The *Bacillus subtilis* conjugative transposon ICE*Bs1* mobilizes plasmids lacking dedicated mobilization functions

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Abstract

Integrative and conjugative elements (ICEs, a.k.a., conjugative transposons) are mobile elements that are found integrated in a host genome and can excise and transfer to recipient cells via conjugation. ICEs and conjugative plasmids are found in many bacteria and are important agents of horizontal gene transfer and microbial evolution. Conjugative elements are capable of self-transfer, and also capable of mobilizing other DNA elements that are not able to self-transfer. Plasmids that can be mobilized by conjugative elements are generally thought to contain an origin of transfer (*oriT*), from which mobilization initiates, and to encode a mobilization protein (Mob, a relaxase) that nicks a site in *oriT* and covalently attaches to the DNA to be transferred. Plasmids that do not have both an *oriT* and a cognate *mob* are thought to be non-mobilizable. We found that *Bacillus subtilis* carrying the integrative and conjugative element ICE*Bs1* can transfer three different plasmids to recipient bacteria at high frequencies. Strikingly, none of the plasmids contain dedicated mobilization-*oriT* functions. Plasmid mobilization required conjugation proteins of ICE*Bs1*, including the putative coupling protein. In contrast, plasmid mobilization did not require the ICE*Bs1* conjugative relaxase or co-transfer of ICE*Bs1*, indicating that the putative coupling protein likely interacts with the plasmid replicative relaxase and directly targets the plasmid DNA to the ICE*Bs1* conjugation apparatus. These results blur the current categorization of mobilizable versus non-mobilizable plasmids and indicate that conjugative elements play an even more significant role in horizontal gene transfer than previously recognized.

48 **Introduction**

49 Integrative and conjugative elements (ICEs, also known as conjugative transposons)
50 are mobile elements found integrated in a host genome. Under certain conditions, ICEs
51 can excise, circularize, and transfer to recipient cells via conjugation. ICEs and
52 conjugative plasmids are found in many bacterial species and contribute to the
53 acquisition of new traits, including antibiotic resistance.

54 Conjugative elements encode components of a transmembrane conjugation (mating)
55 apparatus (often called the mating pore formation or Mpf complex) used to translocate
56 DNA from donor to recipient. They also encode a relaxase protein that processes the
57 element's DNA by nicking and covalently attaching to the element's origin of transfer
58 (*oriT*) creating a relaxosome complex. An element-encoded coupling protein interacts
59 with the relaxosome complex and the mating machinery to recruit (or couple) the
60 substrate DNA to the mating apparatus and to facilitate transfer of the relaxase and a
61 single strand of DNA to a recipient (1, 18, 42, 46, 49).

62 ICE*Bs1* (Fig. 1) is a conjugative transposon found integrated at the gene for tRNA-
63 Leu2 (*trnS-leu2*) in *Bacillus subtilis* (5, 10). ICE*Bs1* is particularly useful for the study of
64 conjugation. Several of the ICE*Bs1* genes are similar to genes in other ICEs, including
65 Tn*916* (8, 10, 41), the first conjugative transposon identified (17). ICE*Bs1* gene
66 expression is induced during the RecA-mediated SOS response or when the cell sensory
67 protein RapI is expressed and active (5). Analyses of ICE*Bs1* functions are facilitated by
68 the ability to induce ICE*Bs1* gene expression simply by overproduction of RapI from an
69 exogenous promoter, leading to excision of ICE*Bs1* from the chromosome of >90% of
70 the cells in a population (5, 32). Induction of gene expression also leads to nicking of
71 ICE*Bs1* by its relaxase (encoded by *nicK*) at a site in *oriT* (34). Nicking and subsequent
72 unwinding by a host-encoded helicase (PcrA) are required for conjugative transfer and

for autonomous plasmid-like replication of ICE*Bs1* (33). Several ICE*Bs1* genes encode proteins required for transfer from donor to recipient, and high mating efficiencies of 1-10% transconjugants per donor (7, 32, 34) are obtained with *B. subtilis* recipients that do not contain ICE*Bs1* (4). ICE*Bs1* can also transfer to other Gram-positive bacterial species (5).

Many transposons and plasmids are not capable of self-transfer to recipient cells, but can be mobilized by the conjugation machinery of conjugative elements (19, 46). These mobilizable transposons (a.k.a., integrative mobilizable elements) and plasmids typically contain a dedicated *oriT* and encode a cognate relaxase protein (Mob) for mobilization. Mobilizable transposons excise from the genome prior to transfer, whereas the Mob/*oriT*-containing plasmids are autonomous genetic elements with their own replication functions (Rep and *ori*) that are separate from the transfer functions (Mob and *oriT*). Similar to the relaxase and *oriT* of conjugative elements, Mob and *oriT* of mobilizable elements are needed to create a relaxosome that then interacts with a coupling protein that will function to transfer the mobilizable DNA. Coupling proteins are typically encoded by the conjugative element, but can also be encoded by the mobilizable element (11, 46).

We found that *B. subtilis* ICE*Bs1* donors are capable of efficiently mobilizing three different plasmids, pC194, pBS42, and pHP13 (Fig. 2) that replicate by rolling circle replication (RCR) and are typically described as non-mobilizable (30, 43, 44, 46), although very low efficiencies of mobilization of pC194 and a relative of pBS42 by the conjugative transposon Tn*916* have been reported (38, 45). The three plasmids used here contain an origin of replication, but are not known to contain an origin of transfer or mobilization functions. For pBS42, we found that the plasmid replicative relaxase was required for plasmid mobilization. Our results indicate that, similar to the ICE*Bs1*

relaxase (33), the plasmid replicative relaxases may function in both replication and DNA transfer. The plasmid relaxase may facilitate DNA transfer by interacting with the conjugation machinery of ICE*Bs1*. These findings indicate that many more plasmids than previously thought might be readily mobilized and disseminated by conjugative elements.

Materials and Methods

Strains and alleles. *B. subtilis* strains were either cured of ICE*Bs1* (ICE*Bs1*^{0}) (5) or carried one of several derivatives of ICE*Bs1* (Fig. 1B-E). All *B. subtilis* strains are listed in Table 1 and are derived from the lab strain JH642 (*trp phe*; not shown in genotypes in Table 1). Chromosomal alleles and plasmid DNA were introduced into *B. subtilis* by natural transformation (24). Important alleles and plasmids are described below.

