JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2007, p. 402–408 Vol. 45, No. 2
0095-1137/07/$08.00+0 doi:10.1128/JCM.01456-06

Conjugative Transfer of Chromosomally Encoded Antibiotic Resistance from *Helicobacter pylori* to *Campylobacter jejuni*▽

Omar A. Oyarzabal,[1] Roland Rad,[2] and Steffen Backert[3]*

Department of Poultry Science, Auburn University, Auburn, Alabama 36849[1]; Department of Internal Medicine and Gastroenterology, Technical University of Munich, Munich, Germany[2]; and Department of Medical Microbiology, Otto von Guericke University Magdeburg, Leipziger Str. 44, D-39120 Magdeburg, Germany[3]

Received 14 July 2006/Returned for modification 18 September 2006/Accepted 15 November 2006

Many strains of *Helicobacter pylori* are naturally competent for transformation and able to transfer chromosomal DNA among different isolates using a conjugation-like mechanism. In this study, we sought to determine whether *H. pylori* can transfer DNA into *Campylobacter jejuni*, a closely related species of the *Campylobacterales* group. To monitor the transfer, a chromosomally encoded streptomycin resistance cassette prearranged by a specific mutation in the *rpsL* gene of *H. pylori* was used. Mating of the bacteria on plates or in liquid broth medium produced *C. jejuni* progeny containing the streptomycin marker. DNA transfer was unidirectional, from *H. pylori* to *C. jejuni*, and the progeny were genetically identical to *C. jejuni* recipient strains. DNase I treatment reduced but did not eliminate transfer, and DNase I-treated cell supernatants did not transform, ruling out phage transduction. Recombinants also did not occur when the mating bacteria were separated by a membrane, suggesting that DNA transfer requires cell-to-cell contact. Transfer of the streptomycin marker was independent of the *H. pylori comB* transformation system, the *cag* pathogenicity island, and another type IV secretion system called *tfs3*. These findings indicated that a DNase I-resistant, conjugation-like mechanism may contribute to horizontal DNA transfer between different members of the *Campylobacteriales* group. The significance of this DNA uptake by *C. jejuni* in the context of acquiring antibiotic resistance is discussed.

Within the *Epsilonproteobacteria*, the order *Campylobacteriales* comprises two families, the *Helicobacteraceae* and the *Campylobacteraceae*. *Helicobacter pylori* and the enterohepatic species *Helicobacter hepaticus* are classified as cancer-causing microorganisms because the infections they produce can lead to gastric cancer in humans and liver cancer in rodents, respectively. However, not every infection leads to disease development, and *H. pylori* can persist in the human stomach asymptomatically. *Campylobacter jejuni* is one of the main causes of bacterial food-borne illness worldwide. It is also the most common microbial antecedent to Guillain-Barré syndrome and persists as a commensal in avian hosts. The genus *Wolinella* is represented by *Wolinella succinogenes*, a bacterium that persists as a commensal in the gastrointestinal tracts of cattle by utilizing metabolites present in the rumen.

There are presently 11 complete genome sequences available from the *Campylobacteriales* group: six from the *Helicobacteraceae*—three distinct strains of *H. pylori*, 26695, J99, and HPAG1 (2, 3, 44, 53); *Helicobacter acinonychis* (17); *W. succinogenes* DSM 1740 (5); and *H. hepaticus* ATCC 51449 (51)—and five from the *Campylobacteraceae*—*C. jejuni* NCTC 11168 and RM1221, *Campylobacter lari* RM2100, *Campylobacter upsaliensis* RM3195, and *Campylobacter coli* RM2228 (22, 46). The availability of these genomes has allowed the study of genome rearrangements that have taken place since these bacteria diverged from their last common ancestor. These ongoing dynamic processes and the level of genome plasticity have been investigated on an intraspecies level, as well as on an interspecies level (2, 18). It is known that horizontal DNA transfer among bacteria is a major factor contributing to their genetic variability (13, 19, 20, 26, 42). Therefore, elucidating the mechanisms involved in DNA transfer will help us understand the adaptation of *H. pylori* and *C. jejuni* to changing environmental conditions and may have clinical relevance in the area of antibiotic resistance.

The various mechanisms of bacterial DNA transfer include (i) uptake of DNA by genetic transformation (16), (ii) bacteriophage transduction (35), and (iii) conjugative DNA transfer mediated by the so-called type IV secretion system (T4SS) (45). The *H. pylori* chromosome has been shown to encode at least three T4SSs (6, 7), although there is no evidence for either bacteriophage transduction or type IV pilin-like proteins associated with bacterial natural competence in *H. pylori*. The first identified T4SS of *H. pylori* is encoded by the 40-kb cytotoxin-associated gene (*cag*) pathogenicity island (PAI), consisting of up to 31 genes (14). This T4SS represents a major disease-associated determinant for the delivery of virulence factors, such as the CagA protein, into host target cells. The second T4SS of *H. pylori* is the *comB* system, consisting of the *comB2* to *comB4* and *comB6* to *comB10* genes. This system has been shown to mediate the uptake of naked DNA (29). The third T4SS gene cluster found in certain strains of *H. pylori* is a segment named type IV secretion system 3 (*tfs3*), located in one of the two plasticity zones (31). This cluster corresponds to a 16.3-kb DNA segment including up to 16 open reading frames, some of which are homologous to the genes *virB4*, *virB7* to *virB11*, and *virD4* in *Agrobacterium tumefaciens*. No specific function has been ascribed to this putative T4SS.

* Corresponding author. Mailing address: Department of Medical Microbiology, Otto von Guericke University, Leipziger Str. 44, D-39120 Magdeburg, Germany. Phone: 49-391-67-13329. Fax: 49-391-67-290469. E-mail: Steffen.Backert@medizin.uni-magdeburg.de.

▽ Published ahead of print on 29 November 2006.

