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Mobilization of the gonococcal 5.2 kb beta-lactamase plasmid pSJ5.2 into *Escherichia coli* by cointegration with several Gram-conjugative plasmids

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Abstract

We report the mobilization by cointegration of the gonococcal 5.2 kb beta-lactamase plasmid pSJ5.2 in an *Escherichia coli* background. Transfer of pSJ5.2 was measured by filter mating assays with five different conjugative plasmids from Enterobacteriaceae and the gonococcal 41 kb *tet(M)*. Plasmid pSJ5.2 was mobilized to *E. coli* at frequencies of 1.7×10^{-6} , 9.3×10^{-8} and 2.7×10^{-5} by the *tet(M)*, R64 *drd-33* and N3 conjugative plasmids, respectively. Mobilization of pSJ5.2 by the 41 kb *tet(M)* conjugative plasmid resulted in stable Amp^R *E. coli* transconjugants consisting of pSJ5.2 plasmid with an insertion located in the 2.4 kb *Bam*H1- *Bam*H1 fragment. Mobilization of pSJ5.2 by R64*drd-33* and N3 conjugative plasmids involved stable cointegrates as detected by Southern Blot with a DIG-labelled *Pst*I-digested pSJ5.2 probe. Restriction analysis of the R64::pSJ5.2 and N3::pSJ5.2 cointegrates and Southern Blot with the pSJ5.2 probe showed that cointegrates formed by deletion of DNA regions within the 1.8 kb *Bam*H1-*Hind*III fragment of pSJ5.2. The plasmid thus appears to use multiple recombination mechanisms for cointegration with different conjugative plasmids. The complete nucleotide sequence of pSJ5.2 was determined, and will be a useful tool to further investigate the molecular mechanisms leading to its cointegrative transfer.

Keywords

Neisseria gonorrhoeae; Beta-lactamase plasmids; mobilization; cointegration

1.0 Introduction

In *Neisseria gonorrhoeae*, the transfer and dissemination of high-level penicillin resistance is by resistance (R)-plasmids encoding a TEM-1 type Beta-lactamase. These naturally-occurring plasmids, which were initially described in 1976 in clinical isolates of *N. gonorrhoeae* (Ashford et al., 1976;Phillips, 1976;Elwel et al., 1977;Roberts, 1989), are genetically related and have persisted since then (Ray et al., 2005). Three R-plasmids are associated with the worldwide emergence of Penicillinase-producing *N. gonorrhoeae* (PPNG): an "Asian" R-plasmid of 7.4kb in length; and its deletion-derivatives "African" (5.6 kb) and "Toronto" (5.2 kb) (Dillon and

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Yeung, 1989). These R-plasmids possess two major biological characteristics considered of great interest for biotechnological applications and of impact in evolution and dissemination of beta-lactam resistance in *N. gonorrhoeae*: They are mobilizable by conjugation (Sox et al., 1978; Biswas et al., 1980; Guinney and Ito, 1982; Mc Nichol et al., 1983; Ikeda et al., 1986; Roberts and Knapp, 1988; Marquez et al., 1999; Rodríguez-Bonano, 2004) and can replicate efficiently in *Neisseria gonorrhoeae*, *Escherichia coli*, *Salmonella enterica* and *Haemophilus influenzae* (Gilbride and Brunton, 1990; Pagotto and Dillon, 2001).

Since the appearance of the gonococcal beta-lactamase plasmids several studies were performed, which demonstrated that different conjugative plasmids recognize different DNA sequences involved in mobilization (McNichol et al., 1983; Tenover et al., 1985; Piffaretti et al., 1988; Gauthier 1990; Dillon et al., 1991). Furthermore, different mechanisms for mobilization of the gonococcal R-plasmids have been described (Sox et al., 1978; Dillon et al., 1991). Analysis of *cis*-acting sites involved in mobilization had identified only one functional *mob/oriT* region located in the 3.2 kb *Bam*H1-*Hind*III site from the 7.4 kb R-plasmid with the help of the broad-host-range IncP transfer system RK2 (Rodríguez-Bonano and Torres-Bauzá, 2004).