Strains that were used as donors in conjugation experiments contained Pspank(hy)-*rapI* integrated at *amyE*, *amyE*::{(Pspank(hy)-*rapI*) *spc*}, for IPTG-inducible overproduction of RapI that causes induction of ICE*Bs1* gene expression (5). To monitor transfer of ICE*Bs1*, a derivative {ICE*Bs1* Δ(*rapI-phrI*)*342*::*kan*}) (Fig 1B) encoding resistance to kanamycin was typically used, as previously described (5).

ICE*Bs1* Δ(*conG-yddM*)319::*kan* (Fig 1C) is an insertion-deletion that removes several essential conjugation genes and replaces them with the *kan* cassette. The mutation removes 661 bp from the the 3' end of *conG* (of the 2,445 bp *conG* open reading frame), 782 bp from the 5' end of *yddM* (of the 939 bp *yddM* open reading frame) and everything between the two genes (34).

ICE*Bs1* Δ*nicK306* (Fig. 1D) is a 519 bp deletion that disrupts the ICE*Bs1* conjugative relaxase encoded by *nicK*, but leaves a functional *oriT* (34).

For this study, a 1,113 bp unmarked, in-frame ICE*Bs1* deletion Δ*conQ848* (Fig. 1E) was constructed that fuses the first two codons to the last 107 codons of *conQ* (of the 1440 bp *conQ* open reading frame), using the same method described for construction of Δ*nicK306* (34). The Δ*conQ848* allele does not appear to affect the function of *oriT*, which likely overlaps the 3' end of *conQ* and the 5' end of *nicK* (34) (Fig. 1A).

Four truncated derivatives of ICE*Bs1* were inserted at *thrC* to test for complementation of the Δ*conQ848* mutant (Fig. 1F-I). *thrC229*::{(ICE*Bs1*-303 Δ(*conQ-attR*)::*tet*) *mls*} (Fig. 1I) and *thrC229*::{(ICE*Bs1*-1637 Δ(*conQ-attR*)::*cat*) *mls*} (Fig. 1H) did not complement the Δ*conQ848* deletion as they only contain ICE*Bs1* genes upstream of *conQ*, from *int* to *ydcP* (Fig. 1H-I). In contrast, *thrC229*::{(ICE*Bs1*-304 Δ(*ydcS-attR*)::*tet*) *mls*} (Fig. 1F) and *thrC229*::{(ICE*Bs1*-337 Δ(*nicK-attR*)::*cat*) *mls*} (Fig. 1G) both contain wild type *conQ* and complement the Δ*conQ848* mutant. These ICE*Bs1* derivatives are integrated at *thrC* and are unable to excise due to loss of *attR*. Δ(*conQ-attR*)::*tet* and Δ(*conQ-attR*)::*cat* remove sequences starting with the 109th codon of *conQ*. Δ*nicK-attR*::*cat* removes sequences starting immediately downstream of the *conQ* stop codon. Δ(*ydcS-attR*)::*tet* removes sequences starting immediately downstream of the *nicK* stop codon. *tet* was from pDG1513 (22). *cat* was from pGEM-cat (50). All four alleles at *thrC* were derived from *thrC229*::{(ICE*Bs1* Δ(*rapI-phrI*)*342*::*kan*) *mls*} (34) and were constructed using the long-flanking homology PCR method (48) or one-step isothermal DNA assembly (20). Introduction of the Δ*conQ-attR*, Δ*nicK-attR* and Δ*ydcS-attR* alleles into *thrC229*::{(ICE*Bs1* Δ*rapI-phrI*)342::*kan*) *mls*} yielded tetracycline-resistant or chloramphenicol-resistant, kanamycin-sensitive transformants due to replacement of the Δ*rapI-phrI*::*kan* insertion.

Strain CAL89 is streptomycin-resistant (*str-84*) and cured of ICE*Bs1* (ICE*Bs1*^{0}) and was used as the recipient in mating experiments. It also contains a *comK*::*spc* null

mutation that prevents acquisition of DNA by transformation (natural genetic competence). Results from mobilization experiments with different alleles of ICE*Bs1* are summarized in Fig. 1.

Plasmids. Three different plasmids were used, pC194, pBS42, and pHP13 (Fig. 2). All three plasmids use rolling circle replication and express chloramphenicol-resistance in *B. subtilis*. pC194 is 2.9 kb and from *Staphylococcus aureus* (26) (Fig. 2A). pBS42 (6) (Fig. 2B) and pHP13 (23) (Fig. 2C) are 4.8 kb shuttle vectors designed to replicate in *Escherichia coli* and *B. subtilis*. pBS42 has replicons from pBR322 (*E. coli*) and pUB110 (*S. aureus*/*B. subtilis*). pHP13 has replicons from a pUC plasmid (*E. coli*) and pTA1060 (*B. subtilis*). Although pUB110 from *S. aureus* and pTA1060 from *B. subtilis* are mobilizable plasmids, their Mob/*oriT* functions are not present on pBS42 and pHP13 (9, 37, 44). The *'mobU* sequence in pBS42 (Fig. 2B) is a non-functional portion of the 3' end of the *mobU* gene from pUB110.

We constructed two derivatives of pBS42 to test for the requirement of the replicative relaxase in conjugative transfer. In one plasmid, pCAL1738, the plasmid relaxase gene *repU* is disrupted at the *Nsi*I site (Fig. 2B). To allow for plasmid replication in the absence of functional RepU, the inserted DNA fragment contains the replication origin (*oriN*) and the cognate replication initiator gene (*repN*) from plasmid pLS32 of *B. subtilis* subsp. natto (25, 35, 47). *oriN-repN* support bi-directional theta replication (25, 47). As a control, pCAL1737 contains the intact origin of replication and relaxase gene from pBS42 and the *oriN-repN* fragment is inserted in the truncated *mob* (*'mob*) in pBS42 at the *Nsi*I site (Fig. 2B). *B. subtilis* strain CAL1749 contains the control plasmid pCAL1737 and forms smaller colonies and grows 15-20% slower than normal in LB liquid medium supplemented with chloramphenicol. The presence of two active

replicons, *oriU-repU* and *oriN-repN*, on pCAL1737 may affect plasmid stability and cell growth.

pCAL1737 and pCAL1738 were constructed using one-step isothermal DNA assembly (20) to piece together three DNA fragments: the 3.13 kb *Nsi*I-*Nsi*I fragment of pBS42; a PCR product with the 1.64 kb *Nsi*I-*Nsi*I fragment of pBS42; and a PCR product with the 1.22 kb *oriN-repN* region. The assembly reactions were designed to yield plasmids identical to those generated by ligation of the *oriN-repN* fragment into full length pBS42, linearized at the *Nsi*I restriction site in *repU* or in *'mob* (Fig. 2B). The *oriN-repN* sequence was obtained from pDL110 (35) and transcription of *repN* was co-oriented with the disrupted *'mob* and *repU* reading frames. The 1.22 kb insert includes 251 bp upstream and 112 bp downstream of the 861 bp *repN* open reading frame.

