespread use, Tn917 has significant shortcomings. For example, Tn917 has a preference for certain genes (40, 44, 49, 52), an observation likely explained by its preferential insertion near the terminus region in several low-G+C gram-positive bacteria (18).

More recently, the Tn10 and mariner transposons were adapted for in vivo transposition in this organism. Tn10, a transposon isolated from E. coli, was adapted for B. subtilis by fusion of the transposase gene to expression signals appropriate for this bacterium (36). In these systems, the transposon and the specific transposase are cloned on a plasmid with a temperature-sensitive origin of replication for gram-positive hosts. Transposition events are selected by growing the strain containing this plasmid at nonpermissive temperatures (at which the plasmid will not replicate) and selecting for the antibiotic resistance marker found on the transposon.

We and others have successfully used a mini-Tn10 delivery plasmid, pIC333, in many genetic screens. Tn10 has not been analyzed for any “regional biases,” but it is known to have a strong preference for a 6-bp target sequence (20). Therefore, while Tn10 insertion events are distributed throughout the genome, the number of potential insertion sites is reduced. In our studies, we have often found only one or two sites of insertion per gene, and in other cases, we failed to recover insertions in expected targets (5, 16). Moreover, the pIC333 plasmid is unstable in both E. coli and B. subtilis (T. Msadek, personal communication) and care needs be to taken when isolating and working with this plasmid. Recently, an improved and stabilized mini-Tn10 system was constructed for use in B. anthracis and was shown to be fully functional in B. subtilis (50).

In an attempt to develop a more efficient and random system, the mariner transposable element Himar1 was adapted for in vivo transposition in B. subtilis (26). The TnYLB-1 element (consisting of Himar-recognized inverted terminal repeats flanking a Kan’ cassette) and the Himar1 transposase (modified for expression in B. subtilis) were cloned on a delivery vector containing a temperature-sensitive origin of replication. Transposon systems using the mariner element have been applied to a number of species and did not display regional hot spots. Insertions are highly random, since they occur in the small target sequence TA (2, 8, 26, 41, 47).

While these in vivo systems have proven successful, transposition can be biased by cell processes. Many transposons are inhibited from transposition into actively transcribed regions (9, 11, 13, 29). Some elements, like Tn916, show biases for noncoding DNA (32). Presumably, the in vivo architecture of the chromosome and DNA-binding proteins could also inhibit transposition into some regions. Therefore, in vitro transposition systems have been developed for use in E. coli and several are commercially available (e.g., the EZ-Tn5 and HyperMu systems from Epicenter and the GPS-1 genome-priming system from New England BioLabs). These systems use purified transposase and a transposon donor to mutagenize a DNA target of choice (plasmid, cosmid, or chromosomal DNA).
TABLE 1. Strains and primers used in this study

