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Transfer of Conjugative Elements from Rumen and Human *Firmicutes* Bacteria to *Roseburia inulinivorans*▽

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Studies on *Firmicutes* bacteria from the gut are hampered by a lack of gene transfer systems. Here the human colonic anaerobe *Roseburia inulinivorans* A2-194 was shown to be a transfer recipient for two conjugative transposons, Tn*1545* from *Eubacterium cellulosolvens* and Tn*K10* from *Clostridium saccharolyticum* K10.

The two most abundant bacterial phyla that inhabit the human colon are the low-G+C-content gram-positive *Firmicutes* and the gram-negative *Bacteroidetes* (13, 25). Recent progress in understanding the ecological roles of gram-positive *Firmicutes* in the human large intestine (4, 11) may soon be supplemented by draft genome sequences. Bacteria related to *Roseburia* spp. and *Eubacterium rectale* are an important group of *Firmicutes* belonging to the *Clostridium coccoides* cluster (also known as clostridial cluster XIVa) that contribute to polysaccharide breakdown in the colon and are major producers of butyrate (1, 10). This group can comprise up to 10% of the total bacterial diversity in the human colon (1). The species *Roseburia inulinivorans* is metabolically versatile, utilizing inulin, starch, and fucose for growth (9, 19, 22). While progress in understanding gene expression responses in *R. inulinivorans* using transcriptomics is being made (22), genetic manipulation methods would clearly enhance the ability to investigate gene function. Some progress has been made with the introduction of transposon and plasmid vectors in the rumen species *Butyrivibrio fibrisolvens* (5, 14, 20), but very little work with related gram-positive strict anaerobes from the human gut has been reported.

Commensal bacteria are known to harbor antibiotic resistance genes and thus contribute to the reservoir of resistance genes in the gut that can potentially be acquired by pathogenic members of the gut microbial community. Several new tetracycline resistance genes have been identified recently in gram-positive anaerobes isolated from the bovine rumen and human colon (3, 16). The transfer of some of these resistance genes was linked to their carriage on conjugative transposons (CTns), and these elements may offer the potential for genetic manipulation. Tn*B1230*, originally identified in the rumen anaerobe *B. fibrisolvens* (20), is a 45- to 50-kb transposon with some similarity to the *Enterococcus faecalis* transposon Tn*1549* (17). Repeated attempts to transfer Tn*B1230* from *B. fibrisolvens* into *R. inulinivorans* were unsuccessful despite the high frequencies of transfer between *B. fibrisolvens* strains observed previously (20). We have, however, been able to transfer another novel CTn, Tn*K10*, previously identified in a human isolate related to *Clostridium saccharolyticum* (16), into *R. inulinivorans* A2-194, and transfer with Tn*1545*, originally identified in *Streptococcus pneumoniae*, was also successful (8). Details of these transfer experiments are presented below.

Transfer of the CTn Tn*1545*. Tn*1545*, which carries the *tet*(M) gene, was transferable from the rumen *Eubacterium cellulosolvens* strain 5484 to a spontaneous rifampin-resistant mutant of *R. inulinivorans* A2-194 (A2-194^R), at frequencies of 10^{-5} transconjugants per donor cell. Anaerobic filter matings followed the method of Hespell and Whitehead (14), modified as described previously (20). Parent cells were grown anaerobically in M2GSC medium lacking antibiotics overnight to stationary phase prior to mating. Potential transconjugants grew on selective M2GSC agar plates containing rifampin (100 μg ml^{-1}) and tetracycline (10 μg ml^{-1}) for 2 days, and their resistance phenotypes were confirmed by replating selected colonies on double-antibiotic plates. Subsequent stocks of transconjugants were prepared from single colonies. The 16S rRNA gene from each transconjugant was amplified by PCR using eubacterial primers fD1 and rP2 (28), and limited sequencing of the amplicons confirmed that transconjugants were *R. inulinivorans* isolates.