Conjugation and mobilization assays. Cells were grown at 37°C in LB medium, supplemented with chloramphenicol when necessary to select for maintenance of the plasmids. Donor cells were induced for ICE*Bs1* gene expression and conjugation by addition of IPTG for 1 hour. Mixtures of donor and recipient cells were filtered onto nitrocellulose membranes and incubated on agar containing minimal salts as described (32). Cells recovered from the filters after mating were plated onto solid media to select for transconjugants.

Transconjugants containing ICE*Bs1* (*kan*) were resistant to kanamycin (from ICE*Bs1*) and streptomycin (from the recipient). Transconjugants containing a plasmid were resistant to chloramphenicol (from a plasmid) and streptomycin (from recipient). Mating efficiencies were calculated as the percent of transconjugant colony forming units (CFU) recovered per donor CFU present in the original mating mixture plus or minus the standard deviation.

197 **Results**

198 In the course of defining the ICE*Bs1 oriT* (34), we found that plasmids previously
199 described as non-mobilizable appeared to be mobilized by ICE*Bs1*. Based on these
200 preliminary findings, we characterized the mobilization of three plasmids, pC194,
201 pBS42, and pHP13, by ICE*Bs1*. All three plasmids (Fig. 2) use rolling circle replication
202 and express chloramphenicol-resistance in *B. subtilis*. These plasmids do not have a
203 known *oriT* and none contain an intact *mob* gene (21, 30, 44, 46). Thus, pC194, pBS42 and
204 pHP13 are typically described as non-mobilizable.

205 **Mobilization of plasmids by ICE*Bs1***

206 We found that all three plasmids were mobilized by ICE*Bs1* (Table 2, line 1; Fig. 1B).
207 In these experiments, donor strains containing ICE*Bs1* marked with a gene encoding
208 resistance to kanamycin (*kan*), with or without the indicated plasmid (all encoding
209 chloramphenicol resistance), were grown in rich medium (LB) and ICE*Bs1* gene
210 expression was induced by ectopic expression of *rapI* from a fusion to a LacI-
211 repressible-IPTG-inducible promoter (Pspank(hy)-*rapI*) for one hour. Production of
212 active RapI induces ICE*Bs1* gene expression, excision, and conjugation ability (5). The
213 recipients did not contain ICE*Bs1* (ICE*Bs1*^{0}) and were defective in the development of
214 genetic competence (*comK*::*spc*) and hence non-transformable. Activated donors were
215 mixed with recipient cells at a ratio of ~1:1 and mating efficiencies were determined
216 (Materials and Methods).

217 pBS42 and pC194 were transferred with frequencies of ~3% plasmid-containing
218 transconjugants per donor and pHP13 was transferred with a frequency of ~0.07%
219 plasmid-containing transconjugants per donor. Plasmid transfer was dependent on the
220 presence of ICE*Bs1* in the donor as there was no detectable acquisition of
221 chloramphenicol-resistance from cells that did not contain ICE*Bs1* (Table 2, line 2).

Plasmid transfer required components of the ICE*Bs1* mating machinery. An ICE*Bs1* mutant that is missing genes from *conG* to *yddM* {Δ(*conG-yddM*)*319*::*kan*} (Fig. 1C) is defective in ICE*Bs1* conjugation (34). This mutant was incapable of mobilizing all three plasmids tested (Table 2, line 3; Fig. 1C). Together, these results indicate that ICE*Bs1* can mobilize the three plasmids pHP13, pBS42, and pC194, and that mobilization requires at least some of the ICE*Bs1* mating components.

Transfer of ICE*Bs1* itself was not affected by the presence of any of the three plasmids tested. The mating efficiency of ICE*Bs1* from plasmid-free donors was approximately 6% (Table 3, line 1), similar to that reported previously (32). The mating efficiencies of ICE*Bs1* from plasmid-containing donors (Table 3, lines 2-4) were indistinguishable from that from the plasmid-free strain.

Acquisition of both ICE*Bs1* and a plasmid by a single recipient

We analyzed transconjugants that acquired a plasmid to determine the frequency that they also acquired ICE*Bs1*. In experiments analogous to those described above (Table 2), single colonies of plasmid-containing transconjugants (chloramphenicol-resistant) were picked and tested for resistance to kanamycin, indicative of acquisition of ICE*Bs1*. Of the transconjugants acquiring pBS42, pC194, or pHP13, 19%, 45%, and 35%, respectively (of ≥200 transconjugants tested for each plasmid), also acquired ICE*Bs1*. If transconjugants that acquired both the plasmid and ICE*Bs1* received the elements from a single donor, then these relatively high frequencies of co-transfer indicate that once a mating pair is formed, it is likely that both elements will be transferred.

In these mating experiments, the ratio of donor to recipient was approximately 1:1, and it seemed possible a single transconjugant could have acquired ICE*Bs1* from one donor and a plasmid from another. If so, then the frequency of co-transfer should drop

247 if the ratio of donor to recipient is reduced. We repeated the mating experiments
248 described above using a donor to recipient ratio of 1:100, rather than 1:1. Of the
249 transconjugants that acquired pBS42, pC194, or pHP13, 20-60% (of 100 transconjugants
250 tested for each plasmid) also acquired ICE*Bs1*. Furthermore, of the transconjugants that
251 acquired ICE*Bs1*, between 2-20% also acquired pBS42, pC194, or pHP13 (of ≥100
252 transconjugants tested for each plasmid). Together, these results indicate that a single
253 donor is capable of transferring both ICE*Bs1* and a plasmid and that the relatively high
254 frequency of co-transfer indicates that once a mating pair is formed, it is likely that both
255 elements will be transferred.

256 **Plasmid mobilization does not require the ICE*Bs1* relaxase NicK or transfer of**
257 **ICE*Bs1***

258 There are several mechanisms by which plasmids and transposons can be mobilized.
259 Mobilizable elements typically contain an *oriT* and a gene (*mob*) that encodes a
260 conjugative relaxase (40, 46). The *mob* gene product nicks a site in *oriT* and is required
261 for mobilization. Plasmids (or transposons) lacking *mob* functions can sometimes be
262 mobilized by cross-recognition of an *oriT* site on the mobilizable element {e.g., (13)}.
263 Plasmids (or transposons) lacking both *mob* and *oriT* functions can sometimes be
264 mobilized when the plasmid integrates into a conjugative element and is transferred in
265 cis as a co-integrate with the conjugative element {e.g., (12)}. In these situations,
266 mutations in the relaxase gene of the conjugative element prevent transfer of the
267 conjugative element and also prevent mobilization of the plasmid (or transposon). Since
268 the plasmids used here do not contain *mob* and a cognate *oriT*, we tested whether the
269 ICE*Bs1* relaxase was required for mobilization of these plasmids.

