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Genetic Modification of *Sodalis* Species by DNA Transduction

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ABSTRACT Bacteriophages (phages) are ubiquitous in nature. These viruses play a number of central roles in microbial ecology and evolution by, for instance, promoting horizontal gene transfer (HGT) among bacterial species. The ability of phages to mediate HGT through transduction has been widely exploited as an experimental tool for the genetic study of bacteria. As such, bacteriophage P1 represents a prototypical generalized transducing phage with a broad host range that has been extensively employed in the genetic manipulation of *Escherichia coli* and a number of other model bacterial species. Here we demonstrate that P1 is capable of infecting, lysogenizing, and promoting transduction in members of the bacterial genus *Sodalis*, including the maternally inherited insect endosymbiont *Sodalis glossinidius*. While establishing new tools for the genetic study of these bacterial species, our results suggest that P1 may be used to deliver DNA to many Gram-negative endosymbionts in their insect host, thereby circumventing a culturing requirement to genetically manipulate these organisms.

IMPORTANCE A large number of economically important insects maintain intimate associations with maternally inherited endosymbiotic bacteria. Due to the inherent nature of these associations, insect endosymbionts cannot be usually isolated in pure culture or genetically manipulated. Here we use a broad-host-range bacteriophage to deliver exogenous DNA to an insect endosymbiont and a closely related free-living species. Our results suggest that broad-host-range bacteriophages can be used to genetically alter insect endosymbionts in their insect host and, as a result, bypass a culturing requirement to genetically alter these bacteria.

KEYWORDS *Sodalis praecaptivus*, *Sodalis glossinidius*, insect endosymbiont, symbiont, transformation, transduction, genetic modification, plasmid transfer, transposition, bacteriophage P1, gene disruption, mutation, paratransgenesis, bacteriophage transduction, symbiosis

Bacteriophages (phages) are the most abundant and diverse biological entities on the planet. With an estimated population size greater than 1×10^{31} (1), these bacterial viruses play essential ecological and evolutionary functions. Phages control the size of bacterial populations and shape the diversity of microbial communities by modulating the abundance of bacterial lineages and promoting, directly and indirectly, the exchange of genetic information among species (2, 3). Historically, phages have played a central role in the development of molecular biology, enabling, for instance, the establishment of DNA as the genetic material of living cells (4). Today, phages are widely used as tools in the study of bacteria. For instance, generalized transducing phages such as P1 allow the rapid transfer of DNA among bacterial strains, greatly facilitating genetic dissection of biological processes (5).

P1 is a temperate bacteriophage capable of alternating between lytic and lysogenic infection. P1 was initially described in studies involving lysogenic strains of *Escherichia coli* (6). This phage is capable of mediating generalized transduction (7), a property

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that has fostered its adoption as an important experimental tool for the genetic analysis and manipulation of *E. coli* (5, 8). Notably, in addition to its habitual *E. coli* host, P1 can also infect a large number of Gram-negative bacterial species (8–12). This broad host range, along with its well-characterized molecular biology and established experimental procedures, has prompted the use of this phage as an experimental tool for the delivery of DNA to a large number of bacterial species (13–18). Here we establish that P1 is capable of infecting two members of the bacterial genus *Sodalis*, including *Sodalis glossinidius* (19, 20).

Sodalis glossinidius is a maternally inherited, Gram-negative bacterial endosymbiont of tsetse flies (*Glossina* spp.; Diptera: *Glossinidae*). Similar to other insect endosymbionts, *S. glossinidius* exists in a stable, chronic association with its insect host and undergoes a predominantly maternal mode of transmission (21–23). Notably, like other insect endosymbionts, this bacterium has undergone an extensive process of genome degeneration as a result of a recent ecological transition from free-living existence to permanent host association (24, 25). Because this process is accompanied by the loss of metabolic capability and stress response pathways (24–29), *S. glossinidius* has proven refractory to harsh artificial DNA transformation procedures that are commonly employed in model organisms such as *Escherichia coli* (29). Consequently, this bacterium has remained genetically intractable (30).

In this study, we demonstrate that the bacteriophage P1 is capable of infecting, lysogenizing, and promoting transduction in *Sodalis glossinidius*, and its free-living close relative, the plant-associated and opportunistic pathogen *Sodalis praecaptivus* (19, 20). We demonstrate that P1 can be used to mediate generalized transduction of chromosomal and extrachromosomal DNA in *S. praecaptivus*. We use P1 to transduce autonomous replicating phagemids containing an array of reporter genes and Tn*7* transposition systems harboring fluorescent proteins for chromosomal tagging. Finally, we developed a suicide phagemid containing a mariner transposase for random mutagenesis of bacterial strains susceptible to P1 infection. This study establishes a new efficient method for genetic manipulation of *Sodalis* species (Fig. 1) that can be readily adapted to other Gram-negative bacteria. Furthermore, these results provide a potential means for the genetic modification of bacterial endosymbionts, in their insect host, through the use of P1 as a DNA delivery system.

