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Nucleotide Sequence and Characterization of *cdrA*, a Cell Division-Related Gene of *Helicobacter pylori*

HIROAKI TAKEUCHI,[1] MUTSUNORI SHIRAI,[1] JUNKO K. AKADA,[1] MASATAKA TSUDA,[2] AND TERUKO NAKAZAWA[1]*

Department of Microbiology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755-8505,[1] and Department of Biology, Faculty of Science, Okayama University, Okayama 700-8530,[2] Japan

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We identified cell division-related gene *cdrA* in *Helicobacter pylori* HPK5. The putative gene product, CdrA, is a 367-amino-acid polypeptide that exhibited a high level of homology to conserved hypothetical ATP-binding protein HP0066 of *H. pylori* 26695, except in the N-terminal region, and showed some similarity to the FtsK/SpoIIIE family proteins. We isolated a *cdrA*-disrupted mutant by allelic exchange mutagenesis. Because of the low transformation frequency, the possibility that a suppressing mutation would be found in the obtained *cdrA* mutant was discussed. A repressive role for CdrA on cell division was suggested by the observations that the wild-type strain formed filamentous cells in a high-salt level medium at early stationary phase, while a *cdrA*-disrupted mutant did not show such an abnormality. In addition, the wild-type strain adopted coccoid forms in the stationary phase, whereas the *cdrA*-disrupted mutant remained mostly as short rods. Furthermore, the *cdrA*-disrupted mutant regained the filamentation phenotype when the intact *cdrA* gene was introduced by allelic exchange. Taken together, these observations show that the *cdrA* gene plays an important role in the cell growth of *H. pylori*.

Helicobacter pylori, a gram-negative spiral bacterium first isolated in 1983 from a patient with chronic active gastritis (19), is the causative agent in the majority of cases of chronic gastritis, nearly all cases of duodenal ulcers, and most cases of gastric ulcers and is a possible risk factor for gastric adenocarcinoma (4, 19, 23). During the life span of *H. pylori*, either in the stomach or cultured in vitro, the bacteria show different morphological features such as rod-like, spiral, U-shaped, and spherical coccoid forms. Recently, the complete genome sequence of *H. pylori* 26695 was reported (26); the study yielded 12 life cycle- and cell division-related gene homologues including *ftsK* (HP1090) and *ftsH* (HP1069) (13), and it was suggested that the basic mechanisms of replication and cell division are similar to those of *Escherichia coli* (reviewed in reference 9).

The *E. coli* FtsK protein is directly required for septum formation independent of chromosome segregation and shows sequence similarity to the SpoIIIE protein of *Bacillus subtilis* (2). It contains a probable membrane-spanning region at the N terminus and a consensus ATP/GTP-binding sequence in the putative cytoplasmic domain as do other members of the SpoIIIE family, but it is much larger (2, 8). The *B. subtilis spoIIIE* gene is required to complete the final closure of the septum between the prespore and the mother compartment of the sporulating cell and also to complete the transfer of a chromosome from the mother cell to the prespore compartment (10, 12, 29, 30).

In this study, we identified the *cdrA* gene encoding a putative ATP-binding protein possibly involved in cell division. The deduced amino acid sequence was partially homologous to that of one of the conserved hypothetical ATP-binding proteins of *H. pylori* 26695 (HP0066) located in the equivalent region (26).

H. pylori cells were cultivated at 37°C under microaerobic conditions (5% O_2, 15% CO_2, and 80% N_2) in brucella broth (Difco) supplemented with 5% horse serum (brucella medium) or on brucella medium solidified with 1.4% agar (brucella agar) (20). Kanamycin (25 μg/ml) or chloramphenicol (5 μg/ml) was added when appropriate. In addition to the standard brucella medium containing 0.5% NaCl, we used high-salt level brucella medium containing 1% NaCl. Cells subcultured in brucella medium were inoculated into 15 ml of the standard or the high-salt level brucella medium in 50-ml conical flasks, and the mixture was incubated at 37°C for 14 days with shaking under microaerobic conditions. Growth was measured by determining the optical density at 590 nm (OD_{590}) with a spectrophotometer (Spectronic 20A; Shimadzu), and CFUs were determined by triplicate plating on brucella agar. *E. coli* cells were cultivated in L broth at 37°C with shaking, and ampicillin (100 μg/ml), kanamycin (10 μg/ml), and chloramphenicol (5 μg/ml) were added when required. Bacterial strains and plasmids used in this study are listed in Table 1.

SuperCos1-based cosmid pMT5047 was constructed by deleting the 2,160-bp *Nru*I fragment from SuperCos1 (Stratagene). A genomic library of *H. pylori* HPK5 consisting of approximately 350 clones was constructed with pMT5047 by using GIGAPACK II XL packaging extracts (Stratagene). Plasmid pHP802 carrying the urease gene cluster of *H. pylori* UMAB41 (15) was utilized as a probe for colony hybridization, and a cosmid clone containing a 40-kb insert was obtained. After subcloning, pHPT177 carrying the entire urease gene cluster in a 12.8-kb fragment in pBR322 was constructed (Fig. 1). Then the 3.5-kb *Bam*HI-*Eco*RI fragment from pHPT177 containing the region downstream of the urease gene cluster was inserted into pBluescript II KS(−) to construct pS4-1 (Fig. 1). The complete nucleotide sequence of this region was determined by the dideoxynucleotide chain termination method of Sanger et al. (25) with a sequencer (Applied Biosystems; model 373S). DNA manipulations were performed by standard methods (24), and the sequence data were analyzed with the GENETYX program (SDC Software Development).

