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comH, a Novel Gene Essential for Natural Transformation of *Helicobacter pylori*

LEONARD C. SMEETS, JETTA J. E. BIJLSMA, SACHA Y. BOOMKENS, CHRISTINA M. J. E. VANDENBROUCKE-GRAULS, and JOHANNES G. KUSTERS*

Department of Medical Microbiology and Infection Control, Vrije Universiteit, Amsterdam, The Netherlands

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***Helicobacter pylori* is naturally competent for transformation, but the DNA uptake system of this bacterium is only partially characterized, and nothing is known about the regulation of competence in *H. pylori*. To identify other components involved in transformation or competence regulation in this species, we screened a mutant library for competence-deficient mutants. This resulted in the identification of a novel, *Helicobacter*-specific competence gene (*comH*) whose function is essential for transformation of *H. pylori* with chromosomal DNA fragments as well as with plasmids. Complementation of *comH* mutants in *trans* completely restored competence. Unlike other transformation genes of *H. pylori*, *comH* does not belong to a known family of orthologous genes. Moreover, no significant homologs of *comH* were identified in currently available databases of bacterial genome sequences. The *comH* gene codes for a protein with an N-terminal leader sequence and is present in both highly competent and less-efficient transforming *H. pylori* strains. A *comH* homolog was found in *Helicobacter acinonychis* but not in *Helicobacter felis* and *Helicobacter mustelae*.**

Helicobacter pylori is a gram-negative bacterium that colonizes the human stomach and causes chronic gastritis and peptic ulceration. Furthermore, colonization with this organism is associated with the development of gastric neoplasms. More than half of the *H. pylori* strains contain a pathogenicity island, the *cag* region, whose presence has a marked influence on the virulence of the organism.

Gene transfer between *H. pylori* strains is extremely common (24) and can generate novel subtypes during colonization with multiple strains (15, 19). The genetic recombination between *H. pylori* strains includes changes in important virulence markers such as the *cag* status (15). Therefore, horizontal gene transfer and uptake of foreign DNA play an important role in virulence and host adaptation of *H. pylori*. Horizontal gene transfer can occur via conjugation, transduction, or transformation. Most *H. pylori* strains are naturally competent for transformation with linear DNA (27; P. Nedenskov, G. Bukholm, and K. Bovre, Letter, J. Infect. Dis. **161:**365–366, 1990) as well as with plasmids (31). In order to get insight into the characteristics of natural transformation in *H. pylori*, it is necessary to understand the mechanisms involved and their regulation.

When Tomb et al. (25) published the first genomic sequence of *H. pylori*, based on sequence homologies, a number of potential competence genes could be recognized. However, no integral DNA uptake system was identified (25). At present, a role in transformation has been described for only two loci: the *comB* operon (13) and *dprA* (3, 22). To identify other components involved in competence or its regulation, we screened a mutant library for competence-deficient mutants. This resulted in the identification of a novel *H. pylori* competence gene, *comH*. Unlike *comB* and *dprA*, *comH* does not belong to a known family of orthologous genes.

* Corresponding author. Mailing address: Department of Medical Microbiology, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands. Phone: 31 20 4448310. Fax: 31 20 4448318. E-mail: jg.kusters.mm@med.vu.nl.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. Bacterial strains and plasmids are listed in Table 1. *H. pylori*, *Helicobacter mustelae* (kindly provided by T. Ó Cróinín, Our Lady's Hospital for Sick Children, Crumlin, Ireland), and *Helicobacter acinonychis* (kindly provided by A. Bart, Academic Medical Center, Amsterdam, The Netherlands) were grown under microaerobic conditions on Dent plates (9) (Dent supplement; Oxoid) supplemented with 40 mg of 2,3,5-triphenyltetrazolium chloride (Sigma Chemical Co., St. Louis, Mo.) per liter. When appropriate, antibiotics were added in the following concentrations: kanamycin, 20 mg/liter (Sigma); chloramphenicol, 15 mg/liter (Serva, Heidelberg, Germany); clarithromycin, 2 mg/liter (Abbott Laboratories Ltd., Queensborough, United Kingdom). *Helicobacter felis* strains were grown as described by Cattoli et al. (7). *Escherichia coli* ER1793 (14) and DH5α (Clontech, Palo Alto, Calif.) were cultured in Luria-Bertani broth, with 30 mg of kanamycin or 30 mg of chloramphenicol/liter if appropriate. Plasmid pHel2 is an *E. coli*-*H. pylori* shuttle vector that carries the cat_{GC} chloramphenicol resistance gene (12). The pBCα3 suicide vector was derived from the pBC SK+/− plasmid (Stratagene, La Jolla, Calif.) by ligation of the *aphA-3* kanamycin resistance cassette (26) into its unique *Sma*I site (4).