RESULTS

Bacteriophage P1 infects, lysogenizes, and forms phage particles in *Sodalis glossinidius* and *Sodalis praecaptivus*. P1CM*clr*-100(ts) is a thermo-inducible P1 variant harboring a chloramphenicol-resistant marker. P1CM*clr*-100(ts) forms chloramphenicol-resistant lysogens at low temperatures (≤30°C) but produces phage particles at higher temperatures (≥37°C) (31). Consequently, infection of *E. coli* by P1CM*clr*-100 (ts) yields chloramphenicol-resistant lysogens at 30°C. We took advantage of these P1CM*clr*-100(ts) properties to test whether *S. glossinidius* and *S. praecaptivus* were susceptible to P1 infection. We exposed cultures of these bacteria to increasing concentrations of P1CM*clr*-100(ts) phage particles and subsequently plated dilutions on solid medium containing chloramphenicol. We established that, similar to the *E. coli* control (Fig. 2A), exposure to increasing concentrations of P1CM*clr*-100(ts) particles yielded increasing numbers of chloramphenicol-resistant colonies in both *S. glossinidius* (Fig. 2B) and *S. praecaptivus* (Fig. 2C). Importantly, no chloramphenicol-resistant colonies were observed in cultures that were not exposed to P1CM*clr*-100(ts) particles (Fig. 2B and C).

That these colonies were P1 lysogens, as opposed to recombinants harboring only the P1-derived chloramphenicol-resistant marker, was supported by several lines of evidence. First, the presence of a P1 DNA fragment was detected by polymerase chain reaction (PCR) in both chloramphenicol-resistant *S. glossinidius* (Fig. 3A and B) and *S. praecaptivus* clones (Fig. 3C and D), but not in the wild-type strains (Fig. 3A to D, left side). This indicated that chloramphenicol-resistant cells harbor at least part of the P1CM*clr*-100(ts) genome. Second, lysates prepared from *S. glossinidius* and *S.*

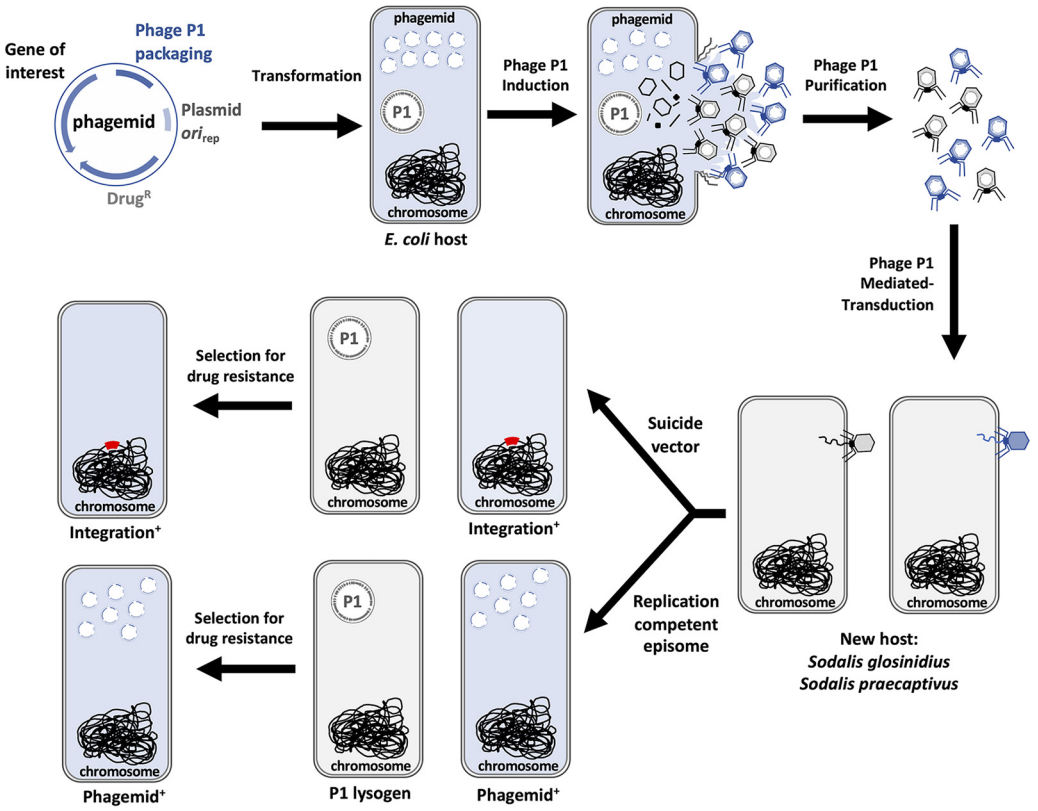

FIG 1 Cartoon representation depicting a workflow of the transduction procedure developed for introduction of phagemids in *Sodalis* species. Following the direction of the arrows, an *E. coli* P1 lysogen host is transformed with a P1 phagemid. The phagemid is packaged following induction of the P1 prophage, and lysates derived from culture supernatant are used to infect a *Sodalis* recipient strain. Cells receiving the phagemid are subsequently isolated on plates containing a selective agent.

praecaptivus chloramphenicol-resistant clones, but not their wild-type counterparts, formed plaques in soft agar cultures of *E. coli* grown at 37°C, a temperature that induces P1CM*clr*-100(ts) lytic replication (31) (Fig. 3E and F). This indicated that chloramphenicol-resistant *S. glossinidius* and *S. praecaptivus* clones can produce phage particles that are lytic to *E. coli* grown at 37°C. Third, the lytic activity of lysates derived from chloramphenicol-resistant *S. praecaptivus* cultures propagated at 37°C was 10,000 times higher than those maintained at 30°C (Fig. 3G). This established that higher titers of phage particles were being produced in *S. praecaptivus* chloramphenicol-resistant clones at a temperature where P1CM*clr*-100(ts) becomes lytic. Finally, lysates derived from *S. glossinidius* and *S. praecaptivus* chloramphenicol-resistant clones, but not their

