Efficient Gene Transfer in Bacterial Cell Chains

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ABSTRACT Horizontal gene transfer contributes to evolution and the acquisition of new traits. In bacteria, horizontal gene transfer is often mediated by conjugative genetic elements that transfer directly from cell to cell. Integrative and conjugative elements (ICEs; also known as conjugative transposons) are mobile genetic elements that reside within a host genome but can excise to form a circle and transfer by conjugation to recipient cells. ICEs contribute to the spread of genes involved in pathogenesis, symbiosis, metabolism, and antibiotic resistance. Despite its importance, little is known about the mechanisms of conjugation in Gram-positive bacteria or how quickly or frequently transconjugants become donors. We visualized the transfer of the integrative and conjugative element ICE*Bs1* from a *Bacillus subtilis* donor to recipient cells in real time using fluorescence microscopy. We found that transfer of DNA from a donor to a recipient appeared to occur at a cell pole or along the lateral cell surface of either cell. Most importantly, we found that when acquired by 1 cell in a chain, ICE*Bs1* spread rapidly from cell to cell within the chain by additional sequential conjugation events. This intrachain conjugation is inherently more efficient than conjugation that is due to chance encounters between individual cells. Many bacterial species, including pathogenic, commensal, symbiotic, and nitrogen-fixing organisms, harbor ICEs and grow in chains, often as parts of microbial communities. It is likely that efficient intrachain spreading is a general feature of conjugative DNA transfer and serves to amplify the number of cells that acquire conjugative mobile genetic elements.

IMPORTANCE Conjugative elements contribute to horizontal gene transfer and the acquisition of new traits. They are largely responsible for spreading antibiotic resistance in bacterial communities. To study the cell biology of conjugation, we visualized conjugative DNA transfer between *Bacillus subtilis* cells in real time using fluorescence microscopy. In contrast to previous predictions that transfer would occur preferentially from the donor cell pole, we found that transfer of DNA from a donor to a recipient appeared to occur at a cell pole or along the lateral cell surface of either cell. Most importantly, we found that when acquired by 1 cell in a chain, the conjugative DNA spread rapidly from cell to cell within the chain through sequential conjugation events. Since many bacterial species grow naturally in chains, this intrachain transfer is likely a common mechanism for accelerating the spread of conjugative elements within microbial communities.

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Horizontal gene transfer is an important factor in evolution, enabling bacteria to acquire new characteristics (1–4). Conjugative plasmids and integrative and conjugative elements (ICEs) are found in many bacterial species and are key mediators of horizontal gene transfer (4–7). ICEs normally reside integrated in the host genome but can excise to form a double-stranded DNA circle. Some and perhaps most ICEs undergo autonomous plasmid-like replication after excision (8, 9). ICEs can mediate their transfer by conjugation to other cells, where they can then integrate into the recipient genome.

ICE*Bs1* (Fig. 1) is an ~20-kbp integrative and conjugative element found integrated in the 3′ end of a leucine-tRNA gene in several strains of *Bacillus subtilis* (10–12). ICE*Bs1* genes required for excision and mating are derepressed during the RecA-dependent SOS response following DNA damage or when the sensory protein RapI is expressed and active (11, 13, 14). Overproduction of RapI causes ICE*Bs1* to excise in >90% of cells in a population (11, 13, 15, 16), greatly facilitating the characterization of this mobile genetic element.

ICE*Bs1* can transfer into various *Bacillus* and *Listeria* species (11) and perhaps other organisms as well.