DNA manipulation. Southern blotting and recombinant DNA techniques were performed according to standard protocols (20) unless stated otherwise. Plasmids were isolated with the QIAprep spin miniprep kit (Qiagen GmbH, Hilden, Germany). Restriction enzymes used in this study were obtained from New England Biolabs Inc. (Beverly, Mass.).

Transformation. Natural transformation of *H. pylori* was performed essentially as described by Wang et al. (31). In brief, 24 h after inoculation bacteria were harvested from their plate and transferred as thick patches onto a fresh plate; after 5 h approximately 1 μg of DNA was added to the patches. After 20 h of incubation, the bacteria were suspended in 120 μl of phosphate-buffered saline and 100-μl portions of appropriate dilutions were spread on selective plates. To calculate a transformation frequency, appropriate dilutions (10^{-6} and 10^{-8}) were plated on nonselective plates. After incubation for 5 days, the colonies were counted.

Electrocompetent *H. pylori* cells were prepared as described for *Campylobacter jejuni* (28). Electroporation was performed on an ECM-600 electroporation system (BTX, San Diego, Calif.) with 50 μl of competent cells and 1 μg of salt-free DNA at 12.5 kV cm^{-1} and 50 μF. The bacteria were suspended in 1 ml of brucella broth containing 2% newborn calf serum and 0.4% Dent supplement immediately after electroporation, plated on nonselective plates within 15 min, and allowed to recover during 7 h of microaerobic incubation. Thereafter they were transferred to selective plates.

Construction and screening of the library. The construction of the *H. pylori* mutant library has been described before (4). Individual mutants of the library were inoculated as patches on kanamycin-agar. After 24 h of growth, the patches were covered with 10 μl of a 25-ng/μl chromosomal DNA solution that confers clarithromycin resistance due to an A-2142-to-G mutation in the 23S ribosomal DNA (8). After another 24 h of growth, the patches were transferred to plates containing clarithromycin.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or characteristics	Reference or source
H. pylori 26695	Wild type	25
H. pylori SPM326	Wild type	17
H. pylori SS1	Wild type	16
H. pylori HPK1	Wild type	27
H. pylori NCTC 11637	Wild type	NCTC[a]
H. pylori J99	Wild type	1
H. pylori ATCC 43504	Wild type	ATCC[b]
H. pylori BR9802	Wild type	30
H. pylori 1061	Wild type	11
1061, SACHA-1	1061, HP1527::*aphA-3*	This study
1061, SACHA-2	1061, HP1527::*aphA-3*	This study
26695, SACHA-1	26695, HP1527::*aphA-3*	This study
H. felis CS1	Wild type	CCUG[c] 28539
H. felis DS4	Wild type	CCUG 28540
H. mustelae NCTC 12198	Wild type	NCTC
H. mustelae NCTC 12032	Wild type	NCTC
H. mustelae F4	Wild type, ferric isolate	T. Ó Cróinín
H. mustelae F8	Wild type, ferric isolate	T. Ó Cróinín
H. mustelae F9	Wild type, ferric isolate	T. Ó Cróinín
H. acinonychis India	Wild type	6
H. acinonychis Sheeba	Wild type	6
H. acinonychis ATCC 12686	Wild type	6
HpC-1527	26695, *rdxA*::HP1527; Mtz^r	This study
HpC-SACHA	26695, HP1527::*aphA-3 rdxA*::HP1527; Mtz^r Km^r	This study
pBC SK$^-$	Phagemid, derivative of pUC19	Stratagene
pRdxA	pBC-SK$^-$; multiple cloning site flanked by the 5′ and 3′ parts of *rdxA*	This study
pRDXA-1527	pRdxA; intact gene HP1527	This study
pSACHA-1	pGEM-T Easy; HP1527::*aphA-3*	This study
pSACHA-2	pGEM-T Easy; HP1527::*aphA-3*	This study
E. coli ER1793	Host strain	14
E. coli DH5α	Host strain	Clontech
pGEM-T Easy	PCR cloning vector; ColE1 Amp^r	Promega
pHel2	*H. pylori-E. coli* shuttle plasmid; ColE1 Cam^r	12
pJMK30	pUC19; Amp^r Km^r	29
pBCα3	*H. pylori* suicide vector; ColE1 Cam^r Km^r	4

[a] NCTC, National Collection of Type Cultures, Colindale, United Kingdom.
[b] ATCC, American Type Culture Collection, Manassas, Va.
[c] CCUG, Culture Collection of the Univerity of Göteborg, Göteborg, Sweden.