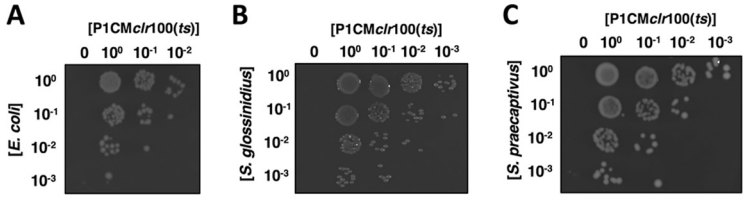

FIG 2 Infection of bacterial strains by phage P1. Lysates derived from an *E. coli* P1CM*clr*-100(ts) lysogen (KL463) were used to infect *E. coli* MG1655 (A), *Sodalis glossinidius* (B), and *Sodalis praecaptivus* (C). Plates depict the formation of chloramphenicol-resistant colonies as functions of the concentration of bacteria (vertical axis) and the concentration of P1CM*clr*-100(ts) lysates (horizontal axis). Note that P1 infection conditions for the strains are different (see Materials and Methods), and images do not reflect efficiency of P1 infection. Images show representative plates of at least three routine experiments.

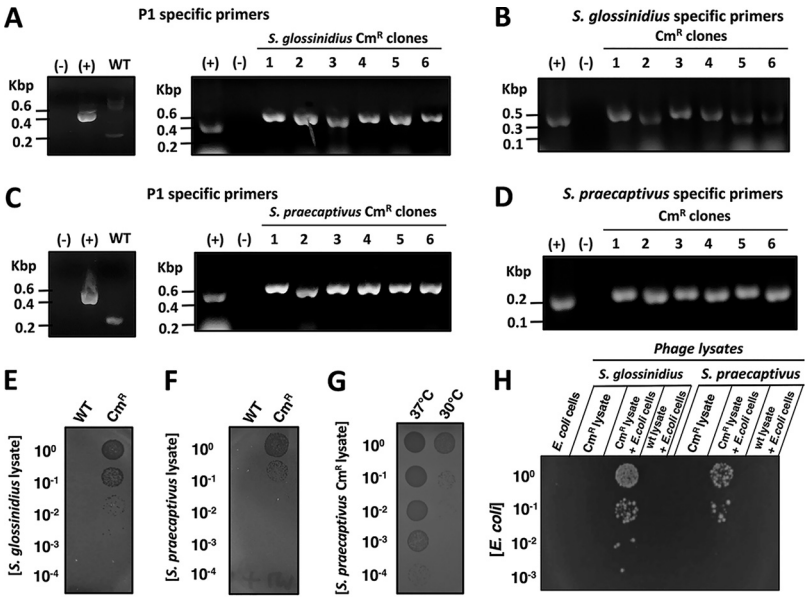

FIG 3 Lysogenization and production of infective phage particles by *Sodalis glossinidius* and *Sodalis praecaptivus* P1 lysogens. (A) Detection of P1 *pacB* gene by PCR and agarose gel electrophoresis in *S. glossinidius* chloramphenicol-resistant clones that emerged following exposure to an *E. coli* P1CM*clr*-100(ts) lysogen (KL463). (B) Detection of an *S. glossinidius*-specific DNA fragment in clones depicted in panel A by PCR and agarose gel electrophoresis. (C) Detection of the P1 *pacB* gene by PCR and agarose gel electrophoresis in *S. praecaptivus* chloramphenicol-resistant clones that emerged following exposure to an *E. coli* P1CM*clr*-100(ts) lysogen (KL463). (D) Detection of an *S. praecaptivus*-specific DNA fragment in clones depicted in panel C by PCR and agarose gel electrophoresis. WT, wild type; (−), no DNA control; (+), positive control. (E to G) Formation of phage plaques on soft agar embedded with *E. coli* MG1655. Soft agar plates were spotted with dilutions of lysates derived from wild-type and *S. glossinidius* chloramphenicol-resistant (Cm^r) P1CM*clr*-100(ts) lysogen (MP1705) (E), wild-type and *S. praecaptivus* Cm^r P1CM*clr*-100(ts) lysogen (MP1703) (F), and *S. praecaptivus* Cm^r P1CM*clr*-100(ts) lysogen (MP1703) grown either at 37°C or 30°C. Plates are representative of the plates of routine experiments. (H) Emergence of Cm^r *E. coli* MG1655 following exposure to lysates derived from wild-type *S. glossinidius*, *S. glossinidius* Cm^r P1CM*clr*-100(ts) lysogen (MP1705), wild-type *S. praecaptivus*, and *S. praecaptivus* Cm^r P1CM*clr*-100(ts) lysogen (MP1703). The plate is representative of the plates of at least three experiments.

wild-type isogenic counterparts, promoted the formation of chloramphenicol-resistant *E. coli* cells at 30°C (Fig. 3H). This indicated that the chloramphenicol-resistant marker can be transduced from *S. glossinidius* and *S. praecaptivus* back to *E. coli*.