Many microbes, including *B. subtilis*, grow in chains, often in communities of cells, e.g., biofilms (17). The presence of conjugative elements in cells can contribute to the formation of such communities, and conjugation in these communities has been observed (18–20). During conjugation, there are potential donors that harbor a mobile element and potential recipients (here simply referred to as donors and recipients, respectively). A recipient that receives a mobile element is called a transconjugant and has the potential to become a donor. Very little is known about the relative orientation of cells during conjugation or how quickly or frequently transconjugants become donors. Some conjugation proteins localize to the cell periphery, predominantly at the poles, leading to the suggestion that DNA transfer occurs predominantly from a donor pole (21–24). However, in the case of ICE*Bs1* from *B. subtilis*, our results indicate otherwise.
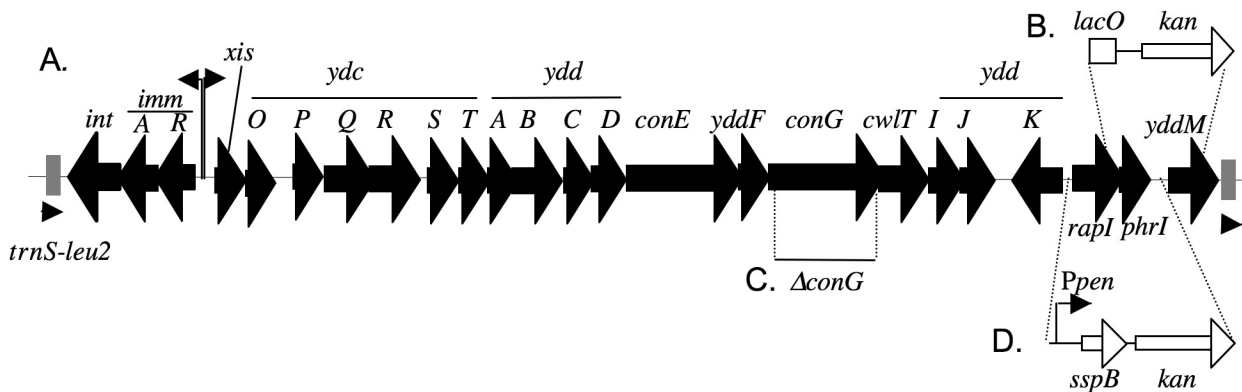
FIG 1 Map of ICE*Bs1* and the constructs used. (A) ICE*Bs1* is ~20 kb and inserted in the 3′ end of *trnS-leu2*. Large arrows indicate open reading frames and orientation. The shaded boxes with small arrowheads underneath at the ends of ICE*Bs1* represent the 60-bp direct repeats. Vertical lines with arrows between *immR* and *xis* represent the two promoters (P*immR*, P*xis*) that are controlled by the transcriptional repressor/activator ImmR. (B) Insertion of the *lacO* array and *kan* and concomitant removal of part of *rapI* through *yddM*. (C) Boundaries of the *conG* deletion. (D) Insertion of *sspB* and *kan* and deletion of *rapI-phrI*.

We visualized the transfer of ICE*Bs1* in living cells in real time using fluorescence microscopy. We found that transfer of ICE*Bs1* from a donor to a recipient appeared to occur at a cell pole or along the lateral cell surface of either cell, in contrast to previous predictions. Furthermore, transconjugants often became donors, and this was especially evident in cell chains. We found that when cells grow in chains, there is efficient and successive transfer to neighboring cells in a chain, likely accelerating the spread of conjugative elements in microbial communities.

RESULTS

***lacO*/LacI-GFP system to visualize conjugative transfer of ICE*Bs1*.** To monitor ICE*Bs1* DNA transfer, we engineered *B. subtilis* strains to distinguish donors from recipients and transconjugants (Fig. 2), using detection systems similar to those previously used to visualize conjugation (25, 26). Recipients did not contain ICE*Bs1* and had a relatively uniform green fluorescence (Fig. 2A) from expression of a green fluorescent protein (GFP) fused to the *Escherichia coli* Lac repressor (LacI-GFP). Donors had a relatively uniform red fluorescence from constitutively expressed mCherry (Fig. 2A). Donors also contained ICE*Bs1* with a *lac* operator array (*lacO*, to which the Lac repressor binds). When ICE*Bs1*::*lacO* transfers to a recipient, LacI-GFP binds the *lacO* array and appears as a green focus in the transconjugant (Fig. 2B). We induced ICE*Bs1* gene expression, excision, and conjugation in donor cells by overproducing RapI. Donors and recipients were mixed and spotted onto agarose pads on a microscope slide. Images of cells were captured every 30 min for up to 3 h and then analyzed. Transconjugants were identified as cells with at least one green (LacI-GFP) focus (Fig. 2). Once a transconjugant was visible, we examined earlier time points to determine the orientation of the cells during the whole time course leading up to the appearance of transconjugants.

Transfer occurs at a cell pole or along the lateral cell surface. The mating efficiency determined microscopically was one transconjugant per 10 to 20 donor cells (~5 to 10%; >5,000 donors visualized), similar to that determined for mating on nitrocellulose paper (8, 11, 15, 16, 27). In the >300 successful mating events visualized, donors and recipients always appeared to be in contact, indicating that mating likely does not occur through an extended pilus, in contrast to conjugation driven by the *E. coli* F factor (28). Mating occurred at either the sides or ends of the rod-shaped recipient cells, indicating that both the lateral and polar surfaces of recipients are receptive to ICE*Bs1* transfer. Many transconjugants contained multiple LacI-GFP foci (Fig. 2B, D, and H). In a small number of mating events, a single donor transferred ICE*Bs1* to multiple recipients (Fig. 2E to H). Multiple transfer events by a single donor are possible because of autonomous plasmid-like replication of ICE*Bs1* after induction (8). Multiple foci in a transconjugant are most likely due to autonomous replication of ICE*Bs1* in the transconjugant and/or transfer of multiple copies from the donor.