Plasmid rescue, sequencing, and sequence analysis. For plasmid rescue chromosomal DNA of the mutants was isolated and restricted with *Hin*dIII (a unique *Hin*dIII restriction site is present on pBCα3 between the *aphA-3* kanamycin and chloramphenicol resistance cassettes; see Fig. 1), self-ligated into circularized *Hin*dIII fragments, and transformed into *E. coli* with selection on chloramphenicol. For the determination of the other point of insertion, the circularized *Hin*dIII fragments were used as a template in an inverse PCR with primers that face outward on the *aphA-3* cassette: AphA3-R and Kana-L (Table 2). PCR products were cloned in the pGEM-T Easy vector (Promega, Madison, Wis.).

Sequence reactions were then performed with the rescued plasmids and the cloned inverse-PCR amplimers with the Thermo-Sequenase premixed cycle sequence kit (Amersham Pharmacia, Uppsala, Sweden) and with standard M13 primers (labeled with Texas red) on an Amersham Vistra 725 sequencer. Data were analyzed with Lasergene software (DNAstar Inc., Madison, Wis.). Se-

TABLE 2. Oligonucleotide primers used in this study

Primer	Annealing site	Primer sequence (5′–3′)[a]
AphA3-R	Gene *aphA-3*, bases 1381–1401	CTGGATGAATTGTTTTAGTAC
Kana-L	Gene *aphA-3*, bases 36–16	TTACCTATCACCTCAAATGG
HP1526rev62	ORF HP1526, bases 62–41	TCCATAAAGCCCTTAGTCAT
HP1527for43	ORF HP1527, bases 43–62	AACCCTCTTCAAGCCCTTGT
HP1527rev1156	ORF HP1527, bases 1156–1137	CCAATTCGCTGGTTTCATAA
HP1529for1110	ORF HP1529, bases 1110–1129	CAAAGTCTCTTCGCGCCAAA
HP1529for1323	ORF HP1529, bases 1323–1342	CCGCTTGAACGAATTGAACG
MetroF	ORF HP0955, bases 751–770	AATTTGAGCATGGGGCAGA
pUC/M13 forward	pGEM-T	GTTTTCCCAGTCACGAC
pUC/M13 reverse	pGEM-T	CAGGAAACAGCTATGAC
rdxAISacI	ORF HP0955, bases 774–789	TTTgagctcATTTATGGTAG
rdxAIXbaI	ORF HP0954, bases 322–342	CACtctagaCTTATAAGACTCC
rdxAIIXhoI	ORF HP0954, bases 384–366	TTGctcgagTGCTTGGCG
rdxAIIKpnI	ORF HP0954, bases 613–635	ATCggtaccAAGTAATCGCATC

[a] Lowercase letters represent restriction sites that were introduced into these primers.

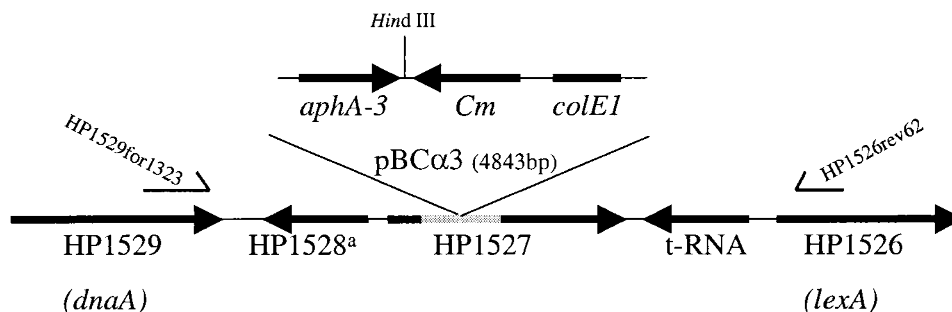

FIG. 1. Schematic representation of the genomic region of HP1527 with the location of the pBCα3 insertion. Depicted are relevant regions of pBCα3 and the *Hin*dIII site used for plasmid rescue, the duplicated chromosomal region (gray), and the primers used for complementation of HP1527 (Table 2). The figure is not drawn to scale. *aphA-3*, kanamycin resistance cassette; *Cm*, chloramphenicol resistance cassette; *colE1*, origin of replication. a, HP1528 is present in strain 26695 only.

quence analysis was performed with the BLAST, version 2.0, algorithm (2) (National Center for Biotechnology Information, Los Alamos, N.Mex.).

