Characterization of Interspecific Plasmid Transfer Mediated by Bacteriophage SP02

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Plasmid pPL1010 is a 7.0-kilobase derivative of plasmid pUB110 that harbors the cohesive end site of the bacteriophage SP02 genome. Plasmid pPL1017 is a 6.8-kilobase derivative of plasmid pC194 that contains the immunity region of bacteriophage φ105 and the cohesive end site of bacteriophage SP02. These plasmids are transducible by bacteriophage SP02 at a frequency of $10^{-2}$ transductants per PFU among mutant derivatives of Bacillus subtilis 168 and have been transferred to other strains of B. subtilis and B. amyloliquefaciens by means of bacteriophage SP02-mediated transduction, with frequencies ranging from $10^{-3}$ to $10^{-7}$ transductants per PFU. The introduced plasmids were stably maintained in nearly all new hosts in the absence of selective pressure. An exception was found in B. subtilis DSM704, which also harbored three cryptic plasmids. Plasmids pPL1010 and pPL1017 were incompatible with a 7.9-kilobase replicon native to strain DSM704. Furthermore, plasmid pPL1017 was processed by strain DSM704 into a ~5.3-kilobase replicon that was compatible with the resident plasmid content of strain DSM704. The use of bacteriophage SP02-mediated plasmid transduction has allowed the identification of Bacillus strains that are susceptible to bacteriophage SP02-mediated genetic transfer but cannot support bacteriophage SP02 lytic infection.

Bacteriophage-mediated interspecific genetic transfer in the genus Bacillus has traditionally been done with generalized transducing bacteriophages such as PBS1 (7, 10, 11, 17), SP-10, SP-15 (18), and CP-51 (15, 19). This paper reports the use of SP02, a temperate bacteriophage which productively infects Bacillus subtilis 168, as a vector for the interspecific transfer of plasmid DNA.

The host range of bacteriophage SP02 encompasses B. subtilis 168 and B. subtilis 3610; much lower efficiencies of plaquing have been reported for strains of B. licheniformis and B. pumilus (2, 7). Such host range determinations classically employ plaque assays which demand that the bacteriophage go through its entire lytic cycle. Barriers to productive infection can be found at the level of phage adsorption, DNA injection, bacteriophage genome replication, enzymatic restriction, and abortive infection. Abortive infection may be brought about by the lack of essential host-coded functions or by antagonistic effects due to functions specified by resident prophages and plasmids (see reference 3 for a review). Thus, the scoring of plaque formation may not always be an appropriate measure for determining the susceptibility of a host to bacteriophage-mediated genetic transfer.

Plasmid pPL1010 is a derivative of plasmid pUB110 that contains a 2.4-kilobase (kb) EcoRI-generated fragment which harbors the cohesive end site of the bacteriophage SP02. This plasmid confers resistance to neomycin (Neo) upon its host (14). Plasmid pPL1017 is a derivative of plasmid pC194 that contains part of the immunity region of bacteriophage φ105, the cohesive end site of bacteriophage SP02, and the chloramphenicol resistance (Cam) determinant of the parent plasmid (Fig. 1) (8, 12). Bacteriophages φ105 and SP02 are unrelated heteroimmune, temperate bacteriophages which productively infect B. subtilis 168 (7, 16). Recombination-proficient strains that are lysogenic for prophage SP02 and harbor either of the “cosmids” pPL1010 or pPL1017 can be induced to yield lysates which exhibit high transducing frequencies for the plasmid-borne markers (12).

The bacterial strains and bacteriophages used are listed in Table 1. Bacteriophages SP02C and φ105C are spontaneous clear-plaque mutants of their wild-type parents. Bayer AG provided strain B. subtilis DSM704 and strain B. amyloliquefaciens DSM7. Taxonomic analysis was performed by previously published methods (5, 16a). The media used include tryptose blood agar base (TBAB), Penassay broth (Difco Laboratories), and M medium (12). The antibiotics neomycin sulfate and chloramphenicol, when used, were added to a final concentration of 10 μg/ml. All incubations were done at 37°C. Liquid cultures were grown with rotary shaking (250 rpm). Bacteriophages were propagated and assayed for infectious and transducing activities were performed as previously described (14). Comparable transducing frequencies were obtained in the presence and absence of 30 μg of DNAse I per ml. Bacterial transformations were performed by the single-step growth procedure of Bott and Wilson (1). Plasmid isolations, restriction endonuclease assays, and agarose gel electrophoresis were performed as previously described (9, 14).