The 5.2 kb Toronto R-plasmid is similar to the Asian 7.4 Kb R-plasmid, except for a 2,270 bp deletion comprising the putative *mob/oriT* region (Pagotto et al., 2000). In spite of this, mobilization of the 5.2 kb R-plasmid has been reported to occur with the help of plasmid pBG791 (Dillon et al., 1991) and the gonococcal 41 kb *tet(M)* conjugative plasmid (Marquez et al., 1999). In both studies, however, no further analysis was done on the transconjugants, and the mechanism for these mobilizations was not determined. Therefore the 5.2 kb R-plasmid is an ideal model to study mobilization either by another functional *oriT* or by cointegrate formation in the gonococcal R-plasmids. Since first isolated in Canada in 1985 during an outbreak, the 5.2 kb R-plasmid appears to be of epidemiologic significance since the isolates have spread worldwide (Marquez et al., 1999; Sarafian et al., 1991; Sarafian et al., 1994; Severence et al., 1988; Yeung et al., 1986). The 5.2 kb R-plasmid was detected between 1987 and 1994 in three different auxotypes of *N. gonorrhoeae* isolated in Puerto Rico and consequently was named pSJ5.2 (Torres-Bauzá, 1995). Preliminary sequence analysis of pSJ5.2 detected *oriT*-like sequences similar to that of the IncN, IncFII, IncW and Inc11 α conjugative plasmids (Zechner et al., 2001; Data not shown). These sites could be activated by the *trans*-acting nickases provided by the conjugative plasmids. Rather, we observed that transfer of pSJ5.2 occurred at a moderate to low frequency by cointegrative mobilization with N3 (IncN), R64 (Inc11 α) and *tet(M)*.

This paper shows the first evidence that the gonococcal 5.2 kb beta-lactamase plasmid pSJ5.2 is mobilized by cointegration with several conjugative plasmids. Stable bacterial transconjugants containing intermediates of plasmid cointegrates were isolated. These isolates, together with the sequence of pSJ5.2 reported in this paper will be useful as a basis for further genetic studies on the full repertoire of the mobilization transfer that allow the horizontal transmission of the gonococcal beta-lactamase plasmids.

2.0 Materials and methods

2.1 Bacterial strains, plasmids, media, and growth conditions

Plasmids and strains used in this study are listed in Table 1. Luria-Bertani (LB) and gonococcal (GC) media were prepared as described on standard protocols (Sambrook and Russell, 2001). Antibiotics were added to the liquid or solid medium at the following concentrations: kanamycin, 50 μ g/ml; chloramphenicol, 35 μ g/ml; tetracycline, 50 μ g/ml (for selection of conjugative plasmids R64 *drd*-33 and N3) and 15 μ g/ml (for selection of the 41kb *tet(M)*)

conjugative plasmid); streptomycin, 300 µg/ml; nalidixic acid, 25 µg/ml; rifampin, 100 µg/ml; and ampicillin, 250 µg/ml.

2.2 Mating experiments

Previous attempts to mobilize pSJ5.2 by different conjugative plasmids resulted in no recovery of transconjugants when using the biparental filter 2-hour mating technique described previously (Guinney and Ito, 1982). The method of Cabezón et al., (1997) with minor modifications from Lejeune et al., (1984) was used to enhance the detection of mobilization events occurring at a low frequency (i.e, recombination leading to plasmid cointegrates) by increasing both the density of cells and duration of mating. Briefly, conjugative plasmids from table 1 were transferred to *E. coli* DH10B carrying pSJ5.2. Double-plasmid donor *E. coli* DH10B and nalidixic acid-resistant recipient *E. coli* DH5α were grown overnight in LB broth with selective antibiotics. Drops of suspensions of donor and recipient cells were mixed in a 1:1 ratio (0.1 ml each) and spotted on a 0.45 µM Millipore filter placed on a LB agar plate without antibiotics. Cells were allowed to conjugate for 8 hours at 37°C.