Construction of site-directed mutants in ORF HP1527. A fragment of open reading frame (ORF) HP1527 was amplified from *H. pylori* strain 1061 with primers HP1527for43 and HP1527rev1156 (Table 2) and cloned into pGEM-T Easy. This HP1527 fragment contains a *Hin*dIII site at base 753 of the ORF that was used for restriction and subsequent ligation with the *aphA-3*-containing *Hin*dIII fragment of pJMK30 (29). The resulting HP1527::*aphA*-3-containing pGEM-T was transformed into *E. coli* DH5α to obtain pSACHA-1 and pSACHA-2. The orientations of *aphA-3* in pSACHA-1 (same direction as the HP1527 ORF) and pSACHA-2 (opposite direction) were determined by PCR with combinations of primers aphA3-R or Kana-L (forward) and HP1527for43 or HP1527rev1156 (reverse) (Table 2) and by sequencing the amplimers. pSACHA-1 and pSACHA-2 were used to create HP1527 mutants in strain 1061 and 26695 by natural transformation (Table 1).

Construction of the *rdxA* vector. The 5′ part of the *rdxA* gene was amplified by PCR with the primers rdxAIXbaI and rdxAISacI (Table 2), and the resulting amplimers were purified. This 5′ fragment of *rdxA* was cloned into the phagemid pBC-SK using the *Xba*I and *Sac*I restriction sites that were introduced by PCR, which resulted in the vector pBC-rdxAI. Subsequently the 3′ part of *rdxA* was amplified by PCR with the primers rdxAIIXhoI and rdxAIIKpnI (Table 2), and the resulting amplimers were purified. The introduction of this 3′ fragment of *rdxA* into pBC-rdxAI, with the aid of the *Xho*I and *Kpn*I restriction sites that were introduced by PCR, gave rise to plasmid pRdxA (Fig. 2).

Complementation analysis with the *rdxA* vector system. The complete gene HP1527 of strain 1061 was amplified by PCR with primers on the flanking genes: HP1526rev62 and HP1529for1323 (Table 2, Fig. 1). This amplimer was cloned in pGEM-T Easy and ligated into the *Eco*RI site of the *rdxA* vector to obtain pRDXA-1527. After being cloned into *E. coli* DH5α, pRDXA-1527 was transformed into *H. pylori* strain 26695; this gave rise to the metronidazole-resistant (Mtz^r) mutant HpC-1527. HpC-1527 was transformed with pSACHA-1. The resulting kanamycin-resistant (Mtz^r Km^r) colonies were tested for the location of the *aphA-3* cassette with a set of PCRs; Apha-L or Apha-3R was used as a forward primer, and reverse primers on the *rdxA* ORF (MetroF) and the HP1529 ORF (HP1529for1323) were chosen (Table 2, Fig. 3).

RESULTS

Screening of the library. Approximately 1,250 mutants from a random *H. pylori* 1061 library were screened for transformation deficiency. Each mutant was inoculated as a small patch and, after 24 h, overlaid with chromosomal DNA that confers clarithromycin resistance. After another 24 h, the patches were transferred to selective plates. In this crude but easy-to-perform screening method 1,200 mutants formed one or more Cla^r colonies and thus proved to be competent. The remaining 50 mutants were subjected to natural transformation by the method of Wang et al. (31). In this test, 3 of the 50 were completely transformation deficient and were selected for further examination.

Plasmid rescue, sequencing, and sequence analysis. The library that was used for this screening was created by chromosomal insertion of pBCα3 suicide plasmids which contain a random fragment of *H. pylori*. This random fragment of DNA recombines into the *H. pylori* chromosome by a single homologous crossover event, which leads to insertion of the complete vector and to a duplication of the DNA fragment of the *H. pylori* chromosome. Thus, each mutant contains one copy of this fragment on each side of the integrated vector. Because of this duplication, the backbone of the pBCα3 vector that interrupts the chromosome has a different insertion point on each side. To determine the first point of insertion, plasmid rescue was performed by restriction with *Hin*dIII and religation of the chromosomal DNA, which restores a pBCα3-based Cam^r plasmid that contains one flanking sequence of the *H. pylori* chromosome (Fig. 1). No suitable restriction endonuclease site was available to obtain a rescue plasmid that contains the other flanking sequence. Therefore, the circularized *Hin*dIII fragments were used as a template in a reverse PCR with primers that face outward on the *aphA-3* resistance cassette. Thus, the chromosomal DNA flanking the *aphA-3* cassette was amplified.

Both flanking sequences revealed the same site of insertion in the three mutants with a chromosomal duplication of 280 bp. Apparently all three mutants were derived from a single pBCα3 vector, either as independent transformants of the same pBCα3 vector or as offspring from a single mutant that divided before storage. The duplicated region flanking both insertion points was aligned with the complete *H. pylori* genomes of strain 26695 (The Institute for Genomic Research, Rockville, Md.; http://www.tigr.org) and strain J99 (AstraZeneca R&D, Boston, Mass.; http://scriabin.astrazeneca-boston.com/hpylori) and was identified as bases 550 to 830 of the ORF designated HP1527 in strain 26695 (JHP1416 in strain J99).