We found that ICE*Bs1* mating occurred either at a donor cell pole or along the lateral surface. We monitored donors surrounded by recipients in various orientations. Of 109 mating events visualized, 81 appeared to occur from the side of the donor (Fig. 2A and B), and 20 appeared to occur from the donor pole (Fig. 2C and D). (In 8 cases, it was difficult to determine the orientation of the donor.) The orientations were determined from the relative positions of cells at the earliest time point, shortly after donors and recipients were mixed and placed on the microscope slide. This ~4:1 ratio corresponds to the approximate ratio of lateral to polar surface area of the rod-shaped bacilli, indicating that mating appears to occur randomly along the donor cell surface. These results contrast with previous predictions that conjugation would occur predominantly at a donor cell pole (23, 24), predictions that were based on observations that some conjugation proteins (including one ICE*Bs1* mating protein) appear concentrated at cell poles (21–24). Occasionally, we observed transfer from a donor that was internal in a chain of cells and flanked at the poles by other donors. That such a cell can serve as a donor is consistent with the conclusion that transfer need not occur at a donor pole.

Rapid and efficient transfer of ICE*Bs1* in cell chains. Like many bacteria, *B. subtilis* often grows in chains (≥4 connected cells). Each cell in a chain is distinct and surrounded by a membrane and cell wall, but the cells remain connected by the polar cell wall. We observed rapid spread of ICE*Bs1* to many cells in a chain when an initial transconjugant was part of the chain. Among 53 cases in which a single cell in a chain initially received ICE*Bs1* from a donor, 43 (81%) of the transconjugants became donors and
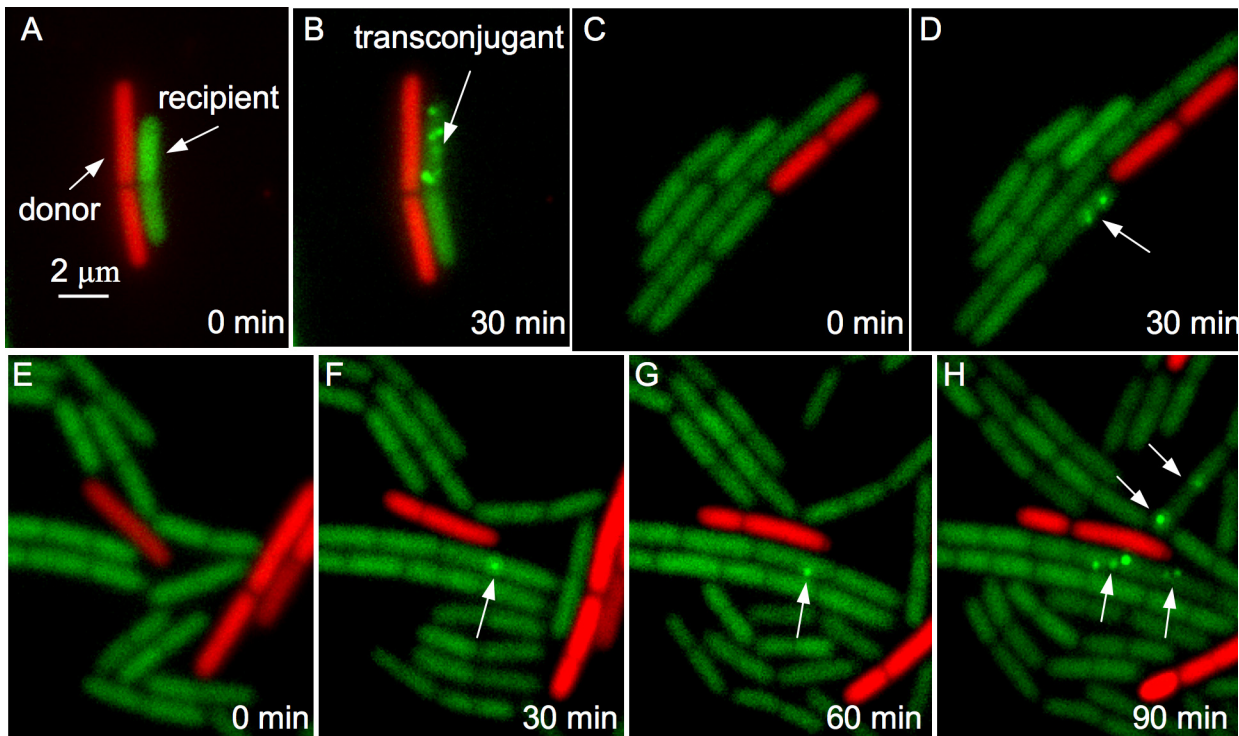
FIG 2 Examples of successful mating pairs between donors that contain ICE*Bs1* with a *lacO* array (red cells, strain AB86) and recipients that express LacI-GFP (green cells, strain MMB849) visualized by fluorescent microscopy. Transconjugants appear as cells with at least one focus of LacI-GFP. The appearance of LacI-GFP foci in the absence of donors is ≤0.01%, indicating that virtually all of the events we visualized were transconjugants. Arrows point to the donor, recipient, and transconjugant, as indicated (A, B), and to some of the foci of LacI-GFP (D, F to H). (A, B) Mating from the side of the donor cell to the side of the recipient. (C, D) Mating from the pole of the donor cell to a pole of the recipient. (E to H) A single donor transfers ICE*Bs1* to two recipients. At the beginning of the time course, before visible transfer (A, C, E), 30 min later (B, D, F), and at successive 30-min time points (G, H). In these examples, the transconjugant has multiple green foci, likely due to replication of ICE*Bs1* in the transconjugant and/or multiple transfer events.