TABLE 1. *Helicobacter pylori* and *Campylobacter jejuni* strains used in this study

Bacterial strain	Genotype[a]									Phenotype[a]				Origin	Reference
	Plasmid	*cag* PAI	*vacA*	*tfs3*	*comB*	*virB11*	*flaA*	*ciaB*	*cdt*	Str^r	Spt^r	Rif^r	Kan^r		
H. pylori															
26695 wt[b]	−	+	+	−	+					−	−	−	−	United Kingdom	53
P1 wt	−	+	+	−	+					−	−	−	−	Germany	43
1061 wt	−	−	+	−	+					−	−	−	−	Canada	25
26695	−	+	+	−	+					−	−	−	−	United Kingdom	This study
P1	−	+	+	−	+					+	−	−	−	Germany	This study
1061	−	−	+	−	+					+	−	−	−	Canada	This study
26695Δ*ureB*	−	+	+	−	+					+	−	−	+	United Kingdom	This study
1061Δ*ureB*	−	−	+	−	+					+	−	−	+	Canada	This study
P1Δ*ureB*	−	+	+	−	+					+	−	−	+	Germany	This study
P1Δ*comB7-10*	−	+	+	−	−					+	−	−	+	Germany	This study
P1Δ*cag*PAI	−	−	+	−	+					+	−	−	+	Germany	This study
C. jejuni															
1543	−					−	+	+	+	−	+	+	−	Germany	This study
ST3046	−					−	+	+	+	−	+	+	−	Germany	This study
81-176	+[c]					+	+	+	+	−	+	+	−	United States	11, 32

[a] +, present; −, absent.
[b] wt, wild type.
[c] Plasmid pVir is approximately 35 kb in size (9).

Although many *H. pylori* strains are naturally competent for transformation (29) and conjugation-like chromosomal-DNA transfer in vitro (33), the mechanisms for genetic exchange among *H. pylori* strains in nature remain enigmatic. The aim of this study was to further characterize the process of conjugative chromosomal DNA transfer in *H. pylori* and to examine whether *H. pylori* is able to transfer DNA into other related bacteria, such as *C. jejuni.* We show here that *H. pylori* is capable of transferring chromosomally encoded streptomycin resistance into *C. jejuni* by a conjugation-like mechanism in a manner independent of the three previously identified T4SSs.

MATERIALS AND METHODS

Bacterial strains. The wild-type and mutant *H. pylori* strains used in this study, as well as their origins, are listed in Table 1. These strains were selected because they do not contain any plasmids. This attribute was verified by plasmid preparations using the Wizard Plus SV Minipreps purification system (Promega) as described by Hofreuter and Hass (28). *H. pylori* was grown on solid or in liquid medium. The solid medium consisted of GC agar plates supplemented with 10% horse serum (Biochrom, Berlin, Germany), 10 μg/ml of vancomycin, 1 μg/ml of nystatin, and 5 μg/ml of trimethoprim (8). Antibiotics were purchased from Sigma-Aldrich (Deisenhofen, Germany). The liquid medium consisted of brain heart infusion (BHI) broth with 10% horse serum. Incubation was performed at 37°C for 2 days in an anaerobic jar under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) generated by CampyGen (Oxoid, Wesel, Germany). *C. jejuni* strains (Table 2) were grown on *Campylobacter* Blood-Free Agar Base containing *Campylobacter* Growth Supplement (Oxoid) at 37°C under microaerophilic conditions for 48 h. *C. jejuni* 81-176 was isolated from an outbreak of campylobacteriosis and has been shown to cause disease in human volunteers (11, 32). Strains 1543 and ST3046 were isolated from feces of patients with diarrhea at the Institute of Medical Microbiology (Magdeburg, Germany).

Mating experiments on GC agar plates. Mating experiments were carried out using plasmid-free *H. pylori* strains. We introduced chromosomal antibiotic resistance markers into various strains using the mating experiments on solid medium described by Kuipers et al. (33). The markers were streptomycin resistance (Str^r) and streptomycin resistance/kanamycin resistance (Str^r/Kan^r). To easily monitor transfer of the Str^r marker, we selected *C. jejuni* strains 1543, ST3046, and 81-176 because they are naturally resistant to spectinomycin (Spt^r) and rifampin (Rif^r) (Table 1). The frequency of DNA transfer was assessed by quantitating the number of Str^r/Spt^r doubly resistant recombinants per parent. Each mating experiment involved one *H. pylori* and one *C. jejuni* strain, represented by A and B, respectively, with mutually exclusive antibiotic resistance markers. After 12 h of growth on GC agar, the bacterial growth was harvested and suspended in 3 ml of BHI broth. The number of bacteria was calculated from a standard curve of optical densities at 600 nm. Matings were assayed by mixing strains A and B containing known resistances (Table 1). Aliquots of 100 μl of the bacterial suspension (~1×10^9 bacteria) from each strain were plated on four GC agar plates in the following order: plate 1, strain A; plate 2, strain B; plates 3 and 4, strain A and strain B together. Plates 3 were supplemented with DNase I (Roche, Mannheim, Germany) and $MgCl_2$ at final concentrations of 200 μg/ml and 2 mM, respectively. In the control experiments, the bacteria were incubated in the presence of 0.1-μm-pore-size membranes (Millipore, Schwalbach, Germany) that blocked cell-to-cell contact between the donor and recipient strains. After incubation overnight, bacteria were harvested and suspended in 1 ml of BHI broth. The suspensions from plates 1 were serially diluted, and 100 μl of 10^{-5}, 10^{-6}, and 10^{-7} dilutions were inoculated on GC agar plates without antibiotics. To exclude the occurrence of spontaneous mutants, aliquots of 200 μl of the undiluted samples from plates 1 and 2 were plated on GC agar plates containing 10 μg per ml each of streptomycin and spectinomycin (GCSS plates). Undiluted suspensions of each individual sample of plates 3 and 4 were inoculated as follows: 200 μl on a GC agar plate without antibiotics and 250, 100, and 25 μl on three GCSS plates. All plates were incubated under microaerophilic conditions for 96 h, after which the colonies were counted. Single colonies from GCSS plates were subcultured onto GC agar plates without antibiotics. To determine the direction of DNA transfer, bacteria were cultured on GC agar plates containing either 10 μg of kanamycin per ml or 10 μg of rifampin per ml.