Construction of site-directed mutants in ORF HP1527 and transformation. To prove that the transformation deficiency of the random mutants was not caused by an unrelated event elsewhere in the genome, site-directed mutants were constructed in strain 1061 by insertion of an *aphA-3* cassette in ORF HP1527. First, a fragment of ORF HP1527 of strain 1061 was amplified by PCR and cloned in the pGEM-T Easy vector. Sequence analysis revealed a *Hin*dIII restriction site in this DNA fragment. This site was used to insert the *aphA-3* cassette, which codes for kanamycin resistance, and the resulting constructs were named pSACHA-1 and pSACHA-2. PCRs with combinations of primers aphA3-R or Kana-L (forward) and HP1527for43 or HP1527rev1156 (reverse) and the sequencing of the amplimers showed that pSACHA-1 has the *aphA-3* gene inserted in the same direction as the HP1527 reading frame and that pSACHA-2 has the gene inserted in the opposite direction. pSACHA-1 and pSACHA-2 were used to create mutants in strain 1061. In addition, a SACHA-1 mutant was made in strain 26695 to confirm that the phenotype caused by disruption of HP1527 is similar in an unrelated strain. Dis-

TABLE 3. Transformation frequencies of wild-type strains 1061 and 26695 and HP1527 mutants with chromosomal DNA and plasmid pHEL2

Strain	Transformation frequency[a] (% of parent frequency) with:	
	Chromosomal DNA (Cla^r) by natural transformation[b]	pHEL2 (Cam^r)
1061 (parent)	3×10^{-6} (100)	10^{-6} (100)
1061, SACHA-1	$<10^{-9}$ (<0.1)	$<10^{-9}$ (<0.1)
1061, SACHA-2	$<10^{-9}$ (<0.1)	$<10^{-9}$ (<0.1)
26695 (parent)	10^{-6} (100)	n.d.[c]
26695, SACHA-1	$<10^{-9}$ (<0.1)	n.d.

[a] Determined as the number of resistant colonies per microgram of DNA per recipient CFU. Data represent the means of two experiments.
[b] Electroporation was successful for each strain.
[c] n.d., not determined.

ruption of ORF HP1527 in each mutant was confirmed by Southern blotting (results not shown).

The competence of these mutants was compared to that of their parental strains (Table 3). Both parental strains transformed at a frequency of at least 10^{-6} with chromosomal DNA conferring clarithromycin resistance. In contrast, no transformants in either the 1061 HP1527 mutants or the 26695 HP1527 mutant were observed. As our transformation system detects a transformation frequency of approximately 10^{-9}, the efficiency of transformation of the mutants is at least 3 log units lower than those of the parental strains. Electroporation of the mutants showed a transformation efficiency comparable to those of the parental strains. The strain 1061 mutants were also tested for their natural transformation competence with *H. pylori* plasmid pHEL2. Again, no transformation was observed (Table 3).

Construction of the *rdxA* vector. For the complementation of HP1527 we developed a replacement vector that would allow for the ectopic integration of DNA into the chromosome of *H. pylori*. As a target for replacement we used gene *rdxA*. Disruption of *rdxA* causes metronidazole resistance in *H. pylori* (11). Thus, insertion of any DNA fragment into *rdxA* will give rise to metronidazole-resistant colonies, and the DNA serves as its own resistance marker to select for the successful integration into *rdxA*. For the construction of the *rdxA* vector, two fragments of the *rdxA* gene were amplified by PCR. With the aid of the restriction sites that were introduced during PCR, the 5′ fragment of the gene was cloned into the first two restriction sites of the multiple cloning site of the phagemid pBC SK− and the 3′ fragment was cloned into the last two restriction sites. This resulted in pRdxA (Fig. 2), a plasmid containing the 5′ and 3′ parts of the *rdxA* gene flanking the remainder of the multiple cloning site, which allows for the introduction of a DNA fragment. Sequencing pRdxA with the M13 forward and M13 reverse primers, located just outside the multiple cloning site, confirmed the correctness of the inserts. Transformation of pRdxA into *H. pylori* yielded metronidazole-resistant colonies, indicating that introduction of the multiple cloning site of pRdxA disrupted the *rdxA* gene (data not shown).

