Use of Salmonella Phage P22 for Transduction in Escherichia coli

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A cosmid (pPR1347) carrying both the rfb gene cluster and the rfc gene of a Salmonella group B serovar has been constructed; Escherichia coli K-12 strains carrying this cosmid produce long-chain O antigens, are sensitive to phage P22, and can be transduced by P22. Some of the benefits of P22 transduction are now available for studying E. coli and potentially other genera.

Transduction was discovered during a search for Escherichia coli K-12-like conjugation in Salmonella enterica (22). In this first demonstration of transduction, one of the strains used, LT2, and the phage P22 (present as a prophage in the other strain used, LT22) became the mainstay of S. enterica genetic analysis. Note that all Salmonella strains are now considered to belong to one species, and the old species names are used as serovar names (8): strains LT2 and LT22 both belong to serovar Typhimurium (part of the group B serogroup). Transduction has been used extensively in bacterial genetics and is very useful, for example, in strain construction. In general, transduction within each bacterial species requires use of a specific phage; for example, phage P1 has been used for transduction in E. coli, and phage P22 has been used for transduction in S. enterica sv. Typhimurium. A significant factor in the development of the genetics of S. enterica has been the ease of use of P22 for transductional crosses. The properties of P22 relevant to transduction have been summarized in a review of the Salmonella linkage map (14). In particular, P22 is stable in storage, high-titer stocks are easily obtained, and high-frequency transduction (HT) and integration-deficient mutants have been isolated (16, 17). Techniques have been developed for focusing transduction on specific parts of the S. enterica chromosome and for transposon use (15).

P22 uses the S. enterica group B polymerized O antigen as a receptor. The O antigen is a component of the outer membrane lipopolysaccharide (LPS) and consists of repeat units of sugar residues covalently linked together to form polysaccharides of various chain lengths. The O antigen requires the rfb gene cluster for synthesis of each of the lipopolysaccharide units and the rfc gene for the subsequent polymerization of the units.

P22 cannot adsorb to and infect E. coli K-12, because K-12 lacks the specific O-antigen receptor required; however, the ability of P22 to replicate within K-12 has been known since the experiments of Botstein and Herskowitz (2) in which an F' factor carrying a P22 prophage was transferred from S. enterica sv. Typhimurium into K-12, resulting in production of mature phage particles (following inactivation of the P22 repressor). We report here construction of a cosmid which carries the group B rfb and rfc genes and confers on K-12 the ability to synthesize group B long-chain O antigen, sensitivity to infection by phage P22, and the capacity for P22-mediated transduction.

Both the rfb gene cluster and the rfc gene have been cloned and are present on different, compatible, replicons. The group B LT2 rfb cluster, cloned in our laboratory (6), is present in cosmid pPR1028 which carries the pSC101 replicon. Plasmid pADE206 (3), a pMB1 replicon plasmid, carries the rfc gene from a Typhimurium strain. When coelectroporated into the E. coli restriction-minus K-12 strain JM109 (21) [recA1 supE44 endA1 hsdR17 gyrA96 relA1 Δ(proAB-lac) thi], these two plasmids were found to confer on JM109 sensitivity to a P22 clear plaque mutant phage (designated CP1/1 of unspecified genotype), whereas electroporation of the rfb-carrying cosmid alone into JM109 did not confer sensitivity to P22. Crude LPS extracts of these JM109 derivatives were prepared by an outer membrane extraction technique as described previously (1) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10), followed by silver staining (19). The results for the JM109 derivative carrying both plasmids (Fig. 1, lane C) show a ladder-like banding pattern characteristic of polymerized O antigens of various chain lengths. In the absence of the rfc gene, the LPS has only one O unit (indicated by an arrow in Fig. 1, lane B) linked to the LPS core oligosaccharide, and clearly the rfc gene cloned by Collins and Hackett (3) is all that is required to effect polymerization. A restriction-modified P22 stock of CP1/1 was prepared by propagating the phage on the P22-sensitive JM109 derivative (in L broth containing 1 mM CaCl₂ and 10 mM MgCl₂) and was used to successfully transduce genetic markers into another P22-sensitive K-12 strain (data not shown). Our next step was to put the rfc gene and the rfb gene cluster into one plasmid, pPR1347 (Fig. 2). A 1.75-kb HindIII fragment from pADE206, carrying the rfc gene, was ligated into the HindIII site of pUC1318 (7) to generate the pPR1346 construct. Digestion of pPR1346 with BamHI yielded the above 1.75-kb rfc fragment flanked by two BamHI sites: this fragment was then inserted into the BamHI-digested pPR1028, replacing the 3-kb Cm⁺ fragment, to give the rfb-rfc-carrying construct pPR1347. When electroporated into JM109, pPR1347 was found to confer on JM109 P22 sensitivity and a typical O-antigen banding pattern on a SDS-polyacrylamide gel (Fig. 1, lane D). Subsequently, pPR1347 was transferred into the K-12 strains C600 (13) (thr-1 leuB6 tonA21 lacY1 supE44 thi-1) and AB3516 (20) (lacZ13 trp-3 his-4 rpsL8 malA1 mlh-1 ilvE316 thi-1 ara-9 ton-1).