<table>
<thead>
<tr>
<th>Strain or primer</th>
<th>Genotype or sequence</th>
<th>Reference, source, or use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW23474</td>
<td>pir-116 hsdR514 endA recA1</td>
<td>CGSC 7838</td>
</tr>
<tr>
<td>HE5101</td>
<td>BW23374(pTn7SX)</td>
<td>This study</td>
</tr>
<tr>
<td><strong>B. subtilis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W168</td>
<td>trpC2</td>
<td>BGSC no. IA1</td>
</tr>
<tr>
<td>ZB703A</td>
<td>trp&quot; phe&quot; SP1722::Tn917::pSK10Δ6</td>
<td>43</td>
</tr>
<tr>
<td>CU1065</td>
<td>W168 att SPβ trpC2</td>
<td>48</td>
</tr>
<tr>
<td><strong>Primers</strong></td>
<td></td>
<td></td>
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<tr>
<td>2510</td>
<td>TCGCCGCCGCGGAGATCCCCCTATGCAAGG</td>
<td>NotI site; creation of pTn7SX</td>
</tr>
<tr>
<td>2511</td>
<td>GGAGCGGGGCTGCCGAATGGCGATTTCG</td>
<td>BssHII site; creation of pTn7SX</td>
</tr>
<tr>
<td>2516</td>
<td>CAATACTTCATATGTAAGTCATCACCC</td>
<td>SpeI site; creation of pTn7SX</td>
</tr>
<tr>
<td>2514</td>
<td>TGAGGCGGGACACAAATAGTGGG</td>
<td>BssHII site; creation of pTn7SX</td>
</tr>
<tr>
<td>2517</td>
<td>AACTGAATCTAAAGTTAGTGC</td>
<td>Screening for pTn7SX clones</td>
</tr>
<tr>
<td>2518</td>
<td>TGTGGCCGGGACACAAATAGTGGG</td>
<td>Screening for pTn7SX clones</td>
</tr>
<tr>
<td>2461</td>
<td>TACATGCCGAGTTATTCGGG</td>
<td>Screening for insertions in gfpTO</td>
</tr>
<tr>
<td>2462</td>
<td>TACATGCCGAGTTATTCGGG</td>
<td>Screening for insertions in gfpTO</td>
</tr>
<tr>
<td>2517</td>
<td>TTGAGACGGCGCATATCCACTCC</td>
<td>Screening for insertions in the pps4 operon</td>
</tr>
<tr>
<td>2509</td>
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</tr>
<tr>
<td>2505</td>
<td>GGCCACCGGTGCGATCGTACANNNNNNNNNGATAT</td>
<td>First-round arbitrary PCR</td>
</tr>
<tr>
<td>2501</td>
<td>CCAGATAGTGAATCTAGTGC</td>
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</tr>
<tr>
<td>2502</td>
<td>CGTAGTACGTGCGATCGTACACC</td>
<td>Second-round arbitrary PCR</td>
</tr>
<tr>
<td>2508</td>
<td>GGCCACCGGTGCGATCGTACACC</td>
<td>Second-round arbitrary PCR</td>
</tr>
</tbody>
</table>

*M MATERIALS AND METHODS

Bacterial strains and culture media. All of the *B. subtilis* and *E. coli* strains and oligonucleotide primers used in this study are listed in Table 1. Bacteria were grown in liquid Luria-Bertani medium (LB) at 37°C with vigorous shaking or on solid medium containing 1.5% Bacto agar (Difco). Screening for auxotrophs and pJPM122::mTn7SX insertions was performed on solid minimal medium (MM) supplemented with 10 μM FeSO₄ (note that this medium contains tryptophan as the strains used are tryptophan auxotrophs). Sporulation proficiency was assayed on Difco sporulation medium (22). Antibiotics were added to the growth medium at the following concentrations: SPEC, 10 μg/ml; neomycin (NEO), 8 μg/ml; kanamycin (KAN), 10 μg/ml; tetracycline, 20 μg/ml; chloramphenicol (CM), 10 μg/ml. Where indicated, 2% xylose or 40 μg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was used. *E. coli* cells were made competent by treatment with ice-cold 0.1 M CaCl₂ (33). Transformation of *B. subtilis* was carried out as described previously (22).

Construction of pTn7SX. The xylA promoter (P₁₅₅₅) was amplified from pXT (14) with Phu polymerase (Stratagene) and primers 2514 (containing a BssHII site) and 2510 (SpeI) and purified through the QIAquick spin column (Qiagen). This P₃₅₅₅₅-PCR product was digested sequentially with BssHII and SpeI (New England BioLabs) and cloned into pGPS2.1 [catalog no. N7121S from New England BioLabs], where the cat gene has been replaced with the aph₃ (Kan') gene; J. C. Huget and J. Peters, unpublished data) digested with the same enzymes. After transformation into *E. coli* BW23474 (*pir*), constructs with P₃₅₅₅₅ in the correct orientation were identified by PCR with 1.1x Thermo-Star PCR Master Mix (ABGene) and primers 2517 (complementary to the Tn7 right region) and 2516. The aph₃ gene was then replaced with the spec gene as follows. The spec gene was amplified from pXT with 1.1x Thermo-Star PCR Master Mix (ABGene) and primers 2511 (containing a BssHII site) and 2510 (NotI), and the product was purified through the QIAquick spin column (Qiagen). This spec PCR product and the plasmid were then sequentially digested with BssHII and NotI, ligated with T4 ligase (Invitrogen), and transformed into *E. coli* BW23474, and clones were selected on LB plus SPEC. Correct clones were confirmed by PCR with primers 2510 and 2516. The resulting plasmid, pTn7SX, was purified with the QIAprep miniprep kit.
(Qiagen) and eluted with 50 μl sterile Milli-Q water. Sequence information for this plasmid is available at http://www.micro.cornell.edu/cal/cal/research/labs/helmann-lab-supplements.cfm.