TABLE 2. Effect of DNase I treatment on the frequency of recombination between *H. pylori* and *C. jejuni* strains

Strain(s)[a]	Treatment with DNase I (200 μg/ml)[b]	Transconjugants	
		Spt^r/Str^r (no. of CFU)	Relative %[c]
H. pylori alone	−	0	0
C. jejuni alone	−	0	0
H. pylori + *C. jejuni*	−	3.4×10^{-7}	100
H. pylori + *C. jejuni*	+	4.4×10^{-8}	13[d]

[a] Plate matings were done in triplicate experiments. *H. pylori* P1 wild type (Str^r); *C. jejuni* 1543 wild type (Spt^r).
[b] +, treated; −, untreated.
[c] Relative to *H. pylori* plus *C. jejuni* without DNase I.
[d] $P < 0.01$ in comparison with *H. pylori* plus *C. jejuni* without DNase I (Mann/Whitney test).

DNA exchange using cell extracts, purified total DNA, or heat-inactivated bacterial cells. Mating experiments of the *C. jejuni* recipient strain with either cell extracts, purified DNA (2 μg), or heat-inactivated *H. pylori* cells were performed as described above. The total DNA of strain P1 was isolated using a genomic-DNA preparation kit (Roche, Mannheim, Germany). Cell extracts from 1×10^9 *H. pylori* cells were prepared by five freeze-thaw steps, followed by passage through a 0.2-μm sterile filter (Roth, Germany). Heat-inactivated *H. pylori* cells were prepared by incubation at 80°C for 10 min. These experiments were done in the presence and absence of 200 μg/ml DNase I and 2 mM $MgCl_2$. After 12 h of incubation, bacteria from each of the suspensions were inoculated on nonselective and selective plates as described above.

Random amplified polymorphic DNA (RAPD) fingerprinting, PCR of bacterial marker genes, and sequencing of the transferred *rpsL* gene. Chromosomal DNA was prepared from wild-type and recombinant bacteria using a genomic-DNA preparation kit (QIAGEN). RAPD PCRs were carried out in 25-μl mixtures that contained 20 ng genomic *H. pylori* or *C. jejuni* DNA, 3 mM $MgCl_2$, 250 μM deoxynucleotide triphosphates, 1 unit of *Taq* polymerase in 1× buffer (QIAGEN), 30 pmol of the RAPD primer D9355 under cycling conditions described previously (1). The PCR products were resolved in 1.0% agarose gels and visualized by staining them with ethidium bromide.

Because the streptomycin resistance in *H. pylori* is mediated by a single point mutation (K43R) in the *rpsL* gene product (21), the *rpsL* gene of the recombinant progeny was sequenced using standard procedures. For this purpose, a 346-bp fragment specific for *H. pylori rpsL* was amplified using the following primers: RpsL-*fwd* (5′-GAA AAG AAA GGA AAA AGG TGG-3′) and RpsL-*rev* (5′-GCT TTA GTC TTT TTA GTC CCG-3′). Chromosomally encoded Str^r clones of *H. pylori* were selected after transformation with the suicide plasmid pEG21, which carries the point mutation in the *rpsL* gene, and by growing clones on GC agar plates containing 10 μg/ml of streptomycin (21, 33).

Genotyping for the presence of *cagA* (*H. pylori*) and *cadF* (*C. jejuni*) genetic markers in the donors, recipients, and recombinants was done by PCR (24). The following primers were used: CagA-*fwd* (5′-AAA GGA TTG TCC CTA CAA GAA GC-3′) and CagA-*rev* (5′-GTA AGC GAT TGC TCT TGC ATC-3′) (a 330-bp fragment), and CadF-*fwd* (5′-TTG AAG GTA ATT TAG ATA TG-3′) and CadF-*rev* (5′-CTA ATA CCT AAA GTT GAA AC-3′) (a 377-bp fragment). The resulting PCR-amplified products were analyzed by standard agarose gel electrophoresis. We also examined the correlation between the conjugation phenotype and the presence of specific T4SS genes of *H. pylori* that may be involved in DNA transfer. Specific knockout mutants were generated by integration of *aphA3* (Kan^r) in the respective chromosomal genes (Table 1) as described previously (8, 33). The integration of the cassette into the genes of interest was confirmed by standard PCR analysis (7, 24, 33).

RESULTS

Chromosomal-DNA transfer between *H. pylori* and *C. jejuni* on GC agar plates. Mating experiments were carried out using plasmid-free *H. pylori* strains (see Materials and Methods) (Table 1). We introduced chromosomal antibiotic resistance markers, such as Str^r or Str^r/Kan^r, into the various strains using procedures described previously (33). To easily monitor the transfer of Str^r, we selected *C. jejuni* strains that are naturally Spt^r/Rif^r (Table 1). Matings were then performed on GC agar plates, and the frequency of DNA transfer was assessed by determining the number of Str^r/Spt^r doubly resistant recombinants per parent. Since no Str^r/Spt^r recombinant was obtained when each strain was incubated alone, the possibility of spontaneous mutation was eliminated. The results showed that *H. pylori* strain P1 and *C. jejuni* strain 1543 gave rise to Str^r/Spt^r doubly resistant recombinants at frequencies of approximately 3×10^{-7} to 4×10^{-7} per donor (Table 2). The addition of 200 μg DNase I per ml decreased this number to approximately 4×10^{-8} to 5×10^{-8} per donor. However, the presence of DNase I was unable to prevent the development of doubly resistant *C. jejuni* recombinants (Table 2). Similar results were obtained for other mating combinations (Table 3). The relative percentage of doubly resistant mutants observed after bacteria mated in the presence or absence of DNase I varied only slightly (10 to 13%) for all of the different combinations. Thus, both DNase I-sensitive and DNase I-resistant events occurred at stable frequencies under the experimental conditions.