transmitted ICE*Bs1* to neighboring cells in the chain, often within 30 min (Fig. 3A to E). It appeared that ICE*Bs1* spread to cells preexisting in the chain before the initial transconjugant divided. In addition, the number of cells in a chain that acquired ICE*Bs1* was greater than 2^n (the number expected from "n" cell divisions), indicating spread by a mechanism other than growth and division of the initial transconjugant. Two types of experiments, described below, verified that spreading through the chains was due to conjugation and not due to replication and segregation of the plasmid form of ICE*Bs1* during cell division, or some unforeseen property of LacI-GFP bound to ICE*Bs1*::*lacO*.

Efficient transfer of ICE*Bs1* in cell chains depends on conjugation. We found that efficient spreading of ICE*Bs1* in cell chains was dependent on conjugation. Null mutations in *conG* (*yddG*) of ICE*Bs1* prevent mating (C. T. Leonetti, M. A. Hamada, S. J. Laurer, A. D. Grossman, and M. B. Berkmen, unpublished results). We used a donor carrying ICE*Bs1*::*lacO* Δ*conG* and a functional copy of *conG*+ elsewhere in the chromosome (see Materials and Methods), permitting the initial transfer of ICE*Bs1*. However, transconjugants that receive ICE*Bs1*::*lacO* Δ*conG* cannot retransfer the element because they lack *conG*. In 26 initial transconjugants that were each part of a chain, there was no detectable spreading of the ICE*Bs1*::*lacO* Δ*conG* mutant to other cells in the chain, other than by cell division and segregation to daughters of the initial transconjugant (Fig. 3F to J). These results indicate that the rapid spreading of wild-type ICE*Bs1* through cells in chains is due to conjugation.

Visualization of horizontal gene transfer using conditional protein degradation. To further confirm that the spreading of ICE*Bs1* in chains was due to conjugation, we observed conjugation using a tracking system based on conditional protein degradation. Recipients expressed a fusion of GFP to a modified SsrA degradation tag (GFP-SsrA*). This fusion protein is rapidly degraded if cells produce SspB (29), a protein that delivers SsrA-tagged proteins to the cellular proteolytic machinery. Recipients did not produce SspB and were green (Fig. 3K). *sspB* was inserted into ICE*Bs1* (ICE*Bs1*::*sspB*, without *lacO*) in a donor strain expressing mCherry (Fig. 3K to N). Transconjugants turn from green to dark due to the instability of GFP-SsrA* in the presence of SspB (29) expressed from newly transferred ICE*Bs1*::*sspB* (Fig. 3K to N). When the initial transconjugant was in a chain of cells, the other cells in the chain (that were not contacting a red donor) also became dark (Fig. 3L to N), indicating the transfer of ICE*Bs1*::*sspB* through the chain. These results also indicate that spreading was not due to growth and division, as once a transconjugant turns dark, all the progeny from division should initially be dark and should not start as green cells that subsequently turn dark. Based on these findings, we conclude that the initial transconjugants become donors and ICE*Bs1* rapidly spreads through cells in chains via efficient conjugation.