Chromosomal DNA isolated from transconjugants was subjected to restriction enzyme digestion, separated by pulsed-field gel electrophoresis (PFGE), Southern blotted, and hybridized to a *tet*(M) probe as described previously (3, 20). Single insertion of Tn*1545* was predicted to give one hybridizing fragment following restriction with HindIII [which cleaves 63 nucleotides into *tet*(M) but not within the probe sequence] and two hybridizing fragments following cleavage at the ClaI site, located at 913 nucleotides in *tet*(M), within the probe sequence. Six different complex hybridization profiles were observed with 12 *R. inulinivorans* transconjugants when the ClaI and HindIII hybridization patterns were compared (Fig. 1, lanes 2, 4, 6, 9, 11, and 12). The lower band (~2.5 kb) present in all transconjugants is due to the presence of an additional ClaI site upstream of *tet*(M), within Tn*1545* (Fig. 1a). Tn*1545* was shown previously to insert into different sites in five *E. cellulosolvens* transconjugants (2) and to target AT-rich regions in the genome (15, 27).

DNA sequences flanking the left end of Tn*1545* were ob-

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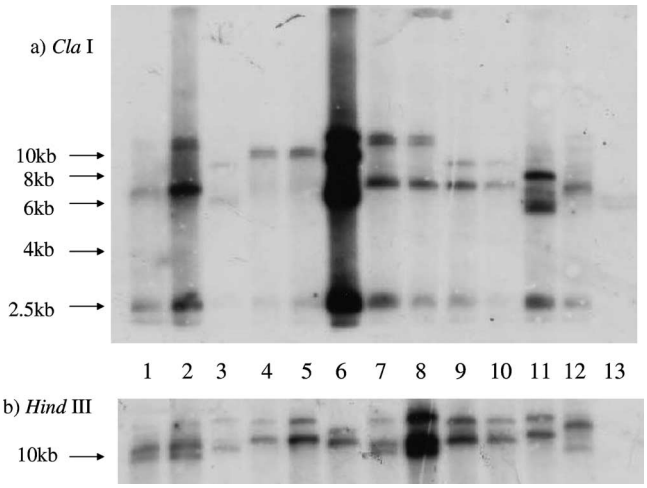

FIG. 1. Southern blot of genomic DNA purified from *R. inulinivorans* A2-194 transconjugants containing Tn*1545*, digested with ClaI (a) or HindIII (b). Lanes 1 to 12, selected transconjugants; lane 13, recipient strain. The sizes of key marker bands are indicated on the left. Unique patterns are represented by transconjugants in lanes 2, 4, 6, 9, 11, and 12.

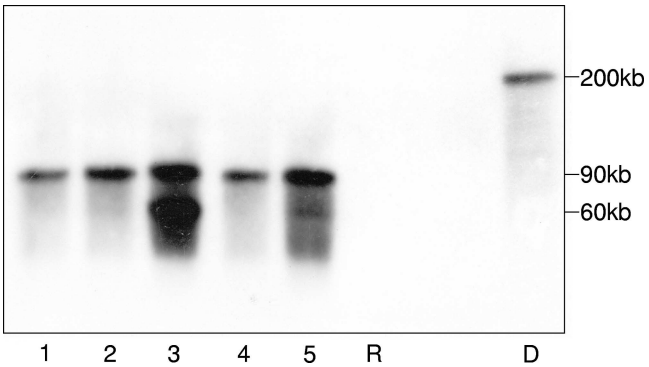

FIG. 2. Southern blot of a representative PFGE gel hybridized to the internal *tet*(32) sequence. R, recipient *R. inulinivorans* A2-194; D, donor *C. saccharolyticum* K10; lanes 1 to 5, representative transconjugants containing Tn*K10*. The sizes of key bands are indicated on the right.

tained for seven transconjugants with six distinct banding patterns. Chromosomal DNA was partially digested with Sau3A, and the 0.5- to 1-kb fraction was ligated into a pBluescript vector (Stratagene) and amplified with M13 specific primers in combination with primers located ~100 bp from each end of Tn*1545* based on the sequence published by Caillaud and Courvalin (6) (Tn1545left, 5′ AGCTCATTCATAAGTAGT 3′; Tn1545right, 5′ CGTGAAGTATCTTCCTACAG 3′). The resulting amplicons were purified and sequenced directly using primer Tn1545left. All seven sequences were identical up to and following the left end of Tn*1545* (6), and the same sequence was also obtained for the donor *E. cellulosolvens* strain. The transferred DNA therefore included DNA in addition to Tn*1545* that was presumably derived from *E. cellulosolvens*. The most plausible explanation is that Tn*1545* became part of a larger conjugal transfer element upon integration into the *E. cellulosolvens* donor strain and that this larger element is able to insert at more than one site upon transfer to *R. inulinivorans*.