270 Using an ICE*Bs1 nicK* null mutant, we found that plasmid mobilization was
271 independent of the ICE*Bs1* relaxase and of ICE*Bs1* transfer. Although the ICE*Bs1*

relaxase encoded by *nicK* is essential for ICE*Bs1* transfer (34), it was not required for plasmid mobilization. There was no detectable decrease in mobilization of pHP13, pBS42, and pC194 from ICE*Bs1* donors lacking *nicK* (Table 4, lines 1, 2; Fig. 1D). In the same experiment, there was no detectable transfer of ICE*Bs1* (<0.00002% transconjugants per donor), as previously reported (34). These results indicate that the ICE*Bs1* relaxase NicK is not needed for plasmid mobilization. Thus, transfer is not occurring by cross-recognition of an *oriT* on the mobilized plasmids by the ICE*Bs1* relaxase. Furthermore, since the relaxase mutant is incapable of transferring ICE*Bs1*, these results demonstrate that plasmid mobilization does not require co-transfer with ICE*Bs1*. Instead, plasmid mobilization by ICE*Bs1* is likely mediated by direct transfer of the plasmid DNA through the ICE*Bs1* conjugation machinery.

The plasmid replicative relaxase RepU is required for pBS42 plasmid mobilization

Since ICE*Bs1*-mediated plasmid mobilization did not require the ICE*Bs1*-conjugative relaxase, it seemed possible that mobilization would require the plasmid replicative relaxase. To test this, we disrupted the relaxase gene (*repU*) of pBS42 by inserting a DNA fragment into the *Nsi*I site in *repU* (Fig. 2B). Since *repU* is needed for replication of pBS42, the inserted fragment contained an origin of replication (*oriN*) and the gene (*repN*) encoding the cognate replication initiator. *oriN-repN* support bi-directional theta replication (25, 47). As a control, we also inserted the *oriN-repN* fragment into the NsiI site in the fragment of *mobU* that is present on pBS42 (Fig 2B).

We found that the replicative relaxase of pBS42 is needed for ICE*Bs1*-mediated mobilization of pBS42. The plasmid with *repU* disrupted, pCAL1738 {pBS42 *repU*::(*oriN-repN*)} was not detectably mobilized by ICE*Bs1* (<10^{-5} % plasmid-containing transconjugants per donor). Transfer of ICE*Bs1* was normal from these donors (~6% ±

297 2% transconjugants/donor) indicating that the ICE*Bs1* transfer machinery was
298 functional. In contrast, the control plasmid pCAL1737 {pBS42 *'mob*::(*oriN-repN*)} in
299 which *repU* is not disrupted was still mobilized (~0.7% ± 0.1% plasmid-containing
300 transconjugants/donor), indicating that the presence of *oriN-repN* did not prevent
301 mobilization. Based on these results, we conclude that the plasmid replicative relaxase
302 RepU is required for pBS42 mobilization and is most likely functioning as both a
303 replicative and a conjugative relaxase.

304 **The putative coupling protein of ICE*Bs1* is required for conjugation and plasmid**
305 **mobilization**

306 Transfer of conjugative elements typically requires a coupling protein, an ATPase
307 that interacts with the relaxosome (relaxase attached to DNA) and the conjugation
308 apparatus, coupling the two complexes and enabling transfer of the relaxase and the
309 covalently attached substrate DNA (36). The coupling proteins typically have an FtsK-
310 like motor domain needed for function and are encoded adjacent to or very near the
311 gene encoding the relaxase (28, 39, 46). *conQ* (previously called *ydcQ*) of ICE*Bs1* (Fig. 1)
312 encodes the putative coupling protein (28).

313 We found that *conQ* was required for transfer of ICE*Bs1*. We made an in-frame
314 deletion in *conQ* and integrated this into ICE*Bs1* (Fig. 1E). Following overproduction of
315 RapI and induction of ICE*Bs1*, the *conQ* null mutant was unable to transfer ICE*Bs1* to
316 recipients ($<10^{-5}$ % transconjugants per donor, CAL848) (Fig. 1E).

317 We also found that the *conQ* deletion does not significantly affect *oriT* function nor
318 was it polar on the downstream genes needed for conjugation. The inability of the *conQ*
319 mutant to transfer was largely relieved when *conQ*, along with all the ICE*Bs1* genes
320 upstream of *conQ* were expressed in trans (Fig. 1G) (mating efficiency of ~1%
321 transconjugants per donor, JT339). The upstream genes were provided in addition to

322 *conQ* because we commonly find that complementation (and presumably protein
323 production) is more efficient when upstream genes are included {e.g., (7)}. The control
324 that provided all the upstream genes, but not a functional *conQ*, was unable to restore
325 conjugation to the ICE*Bs1* Δ*conQ* mutant (<10^{-5} % transconjugants per donor, JT338; Fig.
326 1H). We conclude that *conQ* is required for ICE*Bs1* conjugation, that the Δ*conQ*
327 mutation is not polar on downstream conjugation genes, and that it does not affect *oriT*
328 function or nicking of *oriT* by the ICE*Bs1* relaxase.

329 *conQ* was also required for mobilization of pHP13, pBS42 and pC194, none of which
330 encode their own dedicated coupling protein. When the *conQ* null mutant was used as
331 donor, there was no detectable transfer of any of the three plasmids to recipient cells
332 (Table 4, line 3; Fig. 1E). The inability of the *conQ* mutant to mobilize the plasmids was
333 largely relieved when *conQ*, along with all the ICE*Bs1* genes upstream of and one gene
334 (*nicK*) downstream of *conQ* were expressed from an ectopic locus (Table 4, line 4; Fig.
335 1F). The control that provided all the upstream genes, but not a functional *conQ*, was
336 unable to restore mobilization to the *conQ* mutant (Table 4, line 5; Fig. 1I). Since *nicK* is
337 not needed for plasmid mobilization (Table 4, line 2), these results indicate that the
338 defect in mobilization was due to loss of *conQ* and not a polar effect on downstream
339 genes, and that the putative coupling protein of ICE*Bs1* is likely needed to recruit a
340 plasmid-associated relaxasome complex to the ICE*Bs1* mating machinery.
341
342
343 **Discussion**
344 Experiments described here indicate that ICE*Bs1* of *B. subtilis* is capable of
345 mobilizing at least three different plasmids, pC194, pBS42, and pHP13. Mobilizable
346 plasmids are thought to require dedicated mobilization functions; a conjugative