In *S. glossinidius*, the frequency of chloramphenicol-resistant colonies arising following P1CM*clr*-100(ts) exposure was similar to those observed for the *E. coli* control cells, indicating that P1 infection occurs efficiently in this bacterium. In contrast, chloramphenicol-resistant *S. praecaptivus* colonies emerged at a lower frequency, and higher concentrations of bacterial cells were typically used in P1 infection experiments (see Materials and Methods). Notably, in P1-resistant *Salmonella enterica*, the efficiency of P1 infection can be drastically increased by mutations in either *galU* or *galE* (10). Because these mutations remove the O antigen by truncating the core region of the lipopolysaccharide (LPS) (32, 33), they presumably facilitate access of P1 to its host receptor—conserved structural motifs within the LPS core (8, 10). In particular, while the LPS of *S. praecaptivus* contains structural components attached to its core region, *S. glossinidius* is devoid of such structures (see Fig. S1A in the supplemental material). Nonetheless, the lower infectivity of P1 does not appear to be related to the physical occlusion of the P1 receptor by components present in the outer portion of the *S. praecaptivus* LPS. This is because a mutation in *galU* results in a truncated LPS in *S. praecaptivus* but does not affect P1 infectivity (Fig. S1). Hence, unlike *S. enterica*, this phenotype is not due to the presence of a P1-antagonizing structure(s) in the outer portion

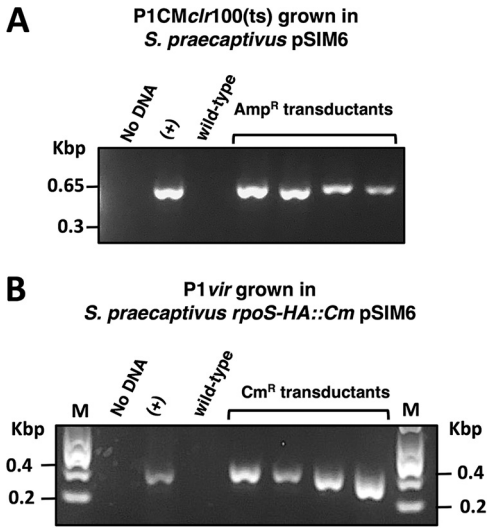

FIG 4 Bacteriophage P1-mediated generalized transduction in *S. praecaptivus*. (A) Detection of a pSIM6 DNA region by PCR and agarose gel electrophoresis in ampicillin-resistant (Amp^r) *S. praecaptivus* transductants following exposure to lysates derived from *S. praecaptivus* Cm^r P1CM*clr*-100(ts) (MP1703) harboring Amp^r plasmid pSIM6. (B) Detection of the *rpoS-HA*::*Cm* chromosomal insertion by PCR and agarose gel electrophoresis in *S. praecaptivus* transductants following exposure to lysates of P1*vir* grown in *S. praecaptivus rpoS-HA*::*Cm* pSIM6 (MP1522) strain.

of *S. praecaptivus* LPS. Taken together, these results indicate that phage P1 is capable of infecting and lysogenize in *S. glossinidius* and *S. praecaptivus*.

P1 generalized transduction in *S. praecaptivus*. During the formation of P1 virions, approximately 0.05 to 0.5% of infective phage particles package random DNA fragments derived from the bacterial host (34). These particles can mediate the transfer of bacterial DNA across P1-susceptible strains through generalized transduction. In the laboratory, generalized transduction of DNA can be identified by virtue of genetic markers that are packaged in these phage particles and transferred between bacterial strains. Accordingly, we sought to determine whether P1 could mediate generalized transduction in *S. praecaptivus*. First, we exposed wild-type *S. praecaptivus* to phage lysates derived from an *S. praecaptivus* P1CM*clr*-100(ts) lysogen harboring the ampicillin-resistant (Amp^r) plasmid pSIM6 (35). Following lysate exposure, we were able to retrieve Amp^r *S. praecaptivus* transductants. Importantly, Amp^r cells were absent from both phage lysates alone and cultures of wild-type *S. praecaptivus* that were not exposed to phage (data not shown). In agreement with the notion that these Amp^r clones were P1 transductants, diagnostic PCR revealed the presence of a pSIM6 fragment in these cells (Fig. 4A).

Next, we attempted to transduce a chromosomal chloramphenicol-resistant marker (*rpoS-HA*::*Cm*) using the P1 lytic strain P1*vir*. (This P1 strain is widely used as a transducing agent in *E. coli* due to its inability to lysogenize cells upon infection and the ease with which transducing lysates can be generated [5, 34].) We infected wild-type *S. praecaptivus* cells with P1*vir* lysates grown in an *S. praecaptivus rpoS-HA*::*Cm* pSIM6 strain. Whereas chloramphenicol-resistant (Cm^r) cells emerged from wild-type *S. praecaptivus* exposed to phage, no Cm^r cells were obtained from phage lysates or cultures of naive wild-type *S. praecaptivus* alone (data not shown). Notably, diagnostic PCR indicated that chloramphenicol-resistant clones were transductants harboring the *rpoS-HA*::*Cm* genetic modification (Fig. 4B). Importantly, all of these *rpoS-HA*::*Cm* transductants were sensitive to ampicillin, indicating that P1*vir* mediated the transduction of a discrete portion of the *S. praecaptivus* genome. Taken together, these results indicate that bacteriophage P1 can be used to mediate generalized transduction in *S. praecaptivus*.