Since the 41 kb *tet(M)* conjugative plasmid cannot be maintained in *E. coli* (Flett et al., 1981; Morse et al., 1986; Sox et al., 1978) intergeneric triparental matings among *N. gonorrhoeae* and two strains of *E. coli* were performed as previously described (Flett et al., 1981) with minor modifications. Overnight cultures of donor *N. gonorrhoeae* strain 15315, intermediate *E. coli* DH10B with pSJ5.2 and recipient rifampicin-resistant *E. coli* HMS174 were grown in LB broth without antibiotics and adjusted to a concentration of 10⁸ cells/ml. Bacterial suspensions were mixed in a 10:1:1 ratio (1 ml: 0.1 ml: 0.1 ml), respectively, washed, suspended in LB broth, and spotted on a 0.45 µM Millipore filter placed on a GC agar plate without antibiotics. Cells were allowed to conjugate for 18–24 hrs at 37°C in a 5% CO₂ environment.

Growth from both biparental and triparental filter matings was scraped from plates with a cotton swab, suspended in LB broth, serially diluted 10-fold with LB broth and spreaded on LB plates containing antibiotics that selected *E. coli* transconjugants (ampicillin and nalidixic acid or rifampin). Transfer and mobilization frequencies were determined by dividing the number of transconjugants by the number of donor cells. Plasmid content of the transconjugants was verified by a rapid cell lysis method (Colony Fast Screen Kit; Epicentre, WI).

2.3 Replica-plating test for transfer

Transconjugants obtained after mobilization of pSJ5.2 with conjugative plasmids R64 and N3 were used as donors in separate conjugation experiments with *E. coli* HMS174. The replica-plating technique was performed as previously described (Winans and Walker, 1985). Briefly, a number of different transconjugants were patched onto a LB plate with no antibiotics using a Cathra replicator (Oxoid Inc., Ontario, Canada). After incubation, each plate was replicated onto another plate containing rifampin and a lawn of recipient *E. coli* HMS174. After incubation to allow any potential transconjugants to form and multiply, the second plate was replicated onto a third plate containing LB with both ampicillin and rifampin. Further analysis of transconjugants was done to screen for plasmid pSJ5.2.

2.4 Non-radioactive probes and hybridization experiments

To confirm the integration of pSJ5.2 during mating assays, a nonradioactive probe consisting of pSJ5.2 was linearized by digestion with *Pst*I. The pSJ5.2 probe was randomly labeled using the Genius nonradioactive labeling and detection kit (Roche Diagnostic Corp., Indianapolis, IN). Southern analysis of both total and restricted DNA was done according to the manufacturer's instructions.

2.5 Genetic manipulation of pSJ5.2 and DNA sequence analysis

The plasmid pSJ5.2 DNA was prepared by ionic exchange columns (Qiagen, Valencia CA) and then purified by electrophoresis on agarose gels as described (Sambrook et al., 1989). Three subclones of pNG2.4 (2.4 kb), pNG1.8 (1.8 kb) and pNG0.9 (0.9 kb) were constructed by cloning the three *Bam*H1-*Hind*III fragments into pGEM7zf (Promega, Madison, WI), and were sequenced by using the M13 forward and reverse universal as initial primers. Sequencing of both strands was performed by the dideoxy chain termination method (Sanger et al., 1977) using the Autoread Sequencing Kit (Amersham Biosciences, Piscataway, NJ) with an ALFexpress Sequencer (Pharmacia). DNA analysis of the sequences was performed using the Visual Cloning 2000 software (Readasoft, Corp., Toronto, Canada) and the ORF Finder program and BLAST homology search from the National Center for Biotechnology Information (NCBI).

3.0 Results and discussion

3.1 Transfer frequencies of pSJ5.2 by different conjugative plasmids

Mobilization frequency of pSJ5.2 was tested using five different conjugative systems listed in Table 1. Self-transfer of pOX38, pSU2007 and R64 *drd*-33 occurred at frequencies of 8.1×10^{-1} , 8.2×10^{-1} and 2×10^{-3} transconjugants per donor cells, respectively, indicating that all have efficient conjugative machineries. The 41 kb *tet*(M) and N3 conjugative plasmids self-transferred at a moderate frequency of 2×10^{-5} and 2.4×10^{-6} , respectively. In contrast, plasmid pSJ5.2 was mobilized at a lower frequency of 8.3×10^{-8} by pOX38, 3.7×10^{-10} by pSU2007 and 9.3×10^{-8} by R64 *drd*-33. The frequency of transmission of pSJ5.2 by N3 and the 41 kb *tet*(M) conjugative plasmids was 2.7×10^{-5} and 1.7×10^{-6} , respectively. The similarity of the transfer frequency obtained by pSJ5.2 and *tet*(M) and by pSJ5.2 and N3 suggests co-transmission of pSJ5.2 and the conjugative plasmid. The low transfer frequency of pSJ5.2 by the conjugative plasmids tested was indicative that mobilization is not taking place via an *oriT* site in pSJ5.2. Furthermore, the Enterobacterial plasmids capable of transferring pSJ5.2 presumably do not share mobilization machinery acting on such *oriT*.