Complementation analysis with the *rdxA* vector system. Complementation of ORF HP1527 was performed to confirm that the competence-deficient phenotype of the mutants was caused by disruption of ORF HP1527 and not by a polar effect on surrounding genes. Gene HP1527 was amplified by PCR with primers located on the flanking genes. Strain 26695 contains a small ORF (HP1528) that overlaps the putative promoter region of HP1527. In order to obtain gene HP1527 only and to avoid problems due to this overlap in strain 26695, gene HP1527 was amplified from strain 1061, which lacks ORF HP1528. The amplimer was cloned in the *rdxA* vector to yield pRdxA-1527 (Fig. 3, left). Because disruption of ORF HP1527 eliminates competence, we performed the complementation of HP1527 as follows. First, pRdxA-1527 was transformed into wild-type *H. pylori* strain 26695. An Mtz^r mutant of 26695 with a second intact HP1527 inserted into the *rdxA* gene, directed opposite to the *rdxA* reading frame, was identified by PCR and called HpC-1527. Next, HpC-1527 was transformed with pSACHA-1, which yielded Mtz^r Km^r transformants with an interruption of either the original or the additional HP1527 ORF. The location of the *aphA-3* insertion was identified with a set of PCRs that demonstrate the presence or absence of the *aphA-3* cassette, both at the original location and in the *rdxA* gene, as shown in Fig. 3. A mutant with the *aphA-3* insertion in the original HP1527 was called HpC-SACHA. We then tested the wild-type 26695 and its derivative HpC-1527, which contains two intact HP1527 genes, and the mutant with the complemented genotype, HpC-SACHA, for their capabilities to transform to clarithromycin resistance. The transformation frequency of HpC-SACHA was identical to the frequency of the parental strain (Table 4). The duplication of gene HP1527 in mutant HpC-1527 had no marked effect on the transformation frequency.

Distribution of HP1527 in the genus *Helicobacter*. The nine wild-type *H. pylori* strains from Table 1 were tested for the presence of *comH* on a Southern blot probed with a *comH* fragment (bases 62 to 1156) of strain 1061, and *comH* was demonstrated in all of them (data not shown). These nine strains included both highly competent strains and strains with relatively low competence such as SS1. The same *comH* fragment also hybridized to three strains of *H. acinonychis* (Table 1, Fig. 4), a species that is closely related to *H. pylori* and that is also naturally transformable (results not shown). However, Southern blotting experiments did not demonstrate sequences homologous to *comH* in two other *Helicobacter* species, *H. felis* (two strains; Table 1) and *H. mustelae* (five strains; Table 1).

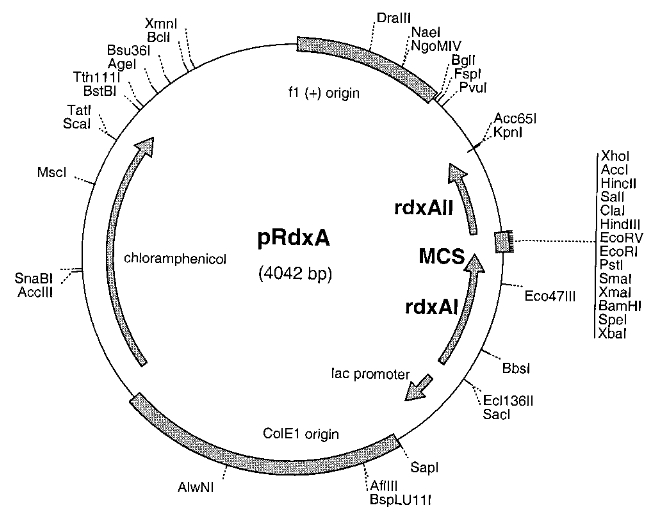

FIG. 2. Map of vector pRdxA. The multiple cloning site (MCS) allows for cloning between two fragments of the *rdxA* gene (rdxAI and rdxAII). Relevant restriction sites are indicated.