Transducing lysates carrying plasmid pPL1010 or pPL1017 can transduce either plasmid at a frequency of $10^{-2}$ transductants per PFU when strains of B. subtilis 168 are used as recipients (12, 14). SP02 (pPL1010) and SP02 (pPL1017) transducing lysates were capable of mediating the transfer of plasmid-borne markers to Bacillus strains other than B. subtilis 168 (Table 2). Bacteriophage SP02 infectious activity could only be demonstrated on the B. subtilis 168 strains BR151 and YB886. Other Bacillus strains susceptible to transduction included B. subtilis DSM704, B. amyloliquefaciens H, B. amyloliquefaciens N, and B. amyloliquefaciens DSM7; however, the level of transduction obtained was $10^{-4}$ to $10^{-5}$ of the transducing activity displayed by the same lysates on strains BR151 (SP02) and YB886 (SP02).

The strains of B. pumilus and B. licheniformis employed did not support SP02 infectious activity. In addition, these strains were not transduced to Neo by SP02 high-frequency
transducing lysates. These data contradict previous information stating that these strains of *B. pumilus* and *B. licheniformis* were capable of supporting a low level of bacteriophage SP02-specified infectivity (2).

All strains found to be susceptible to SP02-mediated transduction, with the exception of DSM704 (see below), normally lacked detectable extrachromosomal DNA as determined by the electrophoretic techniques employed. All Neo' and Cam' transductants harbored plasmid species corresponding to pPL1010 and pPL1017, respectively (Fig. 2 and 3).

The stability with which plasmids pPL1010 and pPL1017 were maintained in various bacilli was determined over a 24-h period in the absence of selective antibiotic pressure. Single-antibiotic-resistant transductant colonies were subcultured (1:10,000 every 12 h) in drug-free Penassay broth, and viable counts were performed on drug-free TBAB plates. A total of 200 of the resultant colonies were then tested on TBAB for resistance to either neomycin or chloramphenicol for pPL1010 and pPL1017, respectively. Plasmid pPL1010 was found to be stably maintained in all new hosts except strain DSM704. Indeed, loss of pPL1010 could not be demonstrated in plasmid-bearing strains subcultured nonselectively for 120 h. Plasmid pPL1017, on the other hand, was not stably maintained by strains BR151, YB886, or any of the other transducible *Bacillus* strains. Typically, there was a 10 to 20% loss of the plasmid-borne Cam' marker during 24 h of nonselective growth. In the strain DSM704, there was a 90 to 100% loss of the Cam' marker over a 24-h period.

Agarose gel electrophoresis of DNA extracted from strain DSM704 revealed three plasmid species with molecular sizes of 5.6, 6.7, and 7.9 kb (Fig. 2). DNA extracted from Neo' DSM704 transductants produced a banding pattern characteristic of that of the three resident plasmids plus a new plasmid band which comigrated with purified pPL1010 plasmid DNA (Fig. 2, lane 3). A single Neo' transductant grown in Penassay broth and then plated on TBAB yielded segregants which were sensitive to neomycin (Neo'). DNA extracted from a Neo' segregant gave the indigenous plasmid banding pattern. Incubating mid-exponential-phase DSM704 cells with an SP02 (pPL1017) transducing lysate yielded Cam' colonies at low frequency (Table 2). Initial screening of several transductants revealed that they carried plasmid pPL1017 (apparently in low copy numbers, judging from the low intensity of the plasmid band) but no longer harbored the 7.9-kb resident plasmid (Fig. 3, lane 3). The plasmid marker was found to be quite unstable in the absence of selective chloramphenicol pressure, and DNA extracted from chloramphenicol-sensitive (Cam') segregants and subjected to