3.2 Phenotypic analysis of the transconjugants generated by mobilization of pSJ5.2 with five conjugative plasmids

About 16 to 20 transconjugants obtained during the mobilization of pSJ5.2 by the pSU2007, pOX38, R64 *drd*-33 and N3 conjugative plasmids were screened for phenotypic analysis. Transconjugants were subcultured on media with antibiotics that allowed selection of pSJ5.2 and both pSJ5.2 with the respective conjugative plasmid. Ampicillin-resistant (amp^R) colonies were no longer recovered on retesting of the 16 transconjugants with conjugative plasmid pSU2007 (data not shown). Two out of 18 amp^R colonies of transconjugants with pOX38 were isolated. However, they were unstable since they were lost during serial transfer without selective pressure (data not shown).

Twenty amp^R transconjugants each from the pSJ5.2 / R64 and pSJ5.2 / N3 matings were recovered. They were all able to grow on tetracycline-containing media plates, a result that indicates transfer of both pSJ5.2 and the conjugative plasmids. Other properties of the pSJ5.2 / R64 and pSJ5.2 / N3 transconjugants screened were: stability through replication and capability of the conjugational transmission of the genes associated with pSJ5.2 and the conjugative plasmids (amp^R / tet^R) by the replica-plating test for transfer described previously (Materials and Methods).

Twenty transconjugants from the triparental cross with the 41 kb *tet*(M) conjugative plasmid were analyzed. Only eight colonies grew in ampicillin when subcultured. These amp^R colonies were stable upon subsequent passages on LB agar without antibiotics. The remaining twelve

colonies were unable to grow on ampicillin or plates containing both ampicillin and tetracycline. The phenotypic characteristics of the transconjugants generated by mobilization of pSJ5.2 with N3, R64 and *tet(M)* conjugative plasmids are consistent with cointegrate formation as an intermediate of conjugal transfer.

3.3 Cointegrates and insertion derivatives of pSJ5.2 formed in *E. coli* during mobilization

DNA samples from randomly selected transconjugants were analyzed on a 1.0% agarose gel (Figure 1A; left panel) and hybridized with a nonradioactive probe from plasmid pSJ5.2 linearized with *Pst*I (right panel). Neither plasmid nor hybridization signal of 5.2 kb in size was observed in the transconjugants (both panels; bottom of gel). The Southern Blot analysis with a pSJ5.2 *Pst*I-digested probe located the pSJ5.2 plasmid integrated within R64 (lanes b, c, f; Top) and N3 (i, j and k; Top). Mobilization of pSJ5.2 by conjugative plasmid N3 resulted in a plasmid larger than N3 based on comparison with the donor (left panel; lanes I, J, K vs lane H at the top of gel). The presence of R64 cointegrates of variable sizes were apparent in the gel (right panel; lanes b, c and f). Variable R64 cointegrates may be the result of random deletion derivatives of the R64::pSJ5.2 cointegrates, however, they were ruled out by a similar *Bam*H1-restriction pattern obtained in previous analysis on twenty (20) transconjugants tested (data not shown). These results confirmed that transfer of pSJ5.2 by conjugative plasmids R64 and N3 involved N3::pSJ5.2 and R64::pSJ5.2 cointegrative intermediates