Transfer of the CTn Tn*K10*. The human gut isolate *C. saccharolyticum* K10 harbors the transmissible tetracycline resistance gene *tet*(O/32/O) and also a *tet*(W) gene (16, 24). Transfer of *tet*(O/32/O) only was detected in matings with the rumen recipient strain *B. fibrisolvens* 2221^{R} (16). *tet*(O/32/O) was therefore postulated to be carried on the CTn Tn*K10*, which had a common insertion site in two transconjugants analyzed (16). Since the two bacteria capable of harboring Tn*K10* belong to clostridial cluster XIVa, this transposon was considered a promising candidate for further experiments on transfer to *R. inulinivorans* A2-194.

Transfer of tetracycline resistance occurred between *C. saccharolyticum* K10 and the recipient *R. inulinivorans* A2-194^{R}, at frequencies of 10^{-6} transconjugants per donor cell. Gene transfer was confirmed by PCR amplification of the *tet*(O/32/O) gene using a primer pair specific for the internal *tet*(32) region (18) [tet (32)for, 5′-AACCGAAGCATACCGCTC-3′; tet(32)rev, 5′-CTC TTTCATAGCCACGCC-3′]. We were unable to estimate the size of Tn*K10* based on PFGE analysis (data not shown). However, sequencing of homologous elements indicated that Tn*K10* is likely to be larger than 21 kb (EMBL database accession number AM419751; M. Rincon, personal communication). The band profile on a PFGE gel was immobilized by Southern blotting, and the blot was hybridized to a radioactively labeled *tet*(O/32/O) probe. The restriction enzyme SalI does not cut internally in *tet*(O/32/O), and a single insertion of Tn*K10* should result in a single hybridizing band. A 90-kb band hybridized to the *tet*(O/32/O) probe in all five of the transconjugants analyzed, with an additional 60-kb band present in two transconjugants (Fig. 2, lanes 3 and 5). This implies that Tn*K10* has a preferred insertion site in *R. inulinivorans* but that insertions into a second site can also occur. A much larger single band of about 200 kb hybridized in the donor genome but was absent in the transconjugants.

Conclusions. The rumen *B. fibrisolvens* and *E. cellulosolvens* and human *R. inulinivorans* isolates studied here all belong to clostridial cluster XIVa (7), the single most abundant phylogenetic group of bacteria resident in both the human colon (25) and the rumen (26). Through this and previous work (21), we have shown that transfer of antibiotic resistance genes occurs in both directions between rumen and human gut representatives of this important group of gut *Firmicutes*. Previous evidence has shown that rumen and human gut representatives of the major gram-negative phylum, the *Bacteroidetes*, can also exchange resistance genes (23). Transfer of Tn*K10* and Tn*1545* between unrelated gut anaerobes was easily detected, but repeated attempts to transfer the rumen CTn Tn*B1230* to *R. inulinivorans* A2-194 were unsuccessful. Interestingly, Tn*B1230* transfers at extremely high frequencies between two phylogenetically distinct strains of *B. fibrisolvens* (12, 20), and the lack of transfer to *R. inulinivorans* A2-194 presumably reflects the restricted host range of Tn*B1230*. In fact, the donor, *B. fibrisolvens* 1.230, is phylogenetically more closely related to *Roseburia* species than to the recipient, *B. fibrisolvens* 2221^{R} (4).

Genetic manipulation of bacteria within clostridial cluster XIVa would enhance the ability to investigate the function of specific genes, and it was hoped that one of the CTns studied here would enable transposon mutagenesis in this group. Tn*K10* showed preferential insertion in *R. inulinivorans* but

remains of interest as a potential delivery system, once more sequence information is available. Although Tn*1545* apparently had multiple insertion sites in the *R. inulinivorans* genome, this was inferred to be due to its transfer as part of a larger element, whose structure and behavior require further investigation. Genomic sequencing of the donor and recipient bacteria may prove the most effective approach to clarifying the transfer mechanisms of Tn*1545* and Tn*K10*.

In conclusion, this work demonstrates conjugal gene transfer in laboratory matings with a strictly anaerobic *Firmicutes* bacterium from the human gastrointestinal tract as the recipient, thus hopefully providing a first step toward genetic manipulation in this important group of bacteria. These experiments emphasize the extent of gene flow between predominant groups of gut bacteria.

Nucleotide sequence accession number. The accession number for the 16S rRNA sequence of *C. saccharolyticum* K10 is EU305624.

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