TABLE 3. Frequency of recombination between different *H. pylori* and *C. jejuni* strains

H. pylori strain (Str^r Kan^r)	Treatment with DNase I (200 μg/ml)[a]	No. of *C. jejuni* transconjugants (Spt^r/Str^r) (CFU)		
		1543	81-176	ST3046
P1Δ*ureB*	−	3.6×10^{-7}	6.8×10^{-8}	2.8×10^{-7}
P1Δ*ureB*	+	4.2×10^{-8}	1.2×10^{-8}	3.3×10^{-8}
26695Δ*ureB*	−	2.3×10^{-7}	8.4×10^{-8}	7.9×10^{-8}
26695Δ*ureB*	+	2.1×10^{-8}	2.2×10^{-8}	6.4×10^{-9}
1061Δ*ureB*	−	1.9×10^{-7}	5.7×10^{-8}	9.5×10^{-8}
1061Δ*ureB*	+	3.1×10^{-8}	7.9×10^{-9}	1.2×10^{-8}

[a] +, treated; −, untreated.

Unidirectional chromosomal-DNA transfer from *H. pylori* into *C. jejuni*. Single recombinant colonies grew only on rifampin-supplemented GC agar plates, but not on kanamycin-containing plates, which suggested that DNA transfer occurred from *H. pylori* into *C. jejuni* and not vice versa (Fig. 1A to F). This unidirectional DNA transfer from *H. pylori* into *C. jejuni* was also confirmed by the growth of the recombinants on blood-free *Campylobacter* selective agar base and by the almost identical RAPD-PCR fingerprinting patterns observed between the recombinants and the parental *C. jejuni* strain (Fig. 1B and E). Moreover, PCR of a 346-bp *rpsL* gene product using *H. pylori*-specific primers confirmed that this gene was successfully transferred from *H. pylori* into the *C. jejuni* recombinants, because it is absent in the corresponding *C. jejuni* parental strains (Fig. 1C and F). Sequencing of these PCR products revealed the *H. pylori rpsL* gene sequence (data not shown). In addition, we performed control PCRs for species-specific genes, including well-known virulence/pathogenicity factors of *H. pylori* (*cagA*) and *C. jejuni* (*cadF*). The results demonstrate the presence of *cadF* in the *C. jejuni* parents and in all recombinants, while *cagA* was present in the *H. pylori* parents but absent in the recombinants (Fig. 1C and F). These data were also in agreement with the identities of strains as described above.

Characterization of the chromosomal-DNA transfer between *H. pylori* and *C. jejuni*. Matings were then carried out in 1 ml BHI broth, which routinely produced fewer recombinants than matings performed on GC plates (Table 4). This suggested that the exchange of chromosomal DNA requires close cell-to-cell contact, somehow reminiscent of bacterial conjugation (6, 33, 45). Indeed, no DNA transfer was detected when parents were separated by a 0.1-μm-pore-size membrane that excluded cell-to-cell contact or when *C. jejuni* was incubated with a cell-free lysate of the parental *H. pylori*. In addition, a relatively low number of *C. jejuni* recombinants resulted from the incubation of *C. jejuni* with purified total DNA from *H. pylori* strain P1, and no DNA transfer was detected when the *H. pylori* parent strain was heat inactivated prior to the mating experiment (Table 4).

Roles of specific T4SS genes in chromosomal-DNA transfer from *H. pylori* into *C. jejuni*. Finally, we examined the correlation between the conjugation phenotype and the presence of