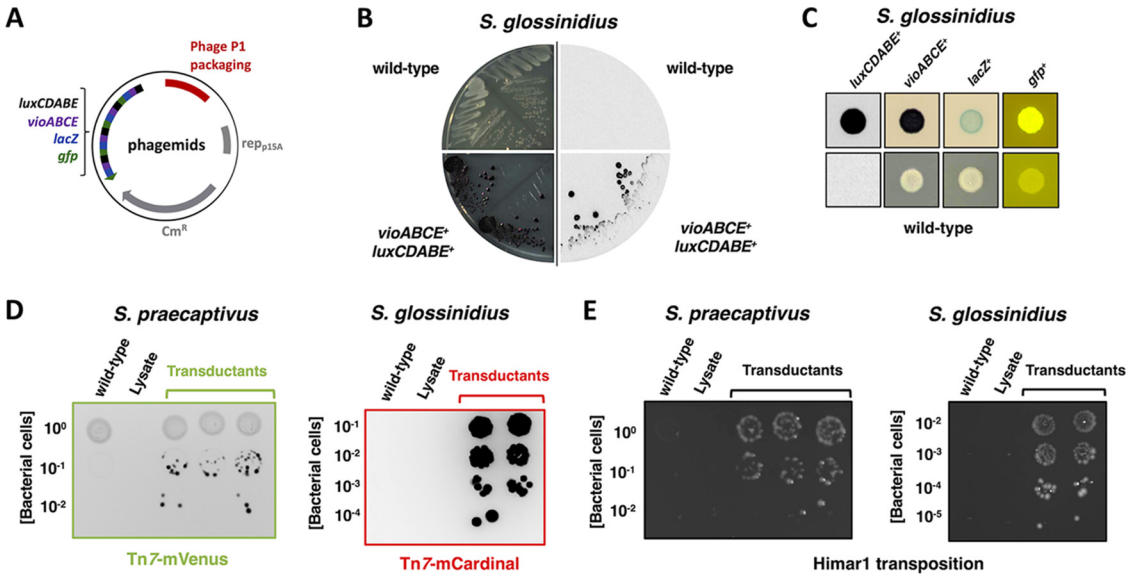

FIG 5 Transduction of P1 phagemids into *Sodalis* species. (A) Schematic representation of replication-competent P1 phagemids encoding a number of phenotypic markers (38). (B) Comparison of wild-type (top quadrants) and *S. glossinidius* transductants (lower quadrants) carrying P1 phagemid BBa_J72114-BBa_J72104 (38). Transductant colonies are purple due to the expression of violacein biosynthetic genes (lower left quadrant), and produce light due to the expression of bioluminescence genes (lower right quadrant). (C) Macrocolonies derived from wild-type *S. glossinidius* (bottom row) and transductants carrying P1 phagemids BBa_J72114-BBa_J72104 (*luxCDABE*$^+$ and *vioABCE*$^+$), BBa_J72114-BBa_J72100 (*lacZ*$^+$), or BBa_J72113-BBa_J72152 (*gfp*$^+$) (top row). (D) Transduction of pP1-Tn*7*-mVenus into *S. praecaptivus* (left-hand side plate) and pP1-Tn*7*-mCardinal into *S. glossinidius* (right-hand side plate). (E) Transduction of phagemid encoding a Himar1 transposition system (pP1-Himar) into *S. praecaptivus* (left-hand side plate) and *S. glossinidius* (right-hand side plate).

Introduction of exogenous DNA in *S. glossinidius* and *S. praecaptivus* by P1-mediated guided transduction. Whereas up to 0.5% of P1 particles can contain random fragments of bacterial host DNA (34), the vast majority of virions harbor P1 DNA. This is because the packaging of P1 genome into phage particles is guided by elements encoded within its DNA sequence (36, 37). Particularly, this packaging element can be cloned into plasmids (to produce phagemids) or incorporated into the bacterial chromosome to increase the frequency of P1-mediated transduction of adjacent DNA (17, 38, 39). Indeed, the P1 packaging element can increase the transduction of linked DNA by 1,600-fold above the levels obtained in generalized transduction (38). Hence, this DNA element can be used to increase the number of transducing particles and, consequently, the efficiency of DNA transfer among bacterial strains that are susceptible to P1 infection.

The lack of genetic tools available for the manipulation of *Sodalis* species, specifically *S. glossinidius*, prompted us to explore P1 as a plasmid DNA delivery tool for these bacteria. As a proof of principle, we used the aforementioned general technique to transfer a number of P1 phagemids (38) (Fig. 5A) into *S. glossinidius*. We were able to recover transductants expressing an array of phenotypic traits encoded in the phagemids. These traits included light production (*luxCDABE* genes), violacein pigment synthesis (*vioABCE*), β-galactosidase activity (*lacZ*), or green fluorescence (*gfp*) (Fig. 5B and C). To expand the tool set available for the modification of *Sodalis* species, we constructed two phagemids for tagging bacterial chromosomes with fluorescent genes at the Tn*7* attachment site (40) and a suicide phagemid encoding a Himar1 transposition system for random mutagenesis (41) (Fig. S2). Following packaging into P1 virions in an *E. coli* P1CM*clr*-100(ts) lysogen, these phagemids were efficiently delivered to *S. glossinidius* and *S. praecaptivus* (Fig. 5D and E). Together, these results establish that bacteriophage P1 can be used to efficiently deliver replication-competent and suicide vectors into *S. glossinidius* and *S. praecaptivus* through a "guided transduction" strategy.