relaxase (Mob) and a cognate *oriT*, that are separate from the replication functions. None of the plasmids used here have dedicated mobilization functions. Mobilization by ICE*Bs1* requires the ICE*Bs1* conjugation machinery and the putative coupling protein, ConQ. In characterized systems, the coupling protein is needed to link the relaxasome complex, which contains a conjugative relaxase attached to the 5' end of a single strand of DNA, to the conjugation machinery (14, 36, 46). Plasmid mobilization by ICE*Bs1* did not require the ICE*Bs1* relaxase encoded by *nicK* indicating that mobilization was not due to cross-recognition of a cryptic *oriT* on the plasmids or co-transfer of ICE*Bs1* and plasmid DNA. Mobilization of pBS42 required the replicative relaxase RepU of pBS42, indicating that this replicative relaxase can also function as a conjugative relaxase. These findings have practical applications for the characterization of ICE*Bs1* genes and the genetic manipulation of heterologous bacteria. In addition, our findings indicate that there is more potential for horizontal gene transfer of non-conjugative plasmids than previously recognized.

Practical applications of plasmid mobilization for genetic studies

Plasmid mobilization can be used to help characterize genes involved in conjugation. For example, some of the genes required for conjugation of ICE*Bs1* were needed for plasmid mobilization, including at least some of the genes encoding the conjugation machinery and the putative coupling protein. Other functions required for ICE*Bs1* conjugation, including the relaxase and the ability to excise from the chromosome (data not shown), were not needed for plasmid mobilization. These differences between transfer of ICE*Bs1* and mobilization of plasmids can be used to help delineate the steps at which different ICE*Bs1* or host gene products act.

Plasmid mobilization by ICE*Bs1* could also be a useful and efficient way to introduce DNA to strains that are difficult to transform. ICE*Bs1* can be transferred to

other organisms (5), and at least some of the plasmids used here are capable of replicating in other organisms. Cloning and genetic manipulations could be done with plasmids in *E. coli* or *B. subtilis* and then the desired plasmids could be mobilized from *B. subtilis* by ICE*Bs1* into other Gram-positive organisms that are suitable recipients for conjugation. Other conjugative elements have been used for plasmid mobilization (16), but the high efficiencies of mobilization by ICE*Bs1* and the ability to easily manipulate *B. subtilis* make mobilization by ICE*Bs1* an attractive system for use with Gram positive bacteria.

Functional relationship between replicative and conjugative relaxases

Duplication of pC194, pBS42, and pHP13 requires a plasmid origin of replication (*ori*) and a cognate replicative relaxase (Rep) that enables rolling circle replication (29). The first steps in production of a substrate for conjugation and for rolling circle replication are similar. Both require a relaxase (a conjugative or replicative relaxase) that nicks a site in an origin (origin of transfer or origin of replication). The relaxase becomes covalently attached to the cognate origin and serves to mark the site for transfer or replication. Following nicking, a helicase is required for unwinding the double-stranded DNA substrate for either conjugation or replication. Many rolling circle replicating plasmids in Gram positive organisms use the host-encoded helicase PcrA for replication (29). Similarly, ICE*Bs1* uses PcrA both for replication and for conjugation, although replication is not required for conjugation (33).

The conjugative relaxases are similar to the replicative relaxases, although they are generally thought to belong to different sub-types of the relaxase family (19, 27, 31). However, recent work demonstrated that the ICE*Bs1* conjugative relaxase NicK also functions as a replicative relaxase using a single origin for both conjugative transfer and replication (33). Results presented here indicate that at least three different replicative

relaxases, from pC194, pBS42 (pUB110), and pHP13 (pTA1060), likely also function as conjugative relaxases. This is in contrast to the prevalent view that mobilizable plasmids have separate replication (Rep/*ori*) and mobilization (Mob/*oriT*) functions (21, 46).

Previous studies found that certain plasmids from *B. thuringiensis* or *B. subtilis* could be mobilized in the absence of mobilization functions (2, 3, 38, 45). For example, the conjugative transposon Tn*916* can mobilize pC194 from *B. subtilis* to *B. thuringiensis* at a low frequency (38), even though pC194 is still described as not being mobilizable. In addition, mobilization of pUB110 by the conjugative transposon Tn*916* from *B. subtilis* into *B. thuringiensis* did not require the pUB110 *mob* gene (45). It was proposed that pUB110 might contain a Tn*916*-like *oriT* that could be recognized by the Tn*916* conjugative relaxase. Based on results presented here, we think a more likely possibility is that the replicative relaxases from pUB110 and pC194 also function as conjugative relaxases and that plasmid mobilization by Tn*916* is likely independent of the Tn*916* relaxase.

Likely mechanism of plasmid mobilization in the absence of dedicated mobilization functions

Plasmid mobilization mediated by ICE*Bs1* probably occurs by a mechanism similar to transfer of ICE*Bs1*. We propose that the plasmid replicative relaxasome, consisting of the replicative relaxase attached to plasmid DNA, and perhaps associated with the helicase PcrA, interacts with the putative coupling protein from ICE*Bs1*, ConQ. This interaction might be analogous to the interactions between coupling proteins and the cognate relaxasomes from ICEs and conjugative plasmids (14, 15, 36). The coupling protein ConQ would then recruit the plasmid relaxasome to the ICE*Bs1* conjugation machinery at the cell membrane. Interactions between the coupling protein and the

helicase PcrA and/or the target DNA could also be involved, either in addition to or instead of interactions with the replicative relaxase. However, the lower efficiency of pHP13 mobilization by ICE*Bs1* as compared to pC194 and pBS42 mobilization argues against mobilization primarily occurring through interactions between the coupling protein and the helicase PcrA. We postulate that the specificity comes from protein-protein interaction between the relaxase and the coupling protein. In this case, the lower efficiency of pHP13 mobilization may be due to a lower affinity of the pHP13 replicative relaxase for the ICE*Bs1* coupling protein and for components of the ICE*Bs1* conjugation machinery

Evolutionary implications

The evolutionary and functional relationship between conjugative and replicative relaxases likely enables direct mobilization of certain rolling circle-replicating plasmids by conjugative elements. Two lines of evidence blur the distinction between conjugative and replicative relaxases. 1) At least three plasmids that are mobilized by the ICE*Bs1* conjugation machinery encode a single relaxase that may mediate both replication and mobilization. 2) The relaxase from ICE*Bs1* is clearly bifunctional, serving as a conjugative relaxase and a replicative relaxase for ICE*Bs1* conjugation and rolling circle replication (33). We suspect that many, and perhaps most, conjugative relaxases can function as replicative relaxases with the cognate *oriT* functioning as an origin of replication. Similarly, many replicative relaxases may function in conjugation. The key distinguishing feature between conjugative and non-conjugative replicative relaxases might be the ability to interact with a coupling protein, necessary for conjugation but not replication.