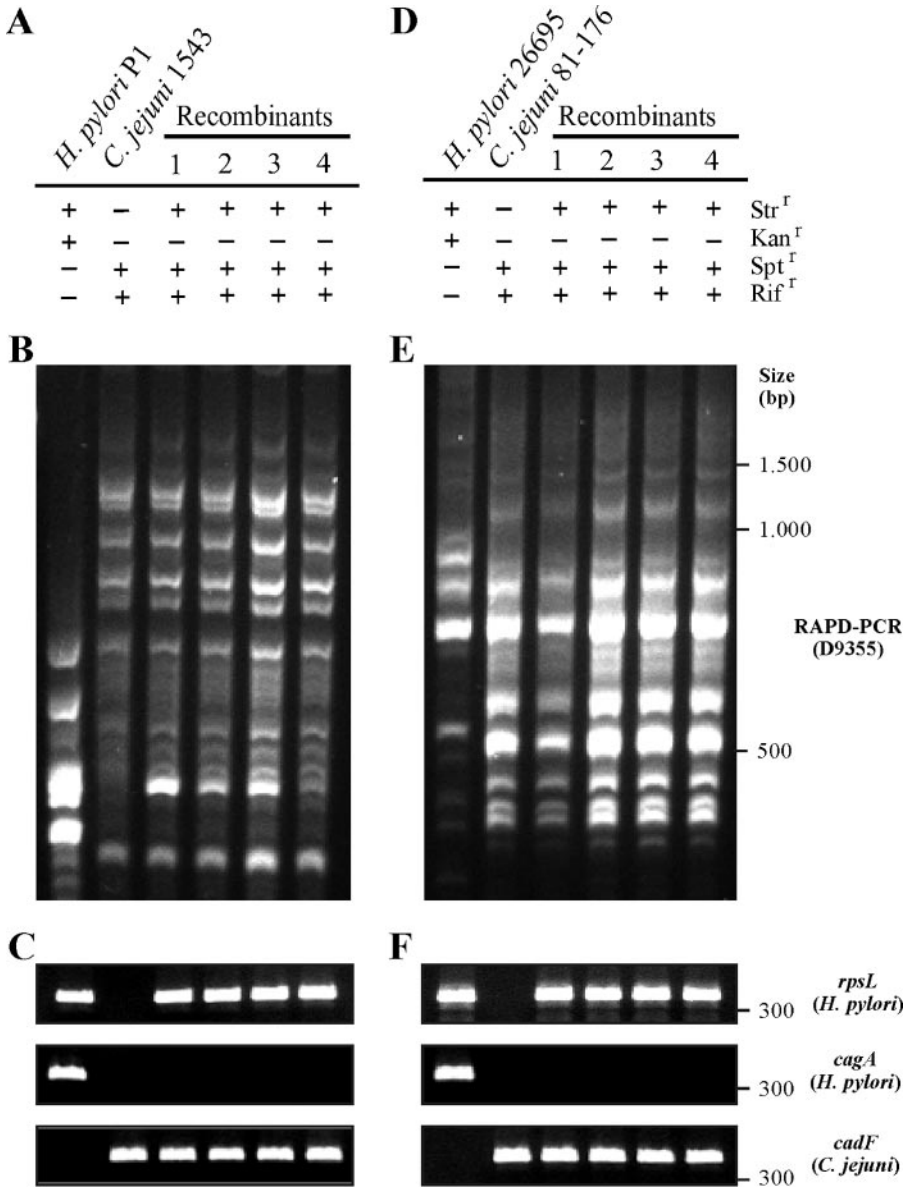

FIG. 1. RAPD-PCR analysis of *H. pylori* and *C. jejuni* recombinants obtained from mating between the indicated parental strains (A and D) using primer D9355 (B and E). Antibiotic resistance phenotypes of selected Str^r/Spt^r doubly resistant recombinants are shown. Agar plates contained 10 μg/ml streptomycin, 10 μg/ml spectinomycin, 8 μg/ml kanamycin, or 10 μg/ml rifampin. A mixture of progeny showed profiles identical to that of the *C. jejuni* parental strain, which is consistent with unidirectional DNA transfer from *H. pylori* into *C. jejuni*. (C and F) Control PCRs for the indicated *H. pylori* and *C. jejuni* genes.

specific T4SS genes that may be involved in the DNA transfer initiated by *H. pylori*. All *H. pylori* strains used in this study lack most of the reported *tfs3* genes (7) (Table 1), which supports the view that *tfs3* is not involved in the DNA transfer. Inactivation of individual *H. pylori comB* genes (Δ*comB7-10*) in the mating donor led to a reduction in, but did not prevent, DNA transfer to the *C. jejuni* recipient (Table 5). Matings of isogenic *H. pylori* mutants lacking the entire *cag* PAI also yielded high numbers of recombinants (Table 5). Taken together, these data suggest that the *cag* PAI T4SS, *tfs3*, and the *comB* system do not play roles in the described conjugative DNA transfer of *H. pylori* into *C. jejuni*.

DISCUSSION

One of the striking characteristics of *H. pylori* is the extensive genetic diversity found among strains (2, 30, 49, 52). The clinical significance of this genetic diversity is highlighted by the identification of unique genetic features in strains with enhanced virulence (39, 47). Interspecies and intraspecies DNA transfers by transformation contribute to the exchange of genetic material in *H. pylori* and play important roles in the adaptation of the pathogen to different environmental conditions. Evidence also suggests that horizontal DNA transfer among *H. pylori* strains can take place via a DNase-resistant,

TABLE 4. Effect of DNase I treatment on the frequency of recombination in which either *H. pylori* cells or culture supernatants were used as an exogenous DNA source[a]

H. pylori sample (Str^r)	Medium	Treatment with DNase I (200 μg/ml)[b]	*C. jejuni* sample (Spt^r)	*C. jejuni* transconjugants	
				Spt^r/Str^r (no. of CFU)	Relative %[c]
Cells	GC agar	−	Cells	3.6×10^{-7}	100
Cells	BHI broth (1 ml)	−	Cells	1.2×10^{-9}	<1
Cells	GC agar	+	Cells	4.7×10^{-8}	13[d]
Cells	GC agar + membrane	−	Cells	0	0
2 μg total DNA	GC agar	−	Cells	2.1×10^{-9}	<1
2 μg total DNA	GC agar	+	Cells	0	0
Cell extract	GC agar	−	Cells	0	0
Cell extract	GC agar	+	Cells	0	0
Heat-inactivated cells	GC agar	−	Cells	0	0

[a] Matings were done in triplicate experiments. *H. pylori* P1 wild type (Str^r); *C. jejuni* 1543 wild type (Spt^r).
[b] +, treated; −, untreated.
[c] Relative to *H. pylori* plus *C. jejuniwithout* DNase I.
[d] $P < 0.01$ in comparison with *H. pylori* plus *C. jejuni* without DNase I (Mann/Whitney test).

conjugation-like mechanism (7, 33). We continued these studies to determine if clinical *H. pylori* isolates were able to transfer DNA into *C. jejuni*.

The chromosomal DNA transfer detected in this study was clearly unidirectional, from *H. pylori* into *C. jejuni*. The way *H. pylori* transferred chromosomally encoded Str^r to *C. jejuni* was independent of the three previously identified T4SSs, and because no Str^r/Spt^r recombinant was obtained when each strain was incubated alone, the possibility of spontaneous mutation was eliminated. The DNA exchange occurred at a frequency of approximately 10^{-7} to 10^{-8} and was largely independent of the extracellular presence of nucleases, such as DNase I. The uptake of naked DNA by natural transformation played a minor role in the DNA transfer, a finding that was corroborated by the fact that cell extracts or purified DNA from parental strains did not induce a high number of recombinant *C. jejuni* organisms. Analysis of Str^r/Spt^r doubly resistant progeny by resistance to secondary antibiotic markers, RAPD-PCR, and gene-specific PCR confirmed that the DNA transfer was unidirectional. The exact integration site of the *H. pylori rpsL* gene into the *C. jejuni* chromosome, however, needs to be identified and characterized in future studies.

Our experiments suggested that the DNA transfer from *H. pylori* to *C. jejuni* was likely to occur by a conjugative mechanism requiring close cell-to-cell contact between the donor and recipient strains. Since cell-free *H. pylori* extracts did not produce recombinants, we were able to exclude phage transduction, thereby providing more evidence for the existence of a conjugative DNA transfer apparatus in *H. pylori*. Similar processes of chromosomal-DNA transfer without the involvement of conjugative plasmids have been described for *Neisseria gonorrhoeae* (15) and *Legionella pneumophila* (38). We do not know if a classical mechanism of mating-pair formation and DNA transfer was involved in our experimental system (45). However, our current findings suggest that mobilization of chromosomal DNA from *H. pylori* occurs in a fashion similar to the high-frequency recombination described for some *Escherichia coli* strains (37, 50).