**Purification of Tn5 proteins.** TnsA and TnsB were purified as previously described (31, 42). The Tn5CasC225V mutation was introduced into TnsC expression vector pRS550 (a kind gift from Nancy Craig), a pCYB1 (New England BioLabs)-based vector, for the expression of a Tn5-cintin-binding domain fusion protein by replacing the SacI-Hind6 fragment of pRS550 with a 904-bp SacI-Hind6 fragment from plasmid pCW15 which carried the TnsABC225V mutation (46), creating pCYB-TnsC*. TnsC* was purified with a cation column as recommended by the manufacturer (New England BioLabs), but the buffer contained 25 mM HEPES (pH 7.5), 1 M NaCl, 1 mM ATP, 10 mM MgCl2, 0.1 mM EDTA, 10 mM 3-[3-cholamidopropyl]-dimethylammonio]-1-propanesulfonate (CHAPS), and 10% glycerol. TnsC was separated from the cintin-binding domain tag by in-between-mediated self-cleavage and stored in 25 mM HEPES (pH 7.5)–1 M NaCl–2.5 mM dithiothreitol–1 mM ATP–10 mM MgCl2–0.1 mM EDTA–10 mM CHAPS–10% glycerol at –80°C. The TnsABC* proteins allowed 10 to 15% conversion of a target plasmid to simple insert products in a 40-min reaction (data not shown).

**In vitro transposition.** Chromosomal DNA was isolated from a 5-ml overnight culture of R. subtilis CU1065 with the GFX Genomic DNA purification kit (Amersham Biosciences) as described by the manufacturer, with the following changes. Cells were resuspended in 200 μl lysis buffer, the optional RNase A treatment was included, and the DNA was eluted in 50 μl sterile Milli-Q water. The in vitro transposition reactions were performed essentially as previously described (4), with the indicated modifications. The 100-/H9262 and/or H9272 treatments were included, and the DNA was eluted in 50 μg/ml bovine serum albumin, 2 mM ATP (pH 7.0), 2.1 mM dithiothreitol, 0.05 mg/ml duramycin, and either 2% xylose or glucose. Insertions within the gfp operon were screened by PCR with primers 2461 and 2462 and 1.1x Thermo-Start PCR Master Mix (ABgene). Duramycin-resistant (Durr) mutants were selected by plating the transformed R. subtilis CU1065 cells on solid LB containing SPEC and xylose. After incubation for 5 h at 37°C, these plates were overlaid with 5 ml of soft LB agar (0.7%) containing SPEC, xylose, and 6 μg/ml duramycin and incubated at 37°C overnight. Clones were checked for insertion of mTn7SX into the gpx operon by PCR with primers 2369 and 1916. MICs were determined as described above, with the following concentrations of duramycin: 0, 1, 1.5, 2, 20, 30, 40, 50, 75, 100, 150, and 200 μg/ml.

**Allelic replacement by long-flanking homology PCR.** The mTn7SX insertions in 10 transposants were replaced with a kan cassette by long-flanking homology PCR as previously described (30). Briefly, regions up- and downstream of the mTn7SX insertion site were amplified and joined with a kan cassette by PCR. The corresponding transposants were transformed with the joined PCR product, and transfectants were selected on LB-KAN and tested for SPEC sensitivity. Kan cassette colonies identified in mTn7SX had been replaced with the kan cassette by double recombination and that no other mTn7SX insertion was present in the genome. Primers used for creation of the joined PCR products are available upon request.

**RESULTS AND DISCUSSION**

**Construction of mTn7SX.** We constructed a Tn7 transposon donor plasmid, pTn7SX, that contains a SPEC resistance cassette and an outward-facing, xylose-inducible promoter, P<sub>lacZ</sub> (Fig. 1). In addition to screening for insertions that disrupt genes, this transposon also allows for upregulation of genes not expressed under the conditions of the experiment or of cryptic genes. B. subtilis P<sub>lacZ</sub> and the spec gene were amplified from pXT and cloned into pGPS2.1ap3·± (a derivative of pGPS2.1 in which the cat gene was replaced with aph3) to create pTn7SX (see Materials and Methods).