The persistence of plasmids in bacterial populations is likely due to benefits they confer on the host cell or to their efficient dissemination to new hosts by horizontal

transfer. Otherwise, the burden placed on the host by the plasmid is thought to result in loss of the plasmid. Based on the lack of a *mob* gene, approximately 60% of 1,730 sequenced plasmids are inferred to be non-mobilizable (46). Because of this inference, it was proposed that persistence of many of these "non-mobilizable" plasmids is due to unknown benefits conferred upon the host (46). The ability of ICE*Bs1* to mobilize three plasmids lacking dedicated *mob* functions indicates that many "non-mobilizable" plasmids may in fact be mobilizable. This could account for the persistence of so many "non-mobilizable" plasmids, indicating that the impact of conjugation on plasmid mobilization and persistence may be much greater than previously thought.

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603 **Table 1. *B. subtilis* strains**[a]

604

Strain	Relevant genotype (reference)
CAL13	ICE*Bs1*^{0} *amyE*::{(Pspank(hy)-*rapI*) *spc*} (34)
CAL89	ICE*Bs1*^{0} *comK*::*spc str-84* (5)
CAL306	Δ*nicK306* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*} (34)
CAL421	Δ(*conG-yddM*)*319*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*} (34)
CAL848	Δ*conQ848* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}
CAL1034	Δ(*conG-yddM*)*319*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pHP13 (*cat mls*)
CAL1392	ICE*Bs1*^{0} *amyE*::{(Pspank(hy)-*rapI*) *spc*}; pBS42 (*cat*)
CAL1393	Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pBS42 (*cat*)
CAL1395	Δ*nicK306* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pBS42 (*cat*)
CAL1396	Δ(*conG-yddM*)*319*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pBS42 (*cat*)
CAL1397	ICE*Bs1*^{0} *amyE*::{(Pspank(hy)-*rapI*) *spc*}; pHP13 (*cat mls*)
CAL1398	Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pHP13 (*cat mls*)
CAL1400	Δ*nicK306* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pHP13 (*cat mls*)
CAL1532	ICE*Bs1*^{0} *amyE*::{(Pspank(hy)-*rapI*) *spc*}; pC194 (*cat*)
CAL1533	Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pC194 (*cat*)
CAL1535	Δ*nicK306* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pC194 (*cat*)
CAL1548	Δ(*conG-yddM*)*319*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pC194 (*cat*)
CAL1590	Δ*conQ848 thrC229*::{(ICE*Bs1-303* Δ*conQ-attR*::*tet*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}
CAL1591	Δ*conQ848* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pBS42 (*cat*)
CAL1592	Δ*conQ848* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pC194 (*cat*)
CAL1593	Δ*conQ848 thrC229*::{(ICE*Bs1-304* Δ*ydcS-attR*::*tet*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pBS42 (*cat*)
CAL1594	Δ*conQ848 thrC229*::{(ICE*Bs1-304* Δ*ydcS-attR*::*tet*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pC194 (*cat*)
CAL1595	Δ*conQ848 thrC229*::{(ICE*Bs1-303* Δ*conQ-attR*::*tet*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pBS42 (*cat*)

CAL1596	Δ*conQ848 thrC229*::{(ICE*Bs1-303* Δ*conQ-attR*::*tet*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pC194 (*cat*)
CAL1597	Δ*conQ848* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pHP13 (*cat mls*)
CAL1598	Δ*conQ848 thrC229*::{ICE*Bs1-304* Δ*ydcS-attR*::*tet*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pHP13 (*cat mls*)
CAL1599	Δ*conQ848 thrC229*::{ICE*Bs1-303* Δ*conQ-attR*::*tet*) *mls* Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*} }; pHP13 (*cat mls*)
CAL1749	Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}; pCAL1737 {pBS42 *cat 'mob*::(*oriN-repN*)}
CAL1751	Δ*(rapI-phrI)342::kan amyE::{(Pspank(hy)-rapI) spc};* pCAL1738 {pBS42 *cat repU*::(*oriN-repN*)}
JMA168	Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*} (5)
JT298	Δ*conQ848 thrC229*::{(ICE*Bs1-304* Δ*ydcS-attR*::*tet*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}
JT338	Δ*conQ848 thrC229*::{ICE*Bs1-1637* Δ*conQ-attR*::*cat*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}
JT339	Δ*conQ848 thrC229*::{ICE*Bs1-337* Δ*nicK-attR*::*cat*) *mls*} Δ(*rapI-phrI*)*342*::*kan amyE*::{(Pspank(hy)-*rapI*) *spc*}

[a] Strains are derived from laboratory strain JH642 and contain *trpC* and *pheA* mutations.

608 **Table 2. Mobilization of plasmids by ICE*Bs1*.**

609

	ICE*Bs1* in donor[a]	Plasmid mobilization (%)[b]		
		pBS42	pC194	pHP13
1	Δ(*rapI-phrI*)::*kan*	2.8 ± 1.6	2.7 ± 1.9	0.068 ± 0.04
2	None	<0.00002	<0.00002	<0.00002
3	Δ(*conG-yddM*)::*kan*	<0.00002	<0.00002	<0.00002

610

611 [a] Donor strains contained the indicated plasmid (chloramphenicol resistant) and
612 *amyE*::{Pspank(hy)-*rapI spc*} for overproduction of RapI to induce ICE*Bs1*. Donor strains
613 contained pBS42, pC194, and pHP13 and ICE*Bs1* Δ(*rapI-phrI*)*342*::*kan* (CAL1393,
614 CAL1533, and CAL1398, respectively); no ICE*Bs1* (CAL1392, CAL1532 and CAL1397,
615 respectively); and ICE*Bs1* Δ(*conG-yddM*)::*kan* (CAL1396, CAL1548 and CAL1034,
616 respectively).

617

618 [b] The recipient strain in the mating experiments was CAL89 (ICE*Bs1*^{0} *comK*::*spc str*).
619 Values for the Δ(*rapI-phrI*)::*kan* donors are the means from 9 (pBS42), 4 (pC194), and 5
620 (pHP13) independent mating assays. Mating assays with donors defective for plasmid
621 mobilization yielded no detectable transconjugants (<0.00002%) in at least two
622 independent experiments. Plasmid mobilization was calculated as percent Cm^R Str^R
623 transconjugant CFU per donor CFU in the original mating mixture plus or minus the
624 standard deviation.