TABLE 5. *comB* and *cag* PAI systems of *H. pylori* are not involved in DNA transfer[a]

H. pylori strain (Str^r)	*C. jejuni* strain (Spt^r/Rif^r)	*C. jejuni* transconjugants	
		Spt^r Str^r (no. of CFU)	Relative %[b]
P1 wild type	1543 wild type	4.3×10^{-7}	100
P1Δ*comB7-10*	1543 wild type	3.5×10^{-7}	81
P1Δ*cag*PAI	1543 wild type	3.7×10^{-7}	86

[a] Matings on GC agar plates were done in triplicate experiments.
[b] Relative to *H. pylori* plus *C. jejuni* without DNase.

Although the natural habitats of *H. pylori* and *C. jejuni* in the gastrointestinal tract are distinct, it is conceivable that they may come into contact with each other. Such contact could occur when *C. jejuni* passes the stomach. *H. pylori*, which is continuously eliminated from the stomach by peristaltic movements and washout with the luminal fluid, could in turn lead to contact of *H. pylori* with *C. jejuni* in the intestine. Thus, we propose that the observed mechanism of DNA exchange may contribute to the heterogeneity of *H. pylori* strains and also other bacteria, such as *Campylobacter*, and may help the bacteria adapt to the hostile milieu of the gastrointestinal tract, where free chromosomal DNA is short-lived. Assuming that the transferred single-stranded DNA is unlikely to be recognized by restriction enzymes (4, 36, 41), this conjugative exchange of genetic material could be a means of overcoming interstrain restrictions. An understanding of how *H. pylori* uses its conjugative transfer system during the course of infection will provide important insights into the evolutionary strategies of this clinically important pathogen.

From the standpoint of the recipient, our findings confirm that *C. jejuni* may use the conjugation-like mechanism to acquire DNA from other species. We do not know if this DNA acquisition helps to enhance genetic diversity, to repair bacterial DNA, or other purposes (12). Apparently, *C. jejuni* can use T2SS- and T4SS-related complexes for DNA uptake (9). Orthologues of the *H. pylori* genes *comB8* to *comB10* have been identified in a plasmid from *C. jejuni* (9), and genes involved in competence in *C. jejuni* were shown to have similarities to the T2SS/T4SS-related proteins (54). However, mutation of *comB7* to *comB10* in this organism led to only a small reduction in transformation frequency. These findings may be important in the transmission of antibiotic resistance. For instance, resis-

tance to tetracycline in *Campylobacter* spp. is common in many countries (40, 48), and although a high level of tetracycline resistance is usually associated with the *tet*(O) gene carried on transmissible plasmids, this gene has also been found in the chromosomes of *C. jejuni* and *C. coli* (23, 34). Recently, the sequencing of two plasmids carrying tetracycline resistance genes, pCC31 from *C. coli* and plasmid pTet from *C. jejuni*, revealed an identity of 94.3% between the plasmids—a remarkably high similarity for plasmids from strains isolated 20 years apart and on different continents. All of the genes present in these plasmids had amino acid similarities to the T4SS of different *Brucella* species. However, the highest homology was to a mating-pair formation gene cluster from *Actinobacillus actinomycetemcomitans*, a periodontal pathogen. Only three genes of unknown function were found on one of the plasmids, two of which have known homologues in *H. pylori* (10).

Besides mediating plasmid transfer, some specific T4SS can mediate DNA secretion in *Neisseria gonorrhoeae* (27) and DNA uptake in *H. pylori* (29). It has been postulated that the mechanism of DNA translocation by the *comB* system might be comparable to the transport of transfer DNA from the cytoplasm of *Agrobacterium tumefaciens* into plant host cells by the VirB/D T4SS or by bacterial plasmid conjugation systems (29). When the correlation between the conjugation phenotype and the presence of specific T4SS genes in *H. pylori* was addressed, we surprisingly found that the *cag* PAI T4SS, the *comB* system, and *tfs3* did not play any role in conjugative DNA transfer. The identification of the *H. pylori* genes involved in the DNA transfer is currently under way in our laboratories.

ACKNOWLEDGMENTS

We are grateful to Rainer Haas and Wolfgang Fischer for providing plasmids pEG21 and P1Δ*comB7-10*.

The work of S.B. is supported through the NBL-3 project (Magdeburger Forschungsverbund PFG4) and Priority Program SPP1150 of the Deutsche Forschungsgemeinschaft (Ba1671/3-2).