DISCUSSION

In the current study, we demonstrate that bacteriophage P1 can infect, lysogenize, and promote transduction in two species of the genus *Sodalis*. We show that P1 can mediate generalized transduction in *S. praecaptivus* (Fig. 4), and we establish that this bacteriophage can be used for the delivery of plasmids and suicide vectors for the genetic manipulation of *S. glossinidius* and *S. praecaptivus* (Fig. 5). While these results constitute a significant advance in the development of genetic modification tools to study these bacterial species, they also clear the way for the implementation of P1-based DNA delivery systems to uncultured *Sodalis* species (42–48) and Gram-negative insect endosymbionts belonging to other genera.

Whereas *S. praecaptivus* can be genetically engineered with relative ease, the ability of P1 to mediate generalized transduction provides a number of applications for the manipulation of this bacterium. For instance, although *S. praecaptivus* can be readily modified by recombineering functions of phage λ (λ-Red) (49–51), the use of this technique has two major drawbacks. First, the expression of recombineering functions can be mutagenic (52). This can potentially produce confounding results in subsequent experiments as phenotypes associated with a particular engineered modification may actually result from secondary mutation(s). Second, typical temperature-sensitive plasmids (rep_{pSC101}^{ts} *ori*) harboring recombineering functions cannot be cured from *S. praecaptivus* by propagating cells at nonpermissive temperatures ($\geq$37°C) in the absence of plasmid selection (our unpublished results). The inability to cure these plasmids can increase the chances of secondary mutations through leaky expression of recombineering functions and hinder the use of plasmids from the same incompatibility group in downstream genetic analyses. Importantly, both of these issues can be overcome by P1-mediated generalized transduction. That is, genomic DNA fragments engineered using λ-Red can be transferred to naive *S. praecaptivus* cells that lack recombineering plasmids and, therefore, have not been exposed to potential mutagenic events (Fig. 4B).

In contrast, the establishment of P1-mediated transduction provides a considerable advancement in our ability to genetically manipulate *S. glossinidius*. This is because *S. glossinidius* is recalcitrant to DNA transformation by standard techniques such as heat shock and electroporation (29, 30, 53). Whereas we have recently developed a method for DNA transfer to *S. glossinidius* via conjugation (30), P1-mediated transduction provides an alternative, simpler method for the introduction of exogenous DNA into this bacterium. Altered chromosomal fragments, replication-competent plasmids carrying an array of functions, and suicide vectors engineered for allelic replacement or containing transposition systems can be quickly transduced into *S. glossinidius* in a simple protocol. Given the large DNA packaging capability of P1 (up to 100 kbp) (5), this bacteriophage can be efficiently used for a variety of applications, including the delivery of bacterial artificial chromosomes (bacterial artificial chromosome [BAC] vectors) or large plasmids encoding multiple genome editing CRISPR systems (54) that are not easily transferred by conjugation (30). Additionally, the P1 packing sequence can be incorporated into DNA fragments used in insertional mutagenesis (39), enabling rapid and efficient combination of mutations via P1 "guided transduction." This approach can greatly facilitate the implementation of several analyses (e.g., complementation and epistasis) to identify and dissect genetic components and pathways governing bacterial behaviors and interactions with eukaryotic hosts.

Beyond the genus *Sodalis*, the results highlighted in this study have potential broad implications for the genetic modification of uncultured Gram-negative insect endosymbionts. In Gram-negative bacteria, the LPS is the major structural constituent of the outer leaflet of the outer membrane. The LPS is composed of a highly conserved lipid A "anchor," a conserved core polysaccharide and, sometimes, a hypervariable outer component designated O antigen (see Fig. S1A in the supplemental material) (76). Bacteriophage P1 has a broad host range, in part, because it recognizes, as its host receptor, structural features of the conserved LPS core (8). In addition to *E. coli* and

several species of the *Gammaproteobacteria*, P1 has been shown to be capable of infecting various members within the *Alpha*, *Beta*-, and *Deltaproteobacteria*, and even bacterial species residing outside the *Proteobacteria* phylum such as *Flavobacterium* sp. strain M64 (8–12).

Notably, a large number of economically important insect species—including several disease vectors of animals and plants—harbor maternally inherited, Gram-negative bacterial endosymbionts (24, 25). However, unlike *S. glossinidius* and a handful of other species, the vast majority of these endosymbionts have not been isolated in pure culture (14, 19, 55–59). This is because these bacteria undergo a process of genome degeneration and size reduction during the course of long-term evolution and specialization within their eukaryotic insect hosts. This process leads to the loss of many physiological functions that are required for replication outside the host (24, 25, 27, 29). Notably, classical protocols of bacterial genetics require the manipulation of large numbers of cells and the subsequent isolation of rare genetic events as bacterial colonies on selective agar plates. Consequently, the implementation of genetics for the study of insect endosymbionts has remained scarce and limited to species that can grow in axenic culture, form colonies on agar plates, and are receptive to exogenous DNA (29, 30).

The ability of bacteriophage P1 to deliver DNA to a large number of bacterial species suggests a clear method in which the requirement for culturing may be bypassed. That is, similar to viral vectors that are commonly utilized in gene therapy in mammalians (60), P1 could be used to deliver DNA to bacterial endosymbionts inside their insect hosts. Specifically, insects could be microinjected (23, 27, 61–63) with P1 virions packaged with recombinant DNA. The establishment of successful P1 infections and subsequent enrichment of transductant endsymbionts to near-homogeneous or clonal populations could be attained by making use of phenotypic markers (Fig. 5) and implementing antibiotic selection regiments in insects (64–69). Whereas this approach would preferentially target recently acquired endosymbionts by virtue of their ability to exist intracellularly and extracellularly in various insect tissues (21–23, 25), ancient obligate intracellular endosymbionts could also be subjected to infection and genetic modification via P1, if they transiently exit host cells (70).