625

626

Table 3. Transfer of ICE*Bs1* is not affected by the presence of plasmids.

	Plasmid (strain)[a]	Percent transfer of ICE*Bs1*[b]
1	None (JMA168)	6.3 ± 3.5
2	pBS42 (CAL1393)	9.9 ± 5.1
3	pC194 (CAL1533)	7.0 ± 3.6
4	pHP13 (CAL1398)	7.3 ± 3.1

[a] Donor strains contained the indicated plasmid, ICE*Bs1* with the Δ(*rapI-phrI*)*342*::*kan* allele and *amyE*::{Pspank(hy)-*rapI spc*} for overproduction of RapI to induce ICE*Bs1*.

[b] The recipient strain in the mating experiments was CAL89 (ICE*Bs1*^{0} *comK*::*spc str*). Transconjugants containing ICE*Bs1* were selected as resistant to kanamycin (from ICE*Bs1*) and streptomycin (from the recipient). Values for percent transfer of ICE*Bs1* are the means from 5 independent mating assays (9 for pBS42) and are calculated as the percent Kan^R Str^R colony-forming units (CFU) per donor CFU in the original mating mixture plus or minus the standard deviation.

Table 4. Plasmid mobilization does not require ICE*Bs1* nickase NicK, but does require the putative coupling protein ConQ.

	ICE*Bs1* in donor[a]	Plasmid mobilization (%)[b]		
		pBS42 (n)	pC194 (n)	pHP13 (n)
1	Δ(*rapI-phrI*)::*kan*	2.8 ± 1.6 (9)	2.7 ± 1.9 (4)	0.068 ± 0.04 (5)
2	Δ*nicK306*	3.6 ± 2.0 (4)	3.2 ± 0.64 (2)	0.15 ± 0.12 (4)
3	Δ*conQ848*	<0.00005 (4)	<0.00005 (3)	<0.00005 (2)
4	Δ*conQ848*/*thrC*::*conQ*$^+$	2.7 ± 1.8 (2)	8.8 ± 5.1 (2)	0.094 ± 0.02 (2)
5	Δ*conQ848*/*thrC*::*conQ*Δ	<0.00005 (2)	<0.00005 (2)	<0.00005 (2)

[a] Donor strains contained the indicated plasmid (chloramphenicol-resistant), *amyE*::{(Pspank(hy)-*rapI*) *spc*}, the ICE*Bs1* Δ(*rapI-phrI*)*342*::*kan* allele (present in all donors but not indicated in lines 2-5), mutations in *nicK* or *conQ* (lines 2-5), and trans-acting ICE*Bs1* genes at *thrC* (lines 4 and 5). Donor strains contained pBS42, pC194, and pHP13 and: ICE*Bs1* Δ(*rapI-phrI*)*342*::*kan* (CAL1393, CAL1533, and CAL1398, respectively, as in Table 2); Δ*nicK306* Δ(*rapI-phrI*)*342*::*kan* (CAL1395, CAL1535, and CAL1400, respectively); Δ*conQ848* Δ(*rapI-phrI*)*342*::*kan* (CAL1591, CAL1592, and CAL1597, respectively); Δ*conQ848* Δ(*rapI-phrI*)*342*::*kan thrC*::*conQ*$^+$ (CAL1593, CAL1594, and CAL1598, respectively); and Δ*conQ848* Δ(*rapI-phrI*)*342*::*kan thrC*::*conQ*Δ (CAL1595, CAL1596, and CAL1599, respectively).

Although plasmid mobilization did not require *nicK*, *nicK* is required for transfer of ICE*Bs1* from plasmid-containing donors. There was no transfer of ICE*Bs1* from plasmid-containing Δ*nicK306* donors (data not shown).

The *thrC*::*conQ*$^+$ (line 4) used for plasmid mobilization contains the ICE*Bs1* genes upstream from and including *conQ* and *nicK* (Fig. 1). Since *nicK* is not needed for mobilization, the defect in the Δ*conQ848* mutant is due to loss of *conQ* and not a polar effect on downstream genes. The *thrC*::*conQ*Δ (*thrC*::{ICE*Bs1* Δ*conQ-attR*::*tet*}) (line 5) contains all ICE*Bs1* genes upstream of *conQ*, but not a functional *conQ* (Fig. 1). This was used as a control for the complementation (line 4).

[b]Percent plasmid mobilization was determined as described for Table 2. Values are the mean from ≥2 independent experiments ± the standard deviation. The number of independent experiments for each strain (n) is indicated in parentheses.

665 **Figure 1. Map of ICE*Bs1* and various mutants.**

666 **A.** Genetic map of ICE*Bs1*. ICE*Bs1* is shown in its linear integrated form. Open
667 arrows indicate open reading frames and the direction of transcription. Gene names are
668 indicated above the arrows. The origin of transfer (*oriT*) is indicated by a thick black line
669 overlapping the 3' end of *conQ* and the 5' end of *nicK*. *oriT* is needed for ICE*Bs1* transfer
670 (34) and replication (33). The small rectangles at the ends of ICE*Bs1* represent the 60 bp
671 direct repeats that contain the site-specific recombination sites in the left and right
672 attachment sites, *attL* and *attR*, that are required for excision of the element from the
673 chromosome.

674 **B-E.** Schematic diagram of deletion mutations in ICE*Bs1* in donor strains. Thin
675 horizontal lines represent the regions of ICE*Bs1* that are present in the donor strains.
676 Gaps in the line represent the regions of ICE*Bs1* that are deleted. Antibiotic-resistance
677 markers that are inserted in some of the alleles are not shown. Δ(*rapIphrI*) (B, D, E) (5)
678 and Δ(*conG-yddM*) (C) (34) are deletion-insertion mutations with a kanamycin-resistance
679 gene inserted (not shown). Δ*nicK* (E) and Δ*conQ* (F) are unmarked in-frame deletion
680 mutations that do not disrupt the expression of downstream genes and do not disrupt
681 *oriT*.

682 **F-I.** Schematic diagram of truncated ICE*Bs1* derivatives integrated at *thrC*. These
683 constructs cannot excise due to deletion of *attR* and were used to provide ICE*Bs1* genes
684 in trans for complementation tests of the ICE*Bs1* Δ*conQ* mutant. Thin horizontal lines
685 represent the regions of ICE*Bs1* that are present at *thrC*.

686 **B-I.** The ability of plasmids to be mobilized or of ICE*Bs1* to be transferred from
687 various donor strains are summarized as + or -. nd indicates not determined.