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\begin{array}{ccc}
\text{Table 1. Bacterial strains, bacteriophages, and plasmids} \\
\hline
\text{Strain, plasmid, or phage} & \text{Relevant properties} & \text{Source} \text{ or reference} \\
\hline
\text{Bacterial strains} & & \\
B. subtilis 168 & trpC2 metB10 lys-3 & B. E. Reilly \\
BR151 & trpC2 metB10 lys-3 & This work \\
B. subtilis 168 & trpC2 metB5 sin-1 & 21 \\
BR151 (SP02) & & \\
B. subtilis 168 & trpC2 metB5 sin-1 & This work \\
YB886 & SP'' Ery' & \\
B. subtilis 168 & trpC2 metB5 sin-1 & D. Stein \\
YB886(7.9') & SP'' Ery' & \\
B. subtilis W23 & Prototroph & 22 \\
RUB815 & Prototroph & Y. Yoneda \\
B. subtilis natto & Prototroph & \\
IAM1212 & & \\
B. subtilis DSM704 & Prototroph & Bayer AG \\
B. amyloliquefaciens & Prototroph & F. E. Young (20) \\
H HSR & & \\
B. amyloliquefaciens & Prototroph & M. A. Courtney \\
N RUD20 & Prototroph & \\
B. amyloliquefaciens & Prototroph & Bayer AG \\
DSM7 & Prototroph & P. S. Lovett \\
B. pumilus NRRL B-3275 & Prototroph & \\
B. licheniformis & Prototroph & M. A. Courtney \\
ATCC 9789 & Prototroph & BGSC \\
B. licheniformis & Prototroph & BGSC \\
ATCC 8480 & Prototroph & \\
B. licheniformis & Prototroph & \\
ATCC 94454 & & \\
\hline
\text{Bacteriophages} & & \\
\delta105C & Clear-plaque mutant of \delta105 & This work \\
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* SP''. Sensitive to infection with phage SPB; Ery', erythromycin resistant; cos, cohesive end site of the genome of bacteriophage SP02. Plasmids were maintained in strain YB886, which is lysogenic for SP02, and under selective antibiotic pressure.

* BGSC. Bacillus Genetic Stock Center, Ohio State University.

* Strain harbors a 7.9-kb cryptic plasmid originating from *B. subtilis* DSM704.
TABLE 2. Bacteriophage SP02-mediated interspecific plasmid transfer

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>Infectious activity PFU/ml</th>
<th>Transductants (no. per ml) obtained with SP02 lysates carrying plasmid</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>pPL1010</td>
</tr>
<tr>
<td>B. subtilis 168</td>
<td>2.9 × 10^{10}</td>
<td>3.8 × 10^{8}</td>
</tr>
<tr>
<td>(SP02)</td>
<td></td>
<td></td>
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<tr>
<td>B. subtilis W23</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td>B. subtilis natto</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>B. subtilis DSM704</td>
<td>2.0 × 10^{3}</td>
<td>2.0 × 10^{4}</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>B. amyloliquefaciens</td>
<td>1.6 × 10^{5}</td>
<td>5.0 × 10^{4}</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. amyloliquefaciens</td>
<td>7.6 × 10^{2}</td>
<td>2.0 × 10^{3}</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSM7</td>
<td>8.0 × 10^{2}</td>
<td>1.0 × 10^{3}</td>
</tr>
</tbody>
</table>

a The titers shown are for an SP02 (pPL1010) transducing lysate. SP02 lysates carrying pPL1017 had comparable infectious activity.

b Transductants harboring plasmid pPL1010 or pPL1017 were selected for on TBAB plates containing neomycin (10 μg/ml) or chloramphenicol (10 μg/ml), respectively.

c The B. subtilis 168 recipient strains were SP02 lysogens of strains BR151 and YB886. Both were transduced by an SP02 (pPL1010) lysate to Neo' at similar frequencies. Two- to three-fold more transductants were usually obtained when the recipients were lysogenic for SP02 than when the recipients were nonlysogenic. Plaque assays were performed on nonlysogenic strain BR151 or YB886.

d The B. licheniformis strains ATCC 8480, ATCC 9789, and ATCC 9945A behaved identically in the infectious and transducing activity assays.

e —, Strain exhibited a natural resistance to chloramphenicol.

Electrophoresis exhibited an apparently restored 7.9-kb resident plasmid but no longer harbored pPL1017 (Fig. 3, lane 5).

Strain DSM704 (pPL1017) was streaked for single colonies and maintained under selective chloramphenicol pressure for several weeks. It yielded isolates whose plasmid DNA gave an altered electrophoretic banding pattern (Fig. 3, lane 4). As in the Cam' segregants, the 7.9-kb plasmid had reappeared and the plasmid pPL1017 band was not in evidence. However, each of several independent isolates harbored a new plasmid species of approximately 5.3 kb. DNA from such a Cam' isolate was used to transform strain YB886. The resulting Cam' transformants were found to carry a 5.3-kb plasmid and exhibited immunity to infection by bacteriophage φ105C (Table 1). The 5.3-kb plasmid was also found to be transducible by bacteriophage SP02 at a frequency of 10^{-2} Cam' transductants per PFU. Restriction analysis revealed that the 5.3-kb plasmid was a derivative of plasmid pPL1017 that had sustained a deletion of approximately 1.5 kb, which included sequences of bacteriophage φ105 and SP02 and plasmid pC194 DNAs (Fig. 1). Several isolates produced identical results (data not shown), and the new deletion plasmid was designated pPL1017A. Interestingly, DNA that was extracted from newly generated strain DSM704(pPL1017) Cam' transductants and used to transform strain YB886 to Cam' yielded transformants which harbored plasmid pPL1017. Apparently, the precise processing of plasmid pPL1017 in a strain DSM704 host requires a minimum period of plasmid residence.