Plasmid content of selected amp^R transconjugants recovered during the transfer of pSJ5.2 with the gonococcal 41 kb *tet(M)* conjugative plasmid was analyzed by agarose gel electrophoresis and hybridization with the *Pst*I-digested pSJ5.2 probe (Figure 1B). Comparison of the pSJ5.2 transconjugants (Lanes Cc, Dd, Ee) with the "wild type" pSJ5.2 (lane Bb) revealed that all acquired an insertion of approx. 800 kb. A band corresponding to a *tet(M)* derivative was also noted in all the pSJ5.2 transconjugants (red arrow at the top of the gel). The *tet(M)* plasmid found in the *E. coli* transconjugants was confirmed by migration on an agarose gel (lane G versus lanes C, D, E, F) and a positive 700 bp "Dutch"-type amplicon obtained by a differential PCR assay as previously described (Xía et al. 1995; results not shown). However, these plasmids were no longer able to self-transfer and the transconjugants did not grow on LB media containing 15 µg/ml of tetracycline (data not shown). These observations suggest that pSJ5.2 is mobilized by the *tet(M)* in the form of amp^R-insertion derivatives in *E. coli*.

3.4 Mapping of the pSJ5.2 cointegrate and insertion derivatives

To determine the site of insertion for pSJ5.2 within conjugative plasmids R64 and N3, plasmid DNA from representative N3::pSJ5.2 and R64::pSJ5.2 cointegrates was purified by alkaline lysis, double digested with *Bam*H1 and *Hind*III, blotted onto nylon membrane, and hybridized with the non-radioactive pSJ5.2-*Pst*I-digested probe. Plasmid DNA from wild type pSJ5.2 was included to determine which segments of the N3::pSJ5.2 and R64::pSJ5.2 differed from the wild type pSJ5.2. The restriction pattern of pSJ5.2 with *Bam*H1-*Hind*III consists of three fragments of 2.4 kb, 1.8 kb and 1.0 kb in size (Figure 2). The results showed that the 2.4 Kb *Bam*H1-*Bam*H1 and the 1.0 Kb *Bam*H1-*Hind*III fragments are conserved in the R64::pSJ5.2 and N3::pSJ5.2 transconjugants, while the 1.8 kb *Bam*H1-*Hind*III band is absent (data not shown). No other pSJ5.2 hybridization signal was detected beyond the 2.4 kb and 1.0 kb limits on the membrane, suggesting that a region within the 1.8 kb band of pSJ5.2 was deleted during fusion with R64 and N3 conjugative plasmids (Figure 2). This in turn may explain the presence of cointegrates of R64 of variable sizes (Figure 1A; top of gel; right panel).

Double digestion with *Bam*H1 and *Hind*III and agarose gel electrophoresis was also performed on the pSJ5.2 insertion derivatives obtained during conjugative transfer with the 41 kb *tet(M)* plasmid. Comparison of the *Bam*H1-*Hind*III restriction fragments of the pSJ5.2 insertion derivatives with the "wild type" pSJ5.2 determined that the insertion is located within the 2.4

kb *Bam*H1-*Bam*H1 fragment. (data not shown). Analysis of the insertion by PCR and sequencing revealed the presence of an Insertion Sequence 1 (*IS*1; unpublished results).

Computer-assisted analysis of plasmid pSJ5.2 did not reveal *IS*-like sequences; however, other transposable elements have been found in the 2.4 kb *Bam*H1- *Bam*H1 fragment from the gonococcal beta-lactamase plasmids giving rise to some R-plasmid insertion variants (Pagotto et al., 2000). Since this fragment contain the beta-lactamase gene and does not contain the putative *mob* / *oriT* region described previously (Rodríguez-Bonano, 2004); we believe that the 2.4 kb *Bam*H1-*Bam*H1 fragment contains a *cis*-acting target site for transposable elements that allows cointegrate formation and transfer of the R-plasmid. Further studies are planned to determine whether *IS*1 was involved in the pSJ5.2 transfer and the source of the transposable element.