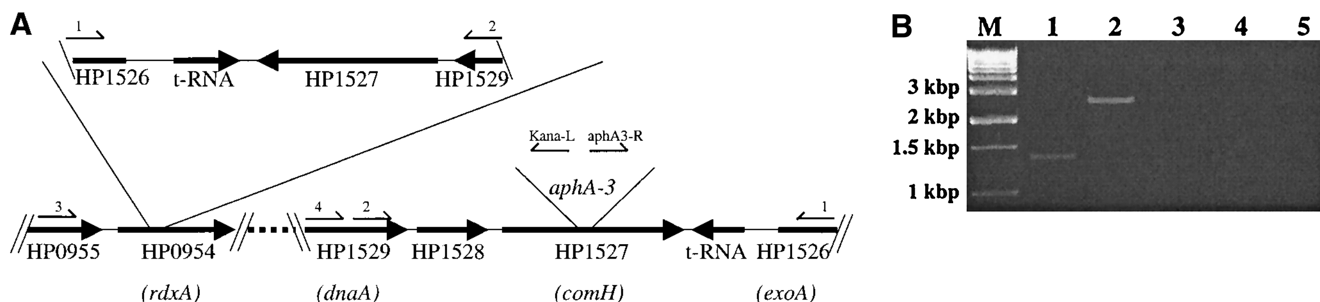

FIG. 3. (A) Schematic representation of complementation mutant HpC-SACHA showing the *rdxA* region with the HP1527 insert (left) and the HP1527 region with the *aphA-3* insert (right). Indicated are the primers used to construct HpC-1527 and to confirm the site of *aphA-3* insertion. Primers 1 (HP1526rev62) and 2 (HP1529for1323) were used to amplify HP1527 from strain 1061 (which lacks ORF HP1528; see text). This HP1527 copy was inserted into the *rdxA* gene of strain 26695. The presence of an uninterrupted HP1527 gene in the *rdxA* gene was confirmed by a PCR with primers 3 (MetroF) and 2. Primers 4 (HP1529for1110) and Kana-L confirmed the *aphA-3* insertion in the original HP1527 gene, while primer 3 did not yield a product with aphA3-R. (B) PCR confirming the HpC-SACHA genotype in the left panel. Lane M, DNA size markers; lane 1, primers 4 and Kana-L confirmed the *aphA-3* insertion in the original HP1527 gene (band at 1,359 bp); lane 2, the presence of an uninterrupted HP1527 gene in the *rdxA* gene was confirmed by a PCR with primers 2 and 3 (2,410 bp); lane 3, primer 3 did not yield a product with aphA3-R, confirming the absence of the *aphA-3* insertion in the complementing gene copy; lane 4, control for lane 1 with HpC-1527 as the template; lane 5, control for lane 2, with the parental strain as the template.

DISCUSSION

To identify elements of the transformation system in *H. pylori*, we screened a random-insertion library for loss of competence. We identified a mutant in ORF HP1527 that was incapable of natural transformation. Site-directed mutants in this ORF showed the same phenotype. Complementation of HP1527 in *trans* completely restored competence, which indicates that the mutation itself rather than a polar effect on surrounding genes causes the transformation deficiency. These results demonstrate that HP1527, an ORF with a heretofore-unknown function, is essential for natural transformation of *H. pylori*. Although the gene might have additional functions, based on the data presented in this paper a gene name in accordance with current nomenclature for competence genes would be appropriate for HP1527. Because the gene has no orthologs (see below), we decided to name HP1527 *comH*, which is, to our knowledge, the first available *com* designation in the alphabet. The *comH* gene is present in all tested *H. pylori* strains, not only in highly competent strains but also in less-efficient transformers, and interruption of this gene completely obliterated the natural-transformation competence for both chromosomal DNA and plasmids. *comH* mutants appear to have a normal growth rate and survival, which suggests that *comH* has no additional household functions.

The organization of the chromosomal region around *comH* differs among strains 26695, J99, and 1061. In all three strains, the *H. pylori exoA* homolog (HP1526) is located downstream of *comH*, separated from *comH* by a tRNA gene that lies in the opposite direction. In the 26695 sequence, upstream of *comH*, are the putative ORF HP1528 and the *dnaA* homolog (HP1529). The small putative ORF HP1528 is absent from 1061 and from the J99 sequence, which indicates that it is not required for competence in *H. pylori*. Because of the large and variable intergenic region between *comH* and the *dnaA* gene, as well as the putative function of *dnaA* in chromosomal replication, cotranscription of *comH* in an operon with this gene is unlikely. Comparison of the ORF *comH* (strain 26695) with the corresponding ORF of strain J99, JHP1416, revealed an amino acid identity of 93% (95% similarity), which is in line with the variation between other genes in *H. pylori*. Sequence analysis with the SignalP program (18) showed that *comH* encodes a product with a presumed transmembrane domain, corresponding to an N-terminal signal peptide for secretion, with a cleavage site between amino acid residues 19 and 20. The putative exported mature protein consists of 460 amino acid residues (52.4 kDa) and has an isoelectric point of 6.35. We did not find sequences that may function as DNA-binding sites in *comH*.