A P22 stock of the HT integration-defective mutant phage H1505/1 int-201 (16) was propagated on C600/pPR1347 and used to transduce the ilv⁺ genetic marker to the recipient

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strain AB3516/pPR1347. Aliquots of an overnight culture of the recipient were mixed with the phage stock (in L broth containing 1 mM CaCl₂ and 10 mM MgCl₂) at a multiplicity of infection of 1, allowing 10-min adsorption at 37°C. Appropriate dilutions were then plated on selective minimal medium plates (without kanamycin selection) and grown for 48 h at 37°C. Of the single colonies obtained from the transduction, 36 were restreaked onto nutrient agar plates, and a single colony from each of these streaks was replica plated onto appropriately supplemented minimal medium plates to test for the selective acquisition of the ilv⁺ phenotype. All the clones tested were positive for ilv⁺, indicating stable maintenance of the ilv⁺ marker. The transduction frequency obtained was 3.3 × 10⁻⁵ transductants per PFU.

A similar transduction assay with the same phage and conditions as described in the above experiment was conducted in S. enterica LT2 strains selecting for transfer of the trp⁺ marker. The donor used, P9329, was a wild-type LT2 isolate (22), and the recipient was strain P9003 (11) [hsdL trpB2 nml H1(b) flaA66 H2(e, n, x) rpsL, yflT404 ilvE452 metE551 metA22 hsdA], which had been lysogenized by the wild-type P22 strain P22 C⁺ (22). The transduction frequency obtained (1.6 × 10⁻³ transductants per PFU) was within the range of frequencies (10⁻⁴ to 10⁻⁵) expected for HT mutants reported by Schmiegler (16). The similarity of the K-12 transduction frequency to that of LT2 indicates that the HT phenotype of the HT1051 int-201 mutant observed during LT2 transduction is also expressed in transduction of K-12 strains.

P22 C⁺, which lysogenizes Salmonella group B strains, gives rise to turbid plaques when plated onto P22-sensitive K-12 strains, suggesting that it can also lysogenize K-12. This result is not surprising, since K-12 permits P22 replication and also has a P22 prophage attachment site (attP22), identical in sequence to the P22 attachment site (attA) in the LT2 chromosome, into which P22 can integrate when attP22 is present on an E. coli F' episome carried by a S. enterica sv. Typhimurium strain (9). The temperate nature of P22 in K-12 was confirmed after cross-streaking AB3516/pPR1347 with P22 C⁺ and purifying presumptive lysogens from the phage lysis zone (by restreaking twice under kanamycin selection for single-colony isolation). As expected for prophage-carrying strains, purified clones were found to release mature phage

FIG. 1. LPS analysis by SDS-PAGE and silver staining of crude cell envelope extracts. Samples were boiled and treated with 1 µl of 25-µg/ml proteinase K prior to loading and electrophoresed on an 18% polyacrylamide gel. The single O unit (linked to the LPS core oligosaccharide) in lane B is indicated by an arrow. Lanes: A, JM109; B, JM109(pPR1028); C, JM109(pPR1028, pADE206); D, JM109 (pPR1347).