3.5 DNA sequence and analysis of pSJ5.2

The sequence of the 7.4 kb Asia-type R-plasmid pJD4 was recently determined, providing the basis for a comparative analysis with the gonococcal beta-lactamases (Pagotto et al., 2000). The 5.2 kb "Torontotype" plasmid was previously assumed to be identical to pJD4 based on comparison of restriction endonuclease map and by partial sequencing comprising only 254 bp of the region surrounding the deletion of the Toronto-type relative to pJD4. Based on these analyses, the 5.2 kb was estimated to be 5,154 bp in size with a deletion of 2,270 bp using as reference the pJD4 (Pagotto et al., 2000). Although many aspects of the "Toronto" type R-plasmid are well characterized through comparison with the parental 7.4 kb "Asian" R-plasmid, the entire sequence is not known and this is the first report of it as a complete replicon unit. The complete sequence provides a valuable tool for elucidating the molecular mechanisms involved in the cointegrative mobilization observed for pSJ5.2 by allowing the design of sequencing primers on the plasmid and flanking the recombination junctions. This approach facilitates the determination of which additional genes or *cis*-acting sites contribute to the lateral transfer of the gonococcal beta-lactamase plasmids.

The complete pSJ5.2 sequence was determined and found to contain 5161 bp. Total G+C content was 38%. The complete nucleotide sequence has been deposited with the GenBank database under accession No. **DO355980**. Computer analysis revealed that pSJ5.2 contains 13 open reading frames that code for proteins of 50 or more amino acids (not shown). Five main open reading frames were assigned a putative function (Figure 2B) through comparison with pJD4 (Accession No. U20374). There are significant differences between pSJ5.2 and pJD4 in the coding frame for the RepB gene. In pJD4, ORF 5 corresponds to coordinates 2479 – 3507 and corresponds to the RepB gene reported previously (Pagotto et al. 2000). However in pSJ5.2, RepB comprises two ORFs at coordinates 2479–2703 (ORF4) and 3004–3510 (ORF5; Figure 3B). Comparative analysis of the nucleotide sequences of RepB in both plasmids revealed the following: deletion of thymine at position 3162 in pJD4 caused a –1 frameshift resulting in incorrect amino acids and a premature UAA stop codon at coordinate 3117 of pSJ5.2. It is unknown whether translational frameshifting can occur at the RepB gene in pSJ5.2 and if this mechanism results in the production of a functional protein product with enzymatic activity. Nevertheless, if translational frameshifting occurs in the RepB from pSJ5.2, an *in vitro* transcription/translation might detect a protein product similar to that detected in pJD4 (Pagotto et al. 2000) rather than two smaller products. Although speculative, it is possible that this (these) protein(s) might play an important role in recombination during plasmid replication. The ORF 5 in pSJ5.2 has significant homology to the conserved domain of the initiator replication protein (NCBI **pfam 01051**) which possesses nicking-closing (topoisomerase 1- like) activity. This or another nicking activity acting within the 1.8 kb *Bam*H1-*Hind*III fragment from pSJ5.2 could account for the deletion observed within this fragment during the recombination of pSJ5.2 with both N3 and R64 conjugative plasmids to form the N3::pSJ5.2 and R64::pSJ5.2 cointegrates.

4.0 Concluding Remarks

Efficient mobilization of the "Asian" 7.4 kb and "African" 5.6 kb beta-lactamase from *N. gonorrhoeae* is due to the presence of a region containing *mobA* / *mobC* and an intergenic *oriT* located in the 3.2 kb *Bam*H1-*Hind*III fragment. The "Toronto" 5.2 kb R-plasmid lacks this mobilization region; nevertheless transfer with the help of the gonococcal 41 kb *tet*(*M*) and IncII α conjugative plasmids has been reported (Marquez et al., 1999; Dillon et al., 1991). Mobilization assays with several conjugative plasmids from Enterobacteriaceae and the gonococcal *tet*(*M*) plasmid revealed that transfer of the 5.2 kb R-plasmid pSJ5.2 occurs by recombination (cointegration) with the conjugative plasmids. Cointegration of pSJ5.2 by *tet*(*M*) involved the acquisition of an Insertion Sequence 1 located in the 2.4 kb *Bam*H1-*Bam*H1 fragment. Another cointegration event was identified with conjugative plasmids N3 and R64 occurring within the 1.8 kb *Bam*H1-*Hind*III fragment from pSJ5.2, resulting in a deletion within the fragment. Stable cointegrates with conjugative plasmids N3 and R64 were recovered for further study. To our knowledge, this is the first report of a naturally-occurring small resistance plasmid that employs different conjugative plasmids for cointegrate formation at different sites for its mobilization. This repertoire of cointegrate events appear rudimentary, yet they are efficient and highly evolved mechanisms for the pSJ5.2 transfer. The sequence of pSJ5.2 was completed and the molecular bases for all the recombination events are currently being studied.