In Southern blotting experiments with an *H. pylori comH* probe, a *comH* homolog was detected in all *H. pylori* and *H. acinonychis* strains but not in *H. mustelae* and *H. felis*. Likewise, the latter two species did not hybridize with the *H. pylori comB* operon in earlier experiments by Hofreuter et al. (13). Because *H. mustelae* is also naturally transformable (data not shown), these results suggest that transformation genes are not conserved among all naturally transformable *Helicobacter* spp. Database sequence similarity searches did not reveal a significant homology of ORF *comH* to any genomic sequence available in GenBank, including naturally transformable species such as

TABLE 4. Transformation frequencies[a] of strain 26695 complementation mutants

Strain (genotype)	Transformation frequency (% of parental frequency)
26695 (parent)	10^{-6} (100)
26695, SACHA-1 (HP1527::*aphA-3*)	$<10^{-9}$ (<0.1)
HpC-1527 (HP1527 *rdxA*::HP1527)	10^{-6} (100)
HpC-SACHA (HP1527::*aphA-3 rdxA*::HP1527)	10^{-6} (100)

[a] Determined as the number of resistant colonies per microgram of DNA per recipient CFU. Data represent the means of two experiments.

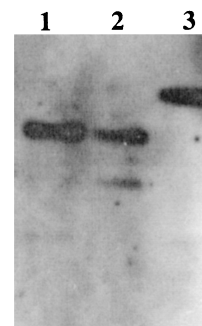

FIG. 4. *H. acinonychis* strains probed with an internal *comH* fragment. Lane 1, strain Sheeba; lane 2, strain India; lane 3, reference strain ATCC 12686.

Bacillus subtilis, *Haemophilus influenzae*, and *C. jejuni*. This indicates that part of the *H. pylori* transformation system is evolutionarily distinct from the systems known from other species. The previously identified *comB* and *dprA* genes, however, have orthologs in other competent bacterial species and even in conjugational plasmids (3, 13, 22).

H. pylori contains many ORFs without an obvious ortholog, and the screening of a random library is therefore a powerful method for the identification of gene function. Although a previous screening of this library revealed 8 unique mutants (4), the present identification of three identical clones indicates that the 1,250 insertion mutants are not all independent. In addition to this, not all pBCα3 insertions inactivate a gene, and only one restriction enzyme was used to create the random fragments for mutagenesis. The present set of mutants is therefore not a comprehensive library of the 1,500 *H. pylori* genes. Indeed, none of the known transformation genes (*comB* operon, *recA*, and *dprA*) were identified in our screening. It is therefore possible that other unrevealed competence genes are present.

In this paper we also describe a new complementation strategy for *H. pylori* based on the *rdxA* vector. This complementation system has obvious advantages over plasmid-based complementation. It produces a stable, single-copy insertion. Furthermore, the *rdxA* vector allows for introduction of DNA into *H. pylori* with an absolute minimum of changes in the genome: it avoids the unknown effects of using additional resistance markers that are unnatural to *H. pylori* and does not introduce remnants of the vector other than a short polylinker sequence. Many clinical isolates of *H. pylori* are metronidazole resistant, which indicates that disruption of *rdxA* does not have a significant effect on the viability of *H. pylori*. A practical advantage of the lack of an additional resistance marker is the reduced length of the DNA fragment that has to be internalized, which enhances the transformation frequency in less-competent strains.

The lack of orthologs makes it difficult to speculate on the role of *comH* in the process of transformation. In general, natural transformation can be divided into the following steps: development of a competent state, DNA binding, DNA uptake, and genomic integration (23). The putative N-terminal secretion signal of the *comH* product suggests that the protein is either anchored in the cytoplasmic membrane or exported to the periplasm and points to a role in the DNA-binding or DNA uptake process, although a function in the development of a competent state cannot be excluded. The results of electroporation experiments that demonstrate a normal recombination in *comH* mutants imply that *comH* is not involved in the recombination that follows uptake of chromosomal fragments. This is in accordance with the finding that *comH* mutants are incapable of plasmid uptake, since RecA-deficient *H. pylori* mutants are still capable of transformation with self-replicating plasmids but not with chromosomal markers (21).

It has become clear from published genomic sequences that *H. pylori* contains relatively few operonic loci. Whereas the *H. pylori comB* competence genes appear to form a small operon, *dprA* and *comH* do not. Organization of competence genes in larger loci and operon structures in other naturally transformable bacteria has been described. In *B. subtilis*, the expression of natural transformation competence is a highly regulated process: competence genes are controlled by a complex signal transduction network that senses environmental changes, and competence is expressed only under specific circumstances (10). In contrast, *H. pylori* can be transformed under standard culture conditions. The lack of operonic organization of competence genes in *H. pylori* could therefore well reflect a relatively loose regulation of competence, as in *Neisseria* spp. (5). The evidence for extensive horizontal gene transfer between *H. pylori* strains and the conserved nature of *comH* and other transformation genes stress the importance of natural transformation for this organism.

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