REFERENCES

1. **Akopyanz, N., N. O. Bukanov, T. U. Westblom, S. Kresovich, and D. E. Berg.** 1992. DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. Nucleic Acids Res. **20:**5137–5142.
2. **Alm, R. A., and T. J. Trust.** 1999. Analysis of the genetic diversity of *Helicobacter pylori*: the tale of two genomes. J. Mol. Med. **77:**834–846.
3. **Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature **397:**176–180.
4. **Aras, R. A., A. J. Small, T. Ando, and M. J. Blaser.** 2002. *Helicobacter pylori* interstrain restriction-modification diversity prevents genome subversion by chromosomal DNA from competing strains. Nucleic Acids Res. **30:**5391–5397.
5. **Baar, C., M. Eppinger, G. Raddatz, J. Simon, C. Lanz, O. Klimmek, R. Nandakumar, R. Gross, A. Rosinus, H. Keller, P. Jagtap, B. Linke, F. Meyer, H. Lederer, and S. C. Schuster.** 2003. Complete genome sequence and analysis of *Wolinella succinogenes*. Proc. Natl. Acad. Sci. USA **20:**11690–11695.
6. **Backert, S., and T. F. Meyer.** 2006. Type IV secretion systems and their effectors in bacterial pathogenesis. Curr. Opin. Microbiol. **9:**207–217.
7. **Backert, S., T. Kwok, and W. König.** 2005. Conjugative plasmid DNA transfer in *Helicobacter pylori* mediated by chromosomally encoded relaxase and TraG-like proteins. Microbiology **151:**3493–3503.
8. **Backert, S., S. Moese, S. Selbach, V. Brinkmann, and T. F. Meyer.** 2001. Phosphorylation of tyrosine 972 of the *Helicobacter pylori* CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. Mol. Microbiol. **42:**631–644.
9. **Bacon, D. J., R. A. Alm, D. H. Burr, L. Hu, D. J. Kopecko, C. P. Ewing, T. J. Trust, and P. Guerry.** 2000. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infect. Immun. **68:**4384–4390.
10. **Batchelor, R. A., B. M. Pearson, L. M. Friis, P. Guerry, and J. M. Wells.** 2004. Nucleotide sequences and comparison of two large conjugative plasmids from different *Campylobacter* species. Microbiology **150:**3507–3517.
11. **Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. J. Blaser.** 1988. Experimental *Campylobacter jejuni* infection in humans. J. Infect. Dis. **157:**472–479.
12. **Chen, I., and D. Dubnau.** 2004. DNA uptake during bacterial transformation. Nat. Rev. Microbiol. **241:**241–249.
13. **Cornelis, G. R., A. Boland, A. P. Boyd, C. Geuijen, M. Iriarte, C. Neyt, M. P. Sory, and I. Stainier.** 1998. The virulence plasmid of *Yersinia*, an antihost genome. Microbiol. Mol. Biol. Rev. **62:**1315–1352.
14. **Covacci, A., J. L. Telford, G. Del Giudice, J. Parsonnet, and R. Rappuoli.** 1999. *Helicobacter pylori* virulence and genetic geography. Science **284:**1328–1333.
15. **Dillard, J. P., and H. S. Seifert.** 2001. A variable genetic island specific for *Neisseria gonorrhoeae* is involved in providing DNA for natural transformation and is found more often in disseminated infection isolates. Mol. Microbiol. **41:**263–277.
16. **Dubnau, D.** 1998. DNA uptake in bacteria. Annu. Rev. Microbiol. **53:**217–244.
17. **Eppinger, M., C. Baar, B. Linz, G. Raddatz, C. Lanz, H. Keller, G. Morelli, H. Gressmann, M. Achtman, and S. C. Schuster.** 2006. Who ate whom? Adaptive *Helicobacter* genomic changes that accompanied a host jump from early humans to large felines. PLOS Genet. **2:**1097–1110.
18. **Eppinger, M., C. Baar, G. Raddatz, D. H. Huson, and S. C. Schuster.** 2004. Comparative analysis of four *Campylobacterales*. Nat. Rev. Microbiol. **2:**872–885.
19. **Faruque, S. M., M. J. Albert, and J. J. Mekalanos.** 1998. Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae*. Microbiol. Mol. Biol. Rev. **62:**1301–1314.
20. **Finlay, B. B., and S. Falkow.** 1997. Common themes in microbial pathogenicity revisited. Microbiol. Mol. Biol. Rev. **61:**136–169.
21. **Fischer, W., D. Schwan, E. Gerland, G. E. Erlenfeld, S. Odenbreit, and R. Haas.** 1999. A plasmid-based vector system for the cloning and expression of *Helicobacter pylori* genes encoding outer membrane proteins. Mol. Gen. Genet. **262:**501–507.
22. **Fouts, D. E., E. F. Mongodin, R. E. Mandrell, W. G. Miller, D. A. Rasko, J. Ravel, L. M. Brinkac, R. T. DeBoy, C. T. Parker, S. C. Daugherty, R. J. Dodson, A. S. Durkin, R. Madupu, S. A. Sullivan, J. U. Shetty, M. A. Ayodeji, A. Shvartsbeyn, M. C. Schatz, J. H. Badger, C. M. Fraser, and K. E. Nelson.** 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *Campylobacter* species. PLOS Biol. **3:**72–85.
23. **Gibreel, A., D. Tracz, L. Nonaka, T. M. Ngo, S. R. Connell, and D. E. Taylor.** 2004. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to *tet*(O)-mediated tetracycline resistance. Antimicrob. Agents Chemother. **48:**3442–3450.
24. **Gieseler, S., B. Konig, W. Konig, and S. Backert.** 2005. Strain-specific expression profiles of virulence genes in *Helicobacter pylori* during infection of gastric epithelial cells and granulocytes. Microbes Infect. **7:**437–447.
25. **Goodwin, A., D. Kersulyte, G. Sisson, S. J. O. Veldhuyzen van Zanten, D. E. Berg, and P. S. Hoffman.** 1998. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdxA*) that encodes an oxygen-insensitive NADPH nitroreductase. Mol. Microbiol. **28:**383–393.
26. **Hacker, J., and J. B. Kaper.** 2000. Pathogenicity islands and the evolution of microbes. Annu. Rev. Microbiol. **54:**641–679.
27. **Hamilton, H. L., N. M. Dominguez, K. J. Schwartz, K. T. Hackett, and J. P. Dillard.** 2005. *Neisseria gonorrhoeae* secretes chromosomal DNA via a novel type IV secretion system. Mol. Microbiol. **55:**1704–1721.
28. **Hofreuter, D., and R. Haas.** 2002. Characterization of two cryptic *Helicobacter pylori* plasmids: a putative source for horizontal gene transfer and gene shuffling. J. Bacteriol. **184:**2755–2766.
29. **Hofreuter, D., S. Odenbreit, and R. Haas.** 2001. Natural transformation competence in *Helicobacter pylori* is mediated by the basic components of a type IV secretion system. Mol. Microbiol. **41:**379–391.
30. **Israel, D. A., N. Salama, U. Krishna, U. M. Rieger, J. C. Atherton, S. Falkow, and R. M. Peek, Jr.** 2001. *Helicobacter pylori* genetic diversity within the gastric niche of a single human host. Proc. Natl. Acad. Sci. USA **98:**14625–14630.
31. **Kersulyte, D., B. Velapatino, A. K. Mukhopadhyay, L. Cahuayme, A. Bussalleu, J. Combe, R. H. Gilman, and D. E. Berg.** 2003. Cluster of type IV secretion genes in *Helicobacter pylori*'s plasticity zone. J. Bacteriol. **185:**3764–3772.
32. **Korlath, J. A., M. T. Osterholm, L. A. Judy, J. C. Forfang, and R. A. Robinson.** 1985. A point-source outbreak of campylobacteriosis associated with consumption of raw milk. J. Infect. Dis. **152:**592–596.
33. **Kuipers, E. J., D. A. Israel, J. G. Kusters, and M. J. Blaser.** 1998. Evidence for a conjugation-like mechanism of DNA transfer in *Helicobacter pylori*. J. Bacteriol. **180:**2901–2905.
34. **Lee, C.-Y., C.-L. Tai, S.-C. Lin, and Y.-T. Chen.** 1994. Occurrence of plas-