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(A) pSJ5.2 cointegrates with conjugative plasmids R64 and N3**Agarose gel electrophoresis****Southern Blot hybridization
with *Pst*I-linearized pSJ5.2** **(B) pSJ5.2 insertion derivatives obtained with tet(M)** **Figure 1.**

Cointegrates and insertion derivatives of pSJ5.2 formed in *E. coli* during mobilization. The left panel on both (A) and (B) refer to the separation of plasmids by agarose gel electrophoresis. The right panel corresponds to Southern Blot hybridization using a *Pst*I-linearized pSJ5.2 probe. (A) Formation of R64::pSJ5.2 and N3::pSJ5.2 cointegrates. Vertical thick lane divides transconjugants obtained with R64 (left) from N3 (right). (B) Plasmid DNA profiles of pSJ5.2 insertion derivatives formed upon transfer of the plasmid by the 41 kb *tetM* conjugative plasmid. Refer text for details. (oc = open circular DNA; ccc = covalently-closed circular DNA).

Frame	strand	Coordinates (bp)	Size (aa)	Putative function	Nucleotide match		Accession
					Identity	E	
ORF 1	+	3908-4438	133	Tn3 resolvase	99 %	0.0	<u>AM040706</u>
ORF 2	+	4621-320	221	TEM-1 Beta lactamase	100 %	0.0	<u>AB194682</u>
ORF 3	-	622-1608	178	replicase	99 %	0.0	<u>M31727</u>
ORF 4	-	2479-2703	74	replicase ¹	99%	10 ⁻¹⁰⁷	<u>NGU20374</u>
ORF 5	-	3004-3510	114	replicase ²	99%	0.0	<u>NGU20374</u>

¹ Similar to coordinates 2479-2703 of pJD4 RepB (Pagotto et al. 2000).
² Similar to coordinates 3117-3462 of pJD4 RepB (Pagotto et al. 2000).

Figure 2.
(A) Linear open reading frame map of pSJ5.2 (below) and comparison with pJD4 (Accession No. **NGU20374**). Maps are not drawn in scale. Arrowheads indicate direction of transcription of open reading frames. Restriction sites for the enzymes used in the study are shown in red. The location of hot-spot sites for pSJ5.2 recombination with conjugative plasmids R64, N3 and tet(M) are indicated in asterisks (Refer text for details). (B) Description of putative ORF products in pSJ5.2.

Table 1.

Plasmids and bacterial strains used in the study

Plasmids	Relevant characteristics/ genotype	Reference or source
Bacterial strains		
<i>N. gonorrhoeae</i> 15315	Donor strain providing the conjugative 41 kb <i>tetM</i>	Morse et al. 1986
<i>E. coli</i> DH10B	Host for pSJ5.2 (amp ^R) and conjugative plasmids / F ⁻ <i>recA1</i> <i>hsdR rpsL</i> (Str ^R)	Invitrogen
<i>E. coli</i> DH5α	Recipient strain for filter mating experiments / F ⁻ <i>recA1</i> <i>endA1</i> <i>gyrA96</i> (Nal ^R)	Invitrogen
<i>E. coli</i> HMS174	Recipient strain for filter mating experiments / F ⁻ <i>recA1</i> <i>hsdR19 rpoB331</i> (Rif ^R)	Yale Stock Collection
Conjugative plasmids		
pSU2007	IncW ⁽¹⁾ , Tra _{R388} ⁽²⁾ , Km ^R , 32 kb	Martínez and De la Cruz, 1998
pOX38Cm	IncFI , Tra _F , Cm ^R , 55 kb	V. Francia (unpublished)
R64 <i>drd-33</i>	IncIIα , Tra _{R64} , Tc ^R , 122 kb	Komano et al. 1990
N3	IncN , Tra _N , Tc ^R , 38 kb	Brown et al. 1984

¹ Incompatibility Group² Transfer System