FIG. 2. Construction of pPR1347. AmpR, KanR, and CmlR denote approximate locations of markers specifying resistance to the antibiotics ampicillin, kanamycin, and chloramphenicol, respectively. The vector sequences within pPR1347 and pPR1028 are derived from the low-copy-number cosmid pPR691 (5) and include the Kan’ marker.
P22 int mutants characteristically do not integrate into the host chromosome but form extrachromosomal unstable “lysocens” (presumably carrying P22 as a nonreplicating plasmid) which are immune and also shed phage (17). The integration-deficient phenotype of HT1051/1.int-201 in K-12 was confirmed by the lysogeny assays described above: of the 20 phage-resistant colonies isolated and examined from the original cross-streak, all were found to have lost their P22-immune phenotype and to segregate phage-free clones. Additionally, none of the 36 isolates from the K-12 transduction experiment showed evidence of carrying phage or prophage (after restreaking twice to avoid carryover of exogenous phage), as determined by plaque assay (phage immunity could not be tested because of loss of the P22 receptor).

The cosmid pPR1347 is unstable, and strains carrying it must be grown in the presence of kanamycin. We have not studied the kinetics of loss of P22 sensitivity after loss of pPR1347, but in a culture of AB3516/pPR1347 grown overnight in the absence of kanamycin from a single colony on a kanamycin-containing plate, approximately 6% of cells are kanamycin resistant. This instability means that not only can plasmid-free transductants be easily obtained, but the transduction frequency can be enhanced by plating on agar without selection for kanamycin resistance. The resulting loss of plasmid (and hence O-antigen receptor) from transductants increases their chances of survival by preventing lysis by phage released from adjacent lysed cells.

The above experiments demonstrate that provided the appropriate receptor is present on the cell surface, E. coli K-12 is a suitable host for P22 infection and replication. P22 propagates well on K-12 strains carrying pPR1347 (provided kanamycin selection is used to maintain the cosmid) and gives similar titers to those observed when propagated on LT2 (10^{10} to 10^{11} PFU/ml in L. broth). It is also shown here that P22 mutants developed specifically for transductional work in S. enterica sv. Typhimurium strains can be effectively used for transduction in K-12.

The bacteriophage P1, used extensively for transduction in E. coli, has been well characterized and developed (18). P22 likewise has been well-developed for genetic analysis in serovar Typhimurium S. enterica, and the work described in this report suggest that many of the methods utilizing P22 in S. enterica may be applicable to E. coli. Under particular circumstances, P22 may therefore provide an appropriate alternative to P1 for genetic manipulation in E. coli.

The ability of pPR1347 to render K-12 sensitive to P22 as described in this report also raises the possibility of using P22-mediated transduction to carry out intergeneric crosses between E. coli and S. enterica group B strains. Restriction-minus mutants for both species exist and could prove very useful in the construction of hybrid strains.

Importantly, the work with K-12 described here illustrates the potential use of P22 for transduction in species or strains other than those of Salmonella group B origin. We have tested P22-mediated transduction only in E. coli K-12, but the availability of the LT2 rfb and rfc genes as a single cosmid construct should facilitate the use of P22 in transduction in other E. coli and S. enterica strains and possibly in other genera. It should be noted that strains carrying prophages of the lambdoid family would generally be suitable for use as transductional recipients, since only one phage, coliphage 21, is known to have the same immune specificity as that of P22. It should also be noted that E. coli K-12 does not produce O antigen and if pPR1347 were introduced into strains which do produce O antigen, there could be interactions between the two O-antigen types such that the S. enterica O antigen would not be expressed: this would of course limit more general application.

The strains required for use of this technique are available from the Salmonella Genetic Stock Centre as a kit which contains P4729 and P4730, both of which carry pPR1347. P4729 is derived from the restriction-minus strain JM109 and is suitable for preparation of E. coli K-12 restriction-modified stocks of P22 and the many P22 derivatives available. P4730, a derivative of the K-12 strain SMRI10 (12), is a lysogen for a temperature-inducible lambda phage which lacks the cos site. On induction, the cosmid pPR1347 is packaged in vivo, and the lysate can be used to transduce pPR1347 to any strain with the lamB receptor. pPR1347 is a large plasmid, and this means of transfer can be advantageous. Note that the lamB clone pAM117 (4) can be used to allow transduction to other strains. Alternatively, pPR1347 extracted from P4729 can be transferred to other strains by electroporation or transformation.

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