We tested this new transposon by performing in vitro transposition into a plasmid carrying a NEO resistance gene and a promoterless cat-lacZ operon (pJM122). After in vitro transposition, gap repair, and linearization, the plasmid was transformed into B. subtilis ZB703A to allow for integration of the plasmid into the SPB prophage carried in this strain. Thirty SPEC-resistant transfectants were selected and screened for
clones were NEO sensitive as a result of the insertion of Tn7neo into the Tn insertions either in the opposite orientation within the lacZ gene or elsewhere in the plasmid. These data confirmed that the transposon is active and that the outward-facing SPEC resistance cassette and the outward-facing xylA promoter (P_{xylA}).

xylose-inducible upregulation of the cat and/or lacZ genes or disruption of the neo gene. Seven clones contained mTn7SX insertions that resulted in the expression of β-galactosidase in the presence of xylose, indicating that the transposon-encoded P_{xylA} promoter was inserted upstream of the lacZ gene. Two of these insertions also exhibited xylose-inducible CM resistance due to insertion of the transposon upstream of the cat gene (thereby activating the cat-lacZ operon fusion). Another 17 clones were NEO sensitive as a result of the insertion of Tn7 into the neo gene. The remaining six clones presumably have Tn7 insertions either in the opposite orientation within the cat or lacZ gene or elsewhere in the plasmid. These data confirmed that the transposon is active and that the outward-facing P_{xylA} promoter is functional and suggest random insertion throughout the target plasmid DNA.

Transposition into B. subtilis chromosomal DNA with mTn7SX. In vitro transposition was carried out with chromosomal DNA isolated from B. subtilis CU1065. After gap repair, this mutagenized chromosomal DNA was used to transform B. subtilis CU1065 and strains carrying transposon insertions were selected on LB agar containing SPEC. We obtained an average of ~57,000 clones per transformation with one in vitro transposition reaction. The B. subtilis genome is about 4,215 kb in size. Thus, this corresponds statistically to one insertion for every 73 bp (or about 13 insertions per gene). Five hundred and one colonies were selected for further characterization. Of these, ~2.6% were sporulation deficient (as monitored on Difco sporulation medium) and ~1.8% were auxotrophs (did not grow on MM). The chromosomal DNA from 30 SPEC-resistant colonies was purified, and the positions of the mTn7SX insertions were mapped by arbitrary PCR and DNA sequencing (see Materials and Methods). These insertions were found throughout the genome (Fig. 2), further highlighting the random nature of transposition. Ten clones were selected, and the transposon was replaced with a kan gene by transformation with a PCR product containing homology to the regions flanking the transposon insertion and an intervening kan cassette. The resulting transformants were all KAN resistant and SPEC sensitive, indicating that there was not a second transposon within these genomes. Taken together, these data show that mutagenesis with this transposon occurs with high efficiency and the resulting insertion events are highly distributed.

While the above reactions were performed with proteins purified in our laboratory (see Materials and Methods), this mTn7SX donor plasmid can also be used with the commercially available GPS-M Mutagenesis System (New England BioLabs). In comparative experiments with the same amount of target chromosomal and donor plasmid DNAs, we found that the efficiency of transposition was similar (we obtained a bank size of 56,800 transposants with the commercial kit).

Isolation of fosfomycin-resistant mutants. We used mTn7SX to select for resistance to fosfomycin, an antibiotic produced by Streptomyces species and used in the treatment of lower urinary tract infections (37). This antibiotic is also effective against methicillin-resistant and vancomycin-resistant strains of Staphylococcus aureus.