DISCUSSION

We used two different methods to visualize conjugative DNA transfer between donor and recipient cells. In one case, we visual-
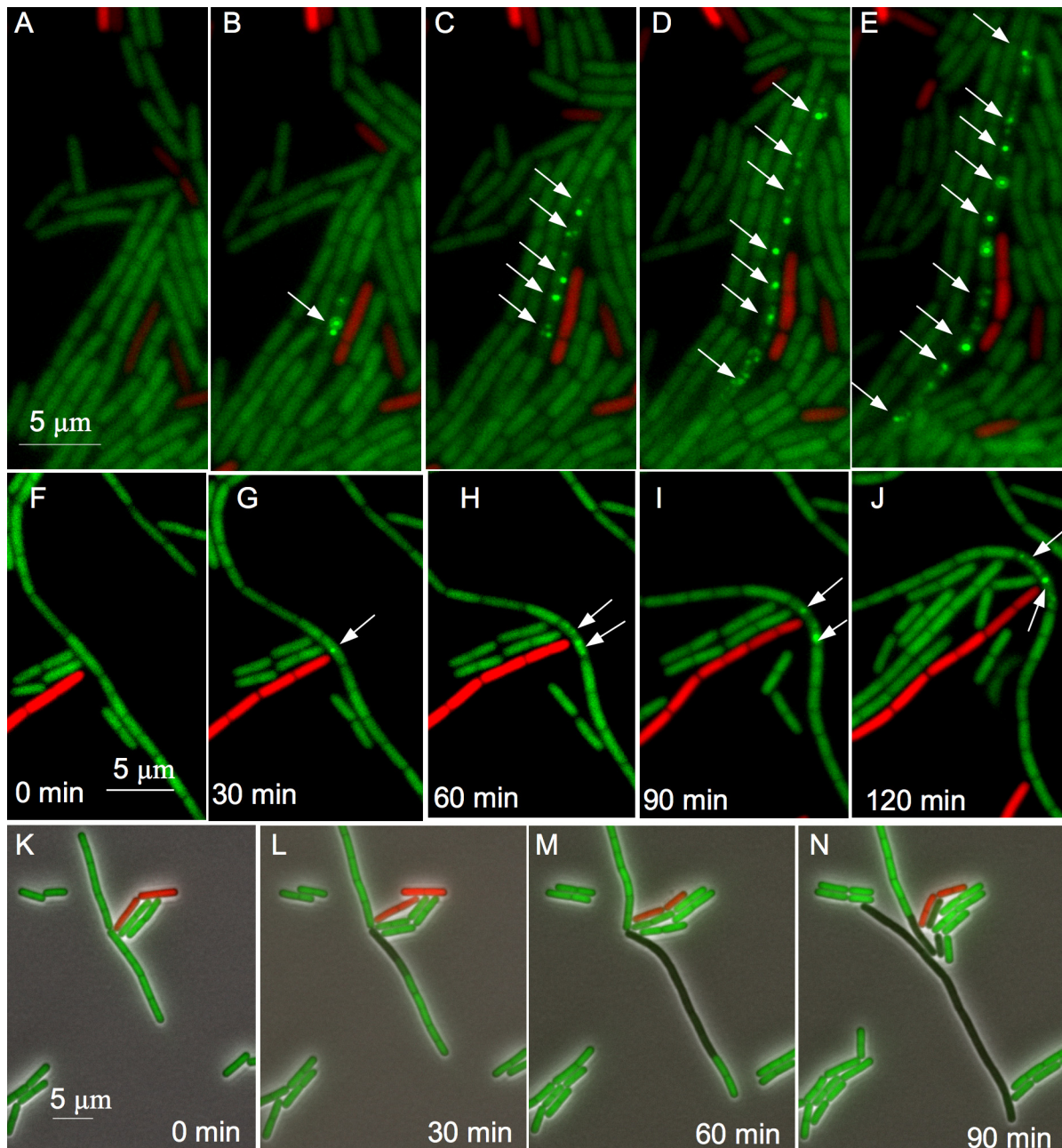
FIG 3 Examples of ICE*Bs1* transferred to cells in chains. A time course is shown for three different matings. In all cases, the first panel of each set (A, F, K) is the first time point (time 0), followed by images of the same field of cells taken at 30-min intervals. Donors are red, and recipients are green. Arrows point to some of the foci of LacI-GFP in transconjugants. (A to E) Spread of ICE*Bs1* through a chain of cells. Donors contained ICE*Bs1* with a *lacO* array (red cells, strain AB86). Recipients expressed LacI-GFP (green cells, strain MMB849). Transconjugants have at least one focus of LacI-GFP. (F to J) Spreading requires conjugation functions. Donors contained ICE*Bs1* with a *lacO* array, a null mutation in *conG* (an ICE*Bs1* gene required for conjugation), and a copy of *conG+* elsewhere in the chromosome (red cells, strain AB101). Recipients and transconjugants were as described above. (K to N) Spreading of ICE*Bs1* through a chain of cells visualized by conditional protein degradation. Images are merges of phase, green (GFP), and red (mCherry). Red donors (strain CAL1391) contained constitutively expressed *sspB* in ICE*Bs1*. Green recipients (strain CAL1379) expressed a *GFP-ssrA** fusion. Transconjugants turned from green to dark due to instability of GFP-SsrA* in the presence of SspB (29) expressed from the newly transferred ICE*Bs1*.