688

689

690 **Figure 2. Plasmid maps.**

691 Schematic diagrams of plasmids pC194 (A), pBS42 (B), and pHP13 (C), all mobilized
692 by ICE*Bs1*. The approximate size of each plasmid is indicated under the plasmid name.
693 Circles represent each plasmid and are not shown to scale. Thin black arrows and gene
694 names within each circle represent functional genes or, in the case of *'mobU* (B), a non-
695 functional 3' fragment of *mobU* (missing the 5'end). Antibiotic resistance genes include
696 *cat* (chloramphenicol resistance) and *mls* (macrolide, lincosamide, and streptogramin
697 resistance). Small open arrows on each circle represent the origin of replication, which
698 contains the site for nicking by the cognate replicative relaxase (encoded by *rep*, *repU*,
699 and *rep60*). The sequences flanking the *nic* sites in each plasmid *ori* are highly
700 conserved. The orientation of the open arrows indicates the direction of leading strand
701 synthesis for rolling circle replication. pBS42 (B) and pHP13 (C) are composites of
702 segments from several plasmids (6, 23). The junctions and sources of the different
703 segments are indicated by the intersecting lines and plasmid names on the perimeter of
704 each circle. For pBS42 (B) the location of two *Nsi*I restriction sites is indicated with a line
705 and an "N". The three plasmids have a 0.98 kb sequence in common that contains *cat*.
706 Other DNA sequences common to all three plasmids are ≤12 bp.
707
708

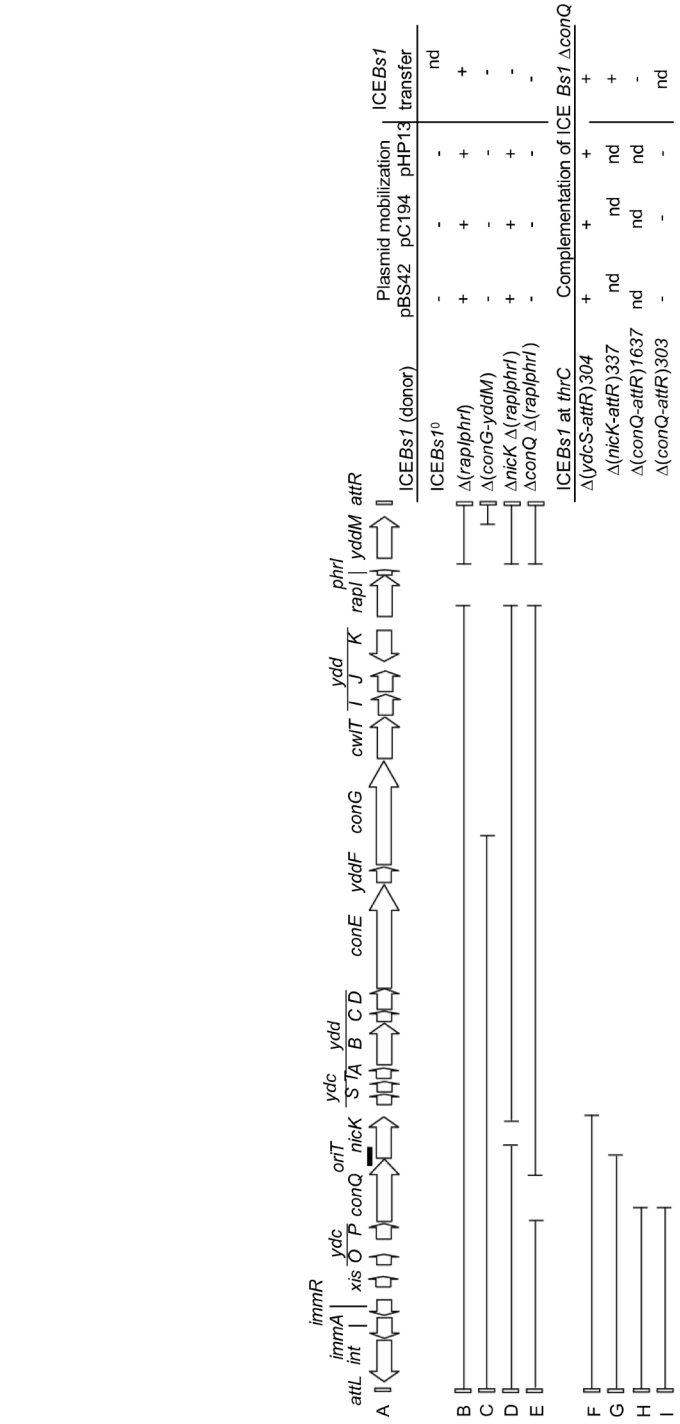
attL int immA immR xis ydcO P conQ oriT nicK ydcS TA ydd B C D conE yddF conG cwlT ydd T J K rapI phrI yddM attR
A
B
C
D
E
F
G
H
I
ICEBs1 (donor)
Plasmid mobilization
pBS42
pC194
pHP13
ICEBs1 transfer
ICEBs1⁰ - - - nd
Δ(rapIphrI) + + + +
Δ(conG-yddM) - - - -
ΔnicK Δ(rapIphrI) + + + -
ΔconQ Δ(rapIphrI) - - - -
ICEBs1 at thrC
Complementation of ICE Bs1 ΔconQ
Δ(ydcS-attR)304 + + + +
Δ(nicK-attR)337 nd nd nd +
Δ(conQ-attR)1637 nd nd nd -
Δ(conQ-attR)303 - - - nd

Figure 1

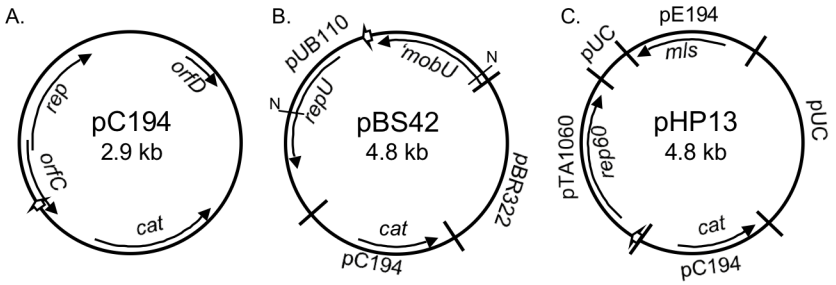
A.
pC194
2.9 kb
rep
orfD
orfC
cat
B.
pUB110
'mobU
N
N
repU
pBS42
4.8 kb
cat
pC194
pBR322
C.
pE194
pUC
mls
pUC
pTA1060
rep60
pHP13
4.8 kb
cat
pC194

Figure 2

710