After transformation of CU1065 with the in vitro transposition reaction mixture, these cells were plated on LB plates containing SPEC, xylose, and 30 μg/ml fosfomycin. Thirty-seven fosfomycin-resistant colonies were isolated and patched to plates containing xylose and 50 μg/ml fosfomycin. All but two of these isolates were resistant to this higher concentration of fosfomycin. The position of the insertion was mapped in 10 of the clones able to grow on the higher fosfomycin concentration, and in each case the transposon was inserted at a...
different position within the glpTQ operon (Fig. 2B). We also mapped the insertions in the two clones that grew only on plates containing 30 μg/ml fosfomycin. In both cases, the transposon was inserted upstream of fosB with Psux44 driving the expression of this gene (Fig. 2B). The remaining 25 clones also grew at the higher fosfomycin concentration, and PCR with primers specific to regions up- and downstream of glpTQ confirmed that all insertions were within this operon. Since some transposons were oriented such that they place the glpQ gene under xylose control, we tested the MICs for these strains in liquid media with and without 2% xylose (Table 2). In these insertions, fosfomycin resistance was not dependent on xylose, indicating that resistance is caused by disruption of either gene. On the other hand, the fosB promoter insertions were sensitive to fosfomycin in the absence of xylose. In this case, resistance is provided by overexpression of the fosB gene.

Glpt is an ABC transporter that actively transports glycerol-3-phosphate to the cytoplasm (27), while the glpQ gene encodes a glycerophosphodiester phosphodiesterase that processes the glycerophosphodiesteros produced from membrane phospholipids into glycerol-3-phosphate and their respective alcohols (25). Fosfomycin enters the cell via the GlpT transporter, where it inactivates MurA, a cytoplasmic enzyme involved in the first step of peptidoglycan biosynthesis (27). It has long been known that mutations in glpT of E. coli result in increased fosfomycin resistance (28). In B. subtilis, the glpT locus was originally mapped by selection for Fosr mutants (28). No Fosr mutants have been mapped to glpQ. However, a glpQ mutant of Enterococcus faecalis was recently found to be more resistant to the class Ia bacteriocin divenicin V41 (6). These authors propose that this mutation results in a change in the fatty acid and phospholipid composition of the membrane, therefore preventing the activity of this membrane-active antibiotic. This would not explain the involvement of GlpQ in resistance to fosfomycin, which acts on a target located in the cytoplasm. In B. subtilis, expression of the glp regulon is induced by the antiterminator GlpP in the presence of glycerol-3-phosphate (38). We speculate that disruption of glpQ results in lower levels of glpT expression, due to reduced levels of glycerol-3-phosphate, and therefore less transport of fosfomycin into the cells.

FosB encodes an enzyme that catalyzes the addition of L-cysteine to fosfomycin, rendering it inactive (37). In B. subtilis, the extracytoplasmic function σ factor σW controls the expression of fosB (7). The transposon mutants isolated in this screen contained insertions in the fosB promoter region, thereby uncoupling the expression of fosB from σW and instead placing this gene under xylose control.

These data highlight the versatility of mTn7SX: in addition to mutations disrupting genes, we also isolated mutations that caused increased resistance due to the upregulation of a resistance gene. This second class of mutations would not be easily detected with conventional transposon screens.

### Isolation of duramycin-resistant mutants

We also used mTn7SX to isolate mutants with increased resistance to the lantibiotic duramycin. Duramycin is a 10-amino-acid tetracyclic peptide produced by Streptovercillatorium cinnamomeum that binds exclusively to ethanolamine phospholipids (23). Duramycin-resistant B. subtilis mutants have little or no phosphatidylethanolamine (PE) and cardiolipin, but the mutant loci were not determined (15).

Our first attempts to isolate Durr mutants by plating the B. subtilis transformants directly on duramycin-containing LB plates failed. Since duramycin resistance appears to be conferred by a change in membrane composition, we hypothesized that our method did not allow for sufficient growth in the absence of selection for the effects of mTn7SX insertions to manifest themselves as changes in the membrane lipid composition. Therefore, we plated the transformed cells on LB agar containing SPEC (for selection of the mutants) and xylose (to allow for upregulation from the Psux44 promoter) and incubated these plates for 5 h to allow time for potential alterations in membrane composition to become manifest. These plates were then overlaid with 0.8% soft LB agar containing SPEC, xylose, and 6 μg/ml duramycin and incubated overnight. We obtained 60 clones resistant to duramycin upon restreaking on plates containing 10 μg/ml duramycin.