Strain DSM704 was transduced to Cam' by using SP02 lysates carrying plasmid pPL1017A. Apparently, the introduction of plasmid pPL1017Δ into DSM704 does not lead to initial loss of the resident 7.9-kb plasmid, as it does in newly generated DSM704(pPL1017) transductants (Fig. 3, lanes 3 and 9). Plasmid pPL1017Δ was not stably maintained in strain DSM704 in the absence of selective chloramphenicol pressure.

The establishment of plasmids pPL1010 and pPL1017 in strain DSM704 has exposed complex plasmid maintenance and incompatibility relationships between the introduced plasmids and the resident replicons of the host. Neither pPL1010 nor pPL1017 was stably maintained in strain DSM704. At least one plasmid (7.9 kb) indigenous to strain DSM704 can destabilize either "cosmid" in a strain YB886 host (data not shown). The 7.9-kb plasmid and pPL1017 apparently cannot coexist in a strain DSM704 host. However, a deletion derivative, plasmid pPL1017A, can be maintained with selective chloramphenicol pressure in the presence of the 7.9-kb plasmid (Fig. 1 and 3). The mechanism by which the 7.9-kb resident plasmid makes a reappearance is not known. It may be that strain DSM704 carrying plasmid pPL1017 also carries the 7.9-kb plasmid in a copy number

FIG. 2. Plasmid content of pPL1010-containing YB886 and DSM704 strains. Rapid plasmid isolation from parent strains, Neo' transductants, and Neo' segregants were performed as previously described (14). All lanes display covalently closed circular monomeric plasmid species extracted from (lane 1) strain YB886(pPL1010) Neo' transductant, (lane 2) strain DSM704 (harboring three naturally occurring cryptic plasmids of approximately 5.6, 6.7, and 7.9 kb), (lane 3) strain DSM704(pPL1010) Neo' transductant, (lane 4) Neo' segregant of strain DSM704(pPL1010), (lane 5) strain YB886 transformed to Neo' with DNA extracted from strain DSM704(pPL1010).

FIG. 3. Plasmid content of pPL1017-containing YB886 and DSM704 strains. Plasmids were prepared as described in the legend to Fig. 2. DNA was extracted from (lane 1) strain YB886(pPL1017) Cam' transductant, (lane 2) strain DSM704, (lane 3) newly generated strain DSM704(pPL1017) Cam' transductant, (lane 4) strain DSM704(pPL1017Δ) Cam' transductant subcultured on TBAB plus chloramphenicol (10 μg/ml) for several weeks, (lane 5) Cam' segregant of strain DSM704(pPL1017), (lane 6) strain YB886 transformed to Cam' with strain DSM704(pPL1017) DNA, (lane 7) strain YB886 transformed to Cam' with strain DSM704(pPL1017Δ) DNA, and (lane 9) strain DSM704(pPL1017Δ) Cam' transductant.
too low to be detected by the electrophoretic technique employed. Alternatively, the extrachromosomal state of the 7.9-kb plasmid may not be compatible with that of plasmid pPL1017. The possibility that the 7.9-kb plasmid can be recombined with the bacterial chromosome or an as a yet undetected replicon has not been investigated. The mechanism of the plasmid processing event is unknown. It is intriguing that several independent deletion events have produced plasmids which are identical to pPL1017Δ in their ability to confer immunity to bacteriophage φ105 infection, transducibility by bacteriophage SP02, and molecular size as determined by electrophoresis. Spontaneous deletions of pC194 derivative plasmids in \textit{B. subtilis} have been reported previously (6).

The results presented in this study demonstrate that plasmids carrying the cohesive end site of bacteriophage SP02 can be introduced, via bacteriophage SP02-mediated transduction, into a variety of different bacilli. In addition, recent results by Flock (4) indicate that bacteriophage φ105 can be used in a similar manner. Thus, it has been possible to identify species that are susceptible to bacteriophage-mediated genetic transfer but cannot support productive viral infection. Furthermore, the system described, in addition to greatly facilitating the transfer of plasmids into incompetent members of the genus \textit{Bacillus}, has allowed plasmid stability and incompatibility studies to be performed in new hosts.

Appreciation is extended to L. Kopec and M. Fisk for rendering excellent technical assistance to M. A. Courtney for contributing valuable discussion on the genetics of plasmids and bacteriophages in \textit{B. subtilis}, and to D. Stein for generously sharing material and data on strain DSM704.

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**LITERATURE CITED**