ized the DNA that was transferred from cell to cell. In the second, we used conditional protein degradation to identify cells that acquired the horizontally transferred element. We found that successful conjugation of the integrative and conjugative element of *B. subtilis*, ICE*Bs1*, occurred with no obvious orientation of the donor and recipient. That is, transfer of DNA from a donor into a

recipient appeared to occur at a cell pole or along the lateral cell surface. Furthermore, when acquired by a cell in a chain of cells, ICE*Bs1* spread rapidly to other cells in the chain through sequential transfer events as transconjugants quickly became donors.

Integration and stable maintenance of ICE*Bs1* in the host chromosome requires repression of ICE*Bs1* gene expression from the

rightward promoter P*xis* (Fig. 1). Derepression of P*xis* leads to expression of genes needed for ICE*Bs1* excision and conjugation (11, 15). The excised circular form of ICE*Bs1* is required for its dissemination to recipients. Our results indicate that soon after receiving ICE*Bs1*, a very high percentage of transconjugants become donors by expressing conjugation genes. The ability of a transconjugant to become a donor is likely influenced by the kinetics of repression of P*xis*, which in turn is influenced by the kinetics of accumulation of the ICE*Bs1* repressor ImmR. ImmR both activates and represses its own expression, creating a homeostatic autoregulatory loop (15). Initially, there is no ImmR in a newly formed transconjugant, permitting transcription from P*xis* and expression of ICE*Bs1* conjugation genes. However, in the absence of an inducing signal, expression and accumulation of ImmR in the transconjugant will eventually repress P*xis*, allowing integration of ICE*Bs1* into the chromosome. This type of regulatory circuit is common in mobile genetic elements, notably in bacteriophage (30, 31), and is important in fate determination for such elements. In ICE*Bs1*, this circuit likely allows switching between an active dissemination mode (excision and gene expression) and a quiescent inactive mode (integration and repression). Our studies indicate that a delay in ICE*Bs1* integration and transcriptional repression in transconjugants contributes to the spread of ICE*Bs1* in cell populations.

Much is known about conjugation and conjugative elements of both Gram-negative and -positive bacteria (4, 32, 33). In most cases, transfer efficiencies of a few percent are considered high. Our results indicate that conjugation efficiencies in cell chains can be >50%. A different mechanism for efficient dispersal of a mobile element has been described for *Streptomyces* ICEs that can exist as stable plasmids. Plasmid spreading through *Streptomyces* mycelia depends on spreading proteins (Spd) and is independent of conjugation proteins (summarized in references 32 and 34). In contrast, transfer of ICE*Bs1* to cells in a chain requires the conjugation machinery and is not due to replication and segregation of the plasmid form of ICE*Bs1*.

Many bacterial species, including pathogenic, commensal, symbiotic, and nitrogen-fixing organisms, grow in chains and harbor conjugative elements. In addition, microbial biofilms are often composed of long chains or aggregates of connected cells (17). It seems likely that efficient intrachain spreading is a general feature of conjugative DNA transfer and probably serves to rapidly amplify the number of cells that acquire conjugative mobile genetic elements. When cells are present in a chain, they are in intimate contact with other cells in a pole-to-pole configuration. The high efficiency of intrachain conjugation is likely due to close and stable cell-cell contact. The high concentration of conjugation proteins at donor cell poles (21–24) might also contribute to the efficient pole-to-pole transfer in cell chains.

MATERIALS AND METHODS

Bacterial strains and alleles. The *B. subtilis* strains used are listed (Table 1). All are derivatives of JH642 and contain *trpC* and *pheA* mutations (not indicated). Strains were constructed by standard procedures using natural transformation (35). Strains cured of (missing) ICE*Bs1* (11) are indicated as ICE*Bs1*°. RapI was overproduced from the xylose-inducible promoter P*xyl* from *amyE*::[(P*xyl-rapI*) *spc*] as described previously (23).