mids and tetracycline resistance among *Campylobacter jejuni* and *Campylobacter coli* isolated from whole market chickens and clinical samples. Int. J. Food Microbiol. **24:**161–170.

35. **Letellier, L., L. Plancon, M. Bonhivers, and P. Boulanger.** 1999. Phage DNA transport across membranes. Res. Microbiol. **150:**499–505.
36. **Lin, L. F., J. Posfai, R. J. Roberts, and H. Kong.** 2001. Comparative genomics of the restriction-modification systems in *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA **98:**2740–2745.
37. **Low, K. B.** 1987. Hfr strains of *Escherichia coli* K-12, p. 1134–1337. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger, (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol 2. ASM Press, Washington, DC.
38. **Miyamoto, H., S. Yoshida, H. Taniguchi, and H. A. Shuman.** 2003. Virulence conversion of *Legionella pneumophila* by conjugal transfer of chromosomal DNA. J. Bacteriol. **185:**6712–6718.
39. **Montecucco, C., and R. Rappuoli.** 2001. Living dangerously: how *Helicobacter pylori* survives in the human stomach. Nat. Rev. Mol. Cell. Biol. **2:**457–466.
40. **Nachamkin, I., J. Engberg, and F. Aarestrup.** 2000. Diagnosis and antimicrobial susceptibility of *Campylobacter* species, p. 45–66. *In* I. Nachamkin, and M. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, DC.
41. **Nobusato, A., I. Uchiyama, and I. Kobayashi.** 2000. Diversity of restriction-modification gene homologues in *Helicobacter pylori*. Gene **259:**89–98.
42. **Ochman, H., J. G. Lawrence, and E. A. Groisman.** 2000. Lateral gene transfer and the nature of bacterial innovation. Nature **405:**299–304.
43. **Odenbreit, S., B. Wieland, and R. Haas.** 1996. Cloning and genetic characterisation of *Helicobacter pylori* catalase and construction of a catalase deficient mutant. J. Bacteriol. **178:**6960–6967.
44. **Oh, J. D., H. Kling-Backhed, M. Giannakis, J. Xu, R. S. Fulton, L. A. Fulton, H. S. Cordum, C. Wang, G. Elliott, J. Edwards, E. R. Mardis, L. G. Engstrand, and J. I. Gordon.** 2006. The complete genome sequence of a chronic atrophic gastritis *Helicobacter pylori* strain: evolution during disease progression. Proc. Natl. Acad. Sci. USA **103:**9999–10004.
45. **Pansegrau, W., and E. Lanka.** 1996. Enzymology of DNA transfer by conjugative mechanisms. Prog. Nucleic Acid Res. Mol. Biol. **54:**197–251.
46. **Parkhill, J., B. W. Wren, K. Mungall, J. M. Ketley, C. Churcher, D. Basham, T. Chillingworth, R. M. Davies, T. Feltwell, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Moule, M. J. Pallen, C. W. Penn, M. A. Quail, M. A. Rajandream, K. M. Rutherford, A. H. M. van Vliet, S. Whitehead, and B. G. Barrell.** 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature **403:**665–668.
47. **Peek, R. M., Jr., and M. J. Blaser.** 2002. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat. Rev. Cancer **2:**28–37.
48. **Pratt, A., and V. Korolik.** 2005. Tetracycline resistance of Australian *Campylobacter jejuni* and *Campylobacter coli* isolates. J. Antimicrob. Chemother. **55:**452–460.
49. **Salama, N., K. Guillemin, T. K. McDaniel, G. Sherlock, L. Tompkins, and S. Falkow.** 2000. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proc. Natl. Acad. Sci. USA **97:**14668–14673.
50. **Smith, G. R.** 1991. Conjugational recombination in *E. coli*: myths and mechanisms. Cell **64:**19–27.
51. **Suerbaum, S., C. Josenhans, T. Sterzenbach, B. Drescher, P. Brandt, M. Bell, M. Droge, B. Fartmann, H. P. Fischer, Z. Ge, A. Horster, R. Holland, K. Klein, J. Konig, L. Macko, G. L. Mendz, G. Nyakatura, D. B. Schauer, Z. Shen, J. Weber, M. Frosch, and J. G. Fox.** 2003. The complete genome sequence of the carcinogenic bacterium *Helicobacter hepaticus*. Proc. Natl. Acad. Sci. USA **100:**7901–7906.
52. **Suerbaum, S., and M. Achtman.** 2004. *Helicobacter pylori*: recombination, population structure and human migrations. Int. J. Med. Microbiol. **294:**133–139.
53. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, and B. A. Dougherty.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539–547.
54. **Wiesner, R. S., D. R. Hendrixson, and V. J. DiRita.** 2000. Natural transformation of *Campylobacter jejuni* requires components of a type II secretion system. J. Bacteriol. **185:**5408–5418.