The results presented in this study pave the way for the development of tractable genetic systems for *S. glossinidius* and, potentially, a myriad of Gram-negative bacterial endosymbionts of insects. While this may empower the use of genetics to study these obscure bacteria, it also has clear translational applications. P1-mediated DNA delivery into insect endosymbionts may allow the engineering of bacterial traits aimed at modifying aspects of insect ecology (29, 30), mitigating their burden on economic activities and human health.

MATERIALS AND METHODS

Microbial strains, phages, plasmids, and growth conditions. Microbial strains, phages, and plasmids used in this study are presented in Table S1 in the supplemental material. Unless indicated, all *Escherichia coli* strains were propagated at 30, 37, or 42°C in Luria-Bertani (LB) broth or agar (1.5% [wt/vol]). *Sodalis glossinidius* was grown at 27°C in brain heart infusion broth supplemented with 10 mM $MgCl_2$ (BHI) or on brain heart infusion agar (1.2% [wt/vol]) supplemented with 10 mM $MgCl_2$ (BHI agar). *S. glossinidius* was also propagated on BHI agar plates supplemented with 10% defibrinated horse blood (BHIB). *Sodalis praecaptivus* was grown at 30, 39, or 42°C in Luria-Bertani broth or agar (1.5% [wt/vol]) lacking sodium chloride. For experiments involving P1 infection or generation of lysates, the growth medium was supplemented with $CaCl_2$ and $MgCl_2$ to a final concentration of 10 mM, respectively. Growth of *S. glossinidius* on BHIB agar plates was carried out under microaerophilic conditions, which was achieved either using BD GasPak EZ Campy Gas Generating sachets or a gas mixture (5% oxygen and 95% CO_2). For all strains, growth in liquid medium was carried out in shaking water bath incubators with aeration (250 rpm). When required, medium was supplemented with ampicillin (100 μg/ml), chloramphenicol (20 μg/ml for *E. coli* or *S. praecaptivus* and 10 μg/ml for *S. glossinidius*), kanamycin (50 μg/ml for *E. coli* and 25 μg/ml for *S. glossinidius* or *S. praecaptivus*). Arabinose was used at a concentration of 0.5 or 1% (wt/vol); 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was used at a concentration of 100 μg/ml.

Lipopolysaccharide extraction and detection. Extraction of lipopolysaccharide (LPS) from *S. glossinidius* and *S. praecaptivus* cultures was carried out as described previously (71). Extracted samples were

separated in a NuPAGE 10% Bis-Tris gel in NuPAGE MES SDS Running (ThermoFisher Scientific). LPS in gels were stained with ProteoSilver silver stain kit (Sigma-Aldrich).

Construction of phagemid pP1-Tn*7*-mCardinal. Oligonucleotide sequences used in this study are presented in Table S2. Phusion high-fidelity DNA polymerase (New England BioLabs) was used in PCRs with primers 469 and 470 and plasmid BBa_J72113-BBa_J72152 (38) as the template. The PCR product was ligated into pMRE-Tn*7*-163 (72), previously digested with SbfI, using NEBuilder HiFi DNA Assembly (New England BioLabs). The integrity of the construct was verified by DNA sequencing and the ability to be efficiently transduced by P1 particles.

Construction of phagemid pP1-Tn*7*-mVenus. Oligonucleotide sequences used in this study are presented in Table S2. Phusion high-fidelity DNA polymerase (New England BioLabs) was used in PCRs with primers 469 and 470 and plasmid BBa_J72113-BBa_J72152 (38) as the template. The PCR product was ligated into pMRE-Tn*7*-166 (72), previously digested with SbfI, using NEBuilder HiFi DNA Assembly (New England BioLabs). The integrity of the construct was verified by DNA sequencing and the ability to be efficiently transduced by P1 particles.

Construction of phagemid pP1-Himar. Oligonucleotide sequences used in this study are presented in Table S2. Phusion high-fidelity DNA polymerase (New England BioLabs) was used in PCRs with primers 475 and 476 and plasmid BBa_J72113-BBa_J72152 (38) as the template. The PCR product was ligated into pMarC9-R6k (73), previously digested with EcoRI and HindIII, using NEBuilder HiFi DNA Assembly (New England BioLabs). The integrity of the construct was verified by DNA sequencing and the ability to be efficiently transduced by P1 particles.

Recombineering procedure for *S. praecaptivus*. Oligonucleotide sequences used in this study are presented in Table S2. An *S. praecaptivus* strain harboring plasmid pSIM6 (35) was grown overnight in LB broth supplemented with 100 μg/ml of ampicillin at 30°C and 250 rpm. Cells were diluted (1:100) in 30 ml of the same medium and grown to an optical density at 600 nm (OD_{600}) between 0.45 and 0.5. The culture flask was then grown in a water bath at 42°C and 250 rpm for 25 min. Cells were immediately transferred to a 50-ml conical tube, collected by centrifugation (7,000 rpm for 2.5 min at 4°C), and resuspended in 40 ml of ice-cold deionized H_2O (dH_2O). Cells were collected again by centrifugation, and this washing procedure was repeated a second time. Finally, cells were resuspended in 150 μl of ice-cold dH_2O. Homologous recombination was obtained by electroporating 70 μl of cell suspension with 10 μl of purified PCR products generated with primers 251 and 252 (*galU*::*Kn*) or primers 84 and 85 (*rpoS-HA*::*Cm*) and plasmids pKD4 and pKD3 (74) as the templates, respectively.