Since the pssA-ybfM-psd operon is required for the synthesis of PE and cardiolipin, we checked whether any of these mutations were at this locus and found that 53 of the 60 clones gave a PCR product consistent with insertion of mTn7SX within this region. We prepared chromosomal DNA from 10 of the 53 putative pssA-ybfM-psd::mTn7SX clones and used this to transform B. subtilis CU1065. Transformants were selected on SPEC, and in all cases subsequent testing revealed that these transformants were resistant to more than 200 μg/ml duramycin. This confirmed that the resistance phenotype was linked to disruption of the pssA operon by mTn7SX. Mapping of the mTn7SX junctions indicated that each insertion was unique, and they were found regularly spaced throughout all three genes of the operon (Fig. 2C). Analysis of chromosomal DNA from the other seven isolates indicated that the Durr phenotype was not linked to the mTn7SX insertion, suggesting that a second mutation (presumably within the pssA-ybfM-psd operon) was responsible for the resistance phenotype.

Phosphatidylserine (PS) synthase (encoded by pssA) catalyzes the formation of PS, the first step in PE synthesis. PS is rapidly converted to PE by PE decarboxylase (encoded by psd).

Therefore, the disruption of these genes results in a lack of PE in the membrane and insensitivity to duramycin (our data; L. Salzberg and J. D. Helmann, unpublished data). The function of YbfM is unknown; however, we have shown that nonpolar mutations within its gene do not result in duramycin resistance (Salzberg and Helmann, unpublished). Therefore, the inser-

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**Table 2. Fosfomycin-resistant mutants used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Orientation of P&lt;sub&gt;sux44&lt;/sub&gt;</th>
<th>MIC (μg/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>M2</td>
<td>Between glpT and glpQ</td>
<td>–</td>
<td>150/150</td>
</tr>
<tr>
<td>M1</td>
<td>glpT::mTn7SX</td>
<td>+</td>
<td>150/150</td>
</tr>
<tr>
<td>M8</td>
<td>glpT::mTn7SX</td>
<td>–</td>
<td>150/150</td>
</tr>
<tr>
<td>M5</td>
<td>glpQ::mTn7SX</td>
<td>+</td>
<td>80/80</td>
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<tr>
<td>M7</td>
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<td>–</td>
<td>80/80</td>
</tr>
<tr>
<td>M18</td>
<td>P&lt;sub&gt;sux44&lt;/sub&gt;-fosB::mTn7SX</td>
<td>+</td>
<td>10/70</td>
</tr>
</tbody>
</table>

<sup>a</sup> +, same orientation as the gene; –, opposite orientation to gene.

<sup>b</sup> MICs for growth in liquid medium were reproducibly higher than on solid medium (see text). The MIC of fosfomycin for strain CU1065 was 30 μg/ml (independent of xylose) under these conditions.
tions that we isolated within this gene are predicted to be polar on the downstream psd gene. Since we obtained multiple unique insertions across the pssA-ybfM-psd operon, we suspect that we have saturated the screen, indicating that there are no other genes within the B. subtilis genome that, when disrupted or overexpressed, result in duramycin resistance.

Conclusion. The development of mTn7SX enables in vitro transposition in the gram-positive bacterium B. subtilis. The addition of the outward-facing, xylose-inducible promoter further improves the system by allowing the identification of “cryptic” or unexpressed genes or simply by ensuring the expression of genes downstream of the insertion. This is analogous to the GeneHunter transposon, based on the EZ-Tn5 system, in which the transposon contains an outward-facing, inducible pTAC promoter (39). Moreover, the observation that some transposants selected in the presence of xylose are unable to grow in medium lacking xylose (data not shown) suggests that this system also generates conditional lethal mutations by insertion of the P_{pTAC} promoter upstream of essential genes. The mTn7SX element has several advantages over in vivo transposon mutagenesis systems. Firstly, there is no need for high-temperature curing of the plasmid, which increases the chance of isolating siblings resulting from outgrowth of the same insertion event. We have not identified any siblings in our experiments. Second, mTn7SX displays no obvious insertion bias or requirement for a target sequence, as highlighted by the random distribution of insertions within the gfpTQ and pssA-ybfM-psd operons and across the genome (Fig. 2). Lastly, Tn7-mediated immunity greatly diminishes the probability of multiple insertions per DNA fragment. This results in libraries of transformants containing a single insertion event per chromosome.

ACKNOWLEDGMENTS

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