(i) ICE*Bs1*::*lacO*/*lacI-gfp*. A deletion-insertion in ICE*Bs1* was made by inserting an array of ~120 Lac operators (*lacO*) (36) along with *kan* (kanamycin resistance), by double crossover, into the region of ICE*Bs1* from bp

TABLE 1 *B. subtilis* strains used

Strain (use)	Relevant genotype
AB86 (donor)	ICE*Bs1* [Δ(*rapI-yddM*)::*lacO kan*]
	amyE::[(P*xyl-rapI*) *spc*]
	cgeD::pMMB1010 (P*pen-mCherry kan cat*)
AB101 (donor)	ICE*Bs1* [Δ(*rapI-yddM*)::*lacO kan conG*Δ(5-805) (unmarked)]
	thrC325::[ICE*Bs1-311* (Δ*attR100*::*tet*) *mls*]
	amyE::[(P*xyl-rapI*) *spc*]
	cgeD::pMMB1010 (P*pen-mCherry kan cat*)
AB110 (donor)	ICE*Bs1* [Δ(*rapI-yddM*)::*lacO kan conG*Δ(5-805) (unmarked)]
	amyE::[(P*xyl-rapI*) *spc*]
	cgeD::pMMB1010 (P*pen-mCherry kan cat*)
CAL1379 (recipient)	ICE*Bs1*° *thrC*::[(Pc-*gfp-ssrA** *mls*]
CAL1391 (donor)	ICE*Bs1-*Δ(*rapI-phrI*)*1366*::[(P*pen-sspB*) *kan*]
	amyE::[(P*xyl-rapI*) *spc*]
	cgeD1388::[(P*pen-mCherry*) *cat*]
MMB849 (recipient)	ICE*Bs1*° *thrC*::(P*pen-lacI*Δ*11-gfpmut2 mls*)

879 (of 1176) at the 5′ end of *rapI* and leaving 156 bp (of 942 bp) at the 3′ end of *yddM* (Fig. 1). This allele, ICE*Bs1* [Δ(*rapI-yddM*)::*lacO kan*], is simply referred to as ICE*Bs1*::*lacO*. The ICE*Bs1*::*lacO* allele used here was present in donor strains in the absence of lacI-GFP. The presence of LacI-GFP (or LacI) interfered with kanamycin resistance, probably by silencing expression of the adjacent *kan* gene.

The *lacO* array contained on a plasmid was previously integrated into ICE*Bs1* by single crossover (23). We found that the single-crossover array was not transferred to recipients during conjugation, necessitating the integration of a *lacO* array by double crossover.

LacI-GFP was produced from *thr*::[P*pen-*(*lacI*Δ*11-gfpmut2*) *mls*], as described previously (37). This construct fuses *lacI-GFP* to a constitutive promoter and is integrated at *thrC* (making the cells threonine auxotrophs). Strains containing this fusion without a *lacO* array have relatively uniform green fluorescence. The presence of a *lacO* array in a cell with LacI-GFP results in a green focus. Strains containing *lacI-GFP* were used as recipients in conjugation experiments with ICE*Bs1*::*lacO* donors.

(ii) Construction and complementation of ΔconG. ΔconG (*yddG*) is an in-frame markerless deletion of codons 5 to 805 (of 815). It was constructed in a manner analogous to that constructed for *conE*Δ(*88-808*) (23). *conG* function was provided *in trans* by *thrC*::[ICE*Bs1-311*(Δ*attR*::*tet*) *mls*], an ICE*Bs1* inserted in *thrC* that is incapable of excision (27). The mating efficiency of this complemented mutant (strain AB101), determined by filter mating (23), is normal.

(iii) P*pen-mCherry* at *cgeD*. We used two different constructs that expressed a version of *mCherry* (38) that was codon optimized for *E. coli* (provided by S. Sandler) from the constitutive promoter P*pen*. P*pen* was obtained from upstream of *lacI*, from a plasmid derived from pSI-1 (35). Plasmid pMMB1010 contains P*pen-mCherry* with a linked *kan* flanked by sequences from *cgeD* in the pGEMcat (35) backbone. This was integrated by single crossover into *cgeD*, selecting for chloramphenicol resistance. This construct was used in donor strains AB86, AB101, and AB110 (Table 1).