Preparation of phage lysates derived from EMG16 P1 lysogens. Lysates from *E. coli* EMG16 harboring selected phagemids were prepared following arabinose induction as described previously (38).

Preparation of stocks of P1*vir* phage lysates. Stocks of P1*vir* phage lysates were prepared by infecting *E. coli* MG1655 (75), as described previously (5).

Preparation of phage lysates derived from P1CM*clr*-100(ts) lysogens. *E. coli* P1CM*clr*-100(ts) lysogens were grown overnight at 30°C. Cultures were diluted (1:100 [vol/vol]) into fresh medium and grown to an OD_{600} value of 0.3 to 0.4. Subsequently, cultures were shifted to 42°C and propagated until extensive cell lysis (3 to 4 h). At times, cultures were allowed to grow at 42°C for 16 h prior to the preparation of lysates. Partially lysed cells were disrupted by vortexing the cultures following the addition of chloroform (1 volume of chloroform per 100 volumes of culture). Cell debris was removed by centrifugation (12 min, 4,000 rpm, room temperature), and the supernatant was passed through a 0.22-μm polyethersulfone membrane filter. *S. praecaptivus* P1CM*clr*-100(ts) lysogens were prepared as described above, except that cells were grown for 2 h at 42°C and 16 h at 37°C prior to lysate preparation. *S. glossinidius* P1CM*clr*-100(ts) lysogens were grown in BHI to an OD_{600} of 0.4. Cultures were heat shocked at 37°C for 2 h. The cultures were treated with chloroform (1 volume of chloroform per 100 volumes of culture) and processed as described for *E. coli* and *S. praecaptivus*.

Infection by P1*vir*, P1CM*clr*-100(ts), and P1 transducing particles. Following overnight growth in LB, *E. coli* cultures were diluted in fresh LB supplemented with 10 mM $CaCl_2$ and $MgCl_2$ to an OD_{600} of 1. One-milliliter aliquots of these cell solutions were incubated for 30 min at 30°C in the presence or absence of various concentrations of P1 lysate. Cells were subsequently collected by centrifugation (1 min, 13,000 rpm, room temperature), and the supernatants were replaced by 1 ml of LB containing 5 mM sodium citrate. Cells were grown for 1 h at 30°C and 250 rpm prior to plating. *S. praecaptivus* cells grown overnight in LB were collected by centrifugation (1 min, 13,000 rpm, room temperature) and resuspended in fresh LB supplemented with 10 mM $CaCl_2$ and $MgCl_2$. One milliliter of these resuspended solutions was incubated for 30 min at 30°C in the presence or absence of various concentrations of P1 lysate. Cells were collected by centrifugation (1 min, 13,000 rpm, room temperature), and the supernatants were replaced by 1 ml of LB containing 5 mM sodium citrate. Cells were plated following 1 h of growth at 30°C and 250 rpm. *S. glossinidius* cells were grown in BHI for 3 to 5 days to an OD_{600} of $\approx$0.5. Cells were collected by centrifugation and concentrated to an OD_{600} of 1. One milliliter of concentrated cultures was incubated for 60 min at 30°C in the presence or absence of various concentrations of P1 lysate. Cells were collected by centrifugation (1 min, 13,000 rpm, room temperature), and the supernatants were replaced by 10 ml of BHI. Cultures were incubated overnight at 27°C overnight with shaking prior to plating.

Curing of pP1-Tn*7* phagemids. Transduction of pP1-Tn*7* phagemids into *S. glossinidius* and *S. praecaptivus* was initially selected on plates containing ampicillin (Fig. 5D; see also Fig. S2 in the supplemental material). Because episomes harboring rep_{pSC101}^{ts} origins of replication are not easily cured from *S. praecaptivus* (see Discussion), the curing of phagemids was performed only in *S. glossinidius*. The strategy used to identify *S. glossinidius* clones lacking phagemids was similar to the one adopted elsewhere (53). Briefly, to identify *S. glossinidius* clones that contained the chloramphenicol-resistant marker at the

Tn*7* attachment site and had lost the ampicillin-resistant plasmid, cultures were propagated in BHI containing chloramphenicol and 1% arabinose. After four passages, cells were diluted and plated. Single colonies were screened for sensitivity to ampicillin. Transposon insertion at the Tn*7* attachment site was verified by PCR with primers 1018 and 1019.

Image acquisition, analysis, and manipulation. DNA agarose gel electrophoresis and bacterial colonies, with the exception of *S. glossinidius* macrocolonies, were detected using an Amersham Imager 680 (GE Healthcare). *S. glossinidius* macrocolonies expressing green fluorescent protein (GFP) were detected using a dark reader (Clare Chemical Research) and documented with an iPhone. When oversaturated, the intensity of signals in images were adjusted across the entire images using Preview (Apple).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, TIF file, 2.4 MB.

FIG S2, TIF file, 2.6 MB.

TABLE S1, XLSX file, 0.01 MB.

TABLE S2, XLSX file, 0.01 MB.

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We declare that we have no conflicts of interest.

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