We also used a P*pen-mCherry* fusion at *cgeD* that is integrated by double crossover. This allele, Δ*cgeD1388*::[(P*pen-mCherry*) *cat*] is an insertion-deletion containing P*pen-mCherry* followed by *cat* (chloramphenicol resistance) inserted between base pairs 160 and 490 of the 1,278-bp *cgeD* open reading frame. The inserted genes are cooriented with *cgeCDE* in the *B. subtilis* chromosome. P*pen-mCherry* was obtained from *B. subtilis* strain MMB1023 containing *cgeD*::[(P*pen-mCherry*) *kan*] as a double crossover from pMMB1010, as described above. *cat* was obtained from pGEMcat (35). The Δ*cgeD1388*::[(P*pen-mCherry*) *cat*] was contained on plasmid pCAL1387 and was introduced into the *B. subtilis* chromosome by transformation and double-crossover homologous recombination. This construct was used in donor strain CAL1391 (Table 1).

(iv) ICE*Bs1*::[(P*pen-sspB*) *kan*] and *gfp-ssrA**. The allele Δ(*rapI-phrI*)*1366*::[(P*pen-sspB*) *kan*] [simply ICE*Bs1*::(P*pen-sspB*)] is an insertion-deletion, removing the region of ICE*Bs1* from 100 bp upstream of the *rapI* open reading frame through the stop codon of *phrI* (Fig. 1). *E. coli sspB* fused to the constitutive promoter P*pen* is inserted in this region, followed by *kan*. The inserted genes are cooriented with downstream *yddM* (Fig. 1). *sspB* with a ribosome-binding site was obtained from pKG1266 (29). *kan* was obtained from pGK67 (39). Δ(*rapI-phrI*)*1366*::[(P*pen-sspB*) *kan*] was constructed as a linear PCR product and introduced into ICE*Bs1* in the *B. subtilis* chromosome by transformation and homologous recombination.

gfp-ssrA* expressed from a constitutive promoter and integrated at *thrC* was described previously (29). Some strains containing insertions in *thrC* also require methionine to grow, likely due to the effects of the insertion at *thrC* on the adjacent *hom* gene, needed for methionine biosynthesis.

Media and growth conditions. *E. coli* and *B. subtilis* cells were grown in LB medium for routine cloning and strain constructions. Strains for experiments were grown in defined S7 minimal salts medium (containing 50 mM MOPS [morpholinepropanesulfonic acid]) supplemented with L-arabinose (1%), phenylalanine (40 μg/ml), tryptophan (40 μg/ml), threonine (200 μg/ml), and methionine (40 μg/ml), as needed. Xylose (1%) was added to induce expression from P*xyl-rapI*.

Live-cell imaging and mating conditions. Donors and recipients were colony purified from frozen (−80°C) stocks on LB plates with the appropriate antibiotic. Cells from a single colony were inoculated into liquid LB medium and grown to an optical density at 600 nm (OD$_{600}$) of ≈0.8 to 1. Cells were then diluted into defined minimal medium with arabinose as the carbon source to an OD$_{600}$ of ~0.02. After at least 3 to 4 generations (OD$_{600}$ of ~0.2), expression of *rapI*, from P*xyl-rapI*, was induced by addition of xylose to the donors. Cells were grown for another hour to allow for ICE*Bs1* gene expression and excision. Donors and recipients were mixed at a ratio of ~1 donor per 10 recipients at a concentration of ~10^8 cells per ml. Two microliters of cells were placed on a slice of agarose (1.5% UltraPure agarose; Invitrogen) dissolved in defined minimal growth medium. The approximate dimensions of the agarose slice were 0.25 mm in height by 15 mm in length by 5 mm in width.

The agarose slice was placed on a standard glass coverslip (VWR), with the cells between the agarose and the coverslip. The agarose slice (with coverslip) was placed in a homemade incubation chamber made by stacking three sealable Gene Frames (ABgene) and mounting them on a standard microscope slide (VWR). Two small pieces of filter paper soaked in water were placed in the edges of the chamber to prevent evaporation and drying of the agarose slice. We found that under these conditions, cells grew and mated successfully. The chamber was mounted on the motorized stage of a Nikon Ti-E inverted microscope placed in the temperature-controlled box (Nikon) at 37°C. Fluorescence was generated using a Nikon Intensilight mercury illuminator through appropriate sets of excitation and emission filters (filter set 49008 for mCherry and filter set 49002 for GFP; Chroma). Acquisition of images was performed using a CoolSnap HQ camera (Photometrics) and processed using NIS-Elements Advanced Research 3.10 software. Typically, 50 to 100 fields of cells of appropriate density were chosen for automated imaging, and images were captured every 30 min for up to 3 h.

ACKNOWLEDGMENTS

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