The nucleotide sequencing of the 3.5-kb insert in pS4-1

* Corresponding author. Mailing address: Department of Microbiology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755-8505, Japan. Phone: 81-836-22-2226. Fax: 81-836-22-2415. E-mail: nakazawa@po.cc.yamaguchi-u.ac.jp.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or characteristics[a]	Source and/or reference
H. pylori		
HPK5	Wild type	Gastric ulcer, 17
CPY2052	Wild type	Gastric ulcer, 17
CPY3401	Wild type	Gastric ulcer, 17
CPY1113	Wild type	Duodenal ulcer
HPKT510	HPK5 derivative; *xylE-kan* in *cdrA*; Km^r	This study
HPKT5L2	HPK5 derivative; *xylE-kan* in *ureF*; Km^r	This study
HPKT510R	HPKT510 derivative; *cat* in *ureF*, intact *cdrA*; Cm^r	This study
E. coli		
NM554	MC1061 *recA13*	Stratagene
DH5α	F^- φ80*dlacZ*ΔM15 Δ(*argF-lac*)*U169 deoR recA1 endA1 hsdR17*(r_K^- m_K^+) *supE44 thi-1 gyrA96 relA1*	GIBCO-BRL
XL1-Blue	*hsdR17 supE44 recA1 endA1 gyrA46 thi relA1 lac*/F′ [*proAB*$^+$ *lacI*^{q} *lacZ*ΔM15::Tn*10*(Tet^r)]	Stratagene
JM105	*endA1 supE sbcB15 thi rpsL* Δ(*lac-proAB*)/F′ [*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	Pharmacia
Plasmids		
pMT5047	SuperCos1 derivative excluding the 2,160-bp *Nru*I fragment; Ap^r	This study
pHP802	8-kb fragment with the urease gene cluster of strain UMAB41; pACYC184 replicon; Ap^r	15
pHPT177	12.8-kb fragment with the urease gene cluster of HPK5; pBR322 replicon; Ap^r	This study
pS4-1	3.5-kb fragment with the *cdrA*, pBluescript II KS(−) replicon; Ap^r	This study
pMT5047	*xylE-kan* (3.13 kb), pBR322 replicon; Ap^r, Km^r	This study
pTA10	*xylE-kan* (3.13 kb) in *cdrA* of pS4-1; Ap^r, Km^r	This study
p177L2	*xylE-kan* (3.13 kb) in *ureF* of pHPT177; Ap^r, Km^r	This study
pTA40	*cat* (1.1 kb) in *ureF* of pHPT177; Ap^r, Cm^r	This study
pBSC103	*cat* (1.2 kb) on pBluescript; Ap^r, Cm^r	D. E. Berg
pBluescript	3.2-kb phagemid vector; Ap^r	Stratagene
pBluescript II KS(−)	2.9-kb cloning vector; Ap^r	Stratagene
pBR322	4.4-kb cloning vector; ColE1 replicon; Ap^r, Tc^r	Takara

[a] Abbreviations: Ap^r, ampicillin resistance; Cm^r, chloramphenicol resistance; Tc^r, tetracycline resistance; Km^r, kanamycin resistance.

revealed an open reading frame (ORF) coding for a 367-amino-acid polypeptide with a molecular weight of 42,500 that was convergent to the urease gene cluster (Fig. 2A). The deduced amino acid sequence showed a high degree of sequence similarity to that of HP0066 of strain 26695, a conserved hypothetical ATP-binding protein with an 831-amino-acid polypeptide at the equivalent location (Fig. 2B) (26). The central region contained a typical ATP-binding motif, and the sequence of the central and C-terminal regions (residues 136 to 152) exhibited 83.6% identity to the equivalent region

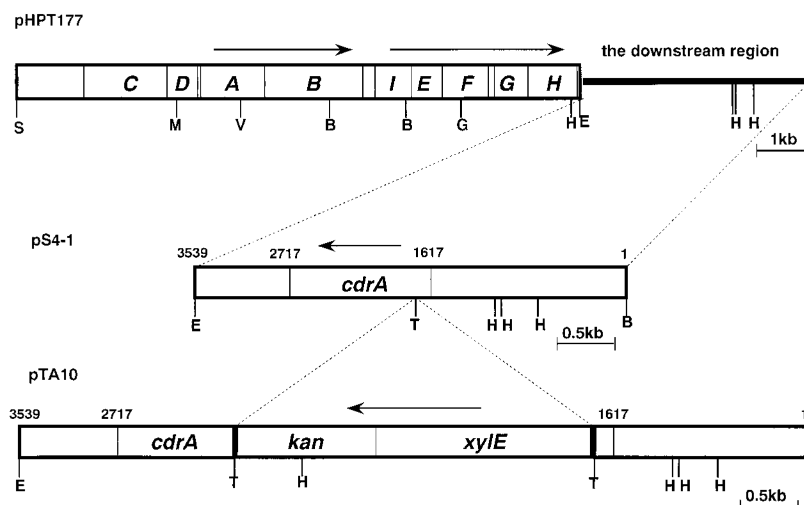

FIG. 1. Restriction maps of the urease gene cluster and the region downstream of this cluster of *H. pylori* HPK5. Plasmid pHPT177 carries a 12.8-kb *Sac*I-*Bam*HI fragment containing the urease operon (*ureA* to *ureH*) and *cdrA*; pS4-1 carries a 3,539-bp *Bam*HI-*Eco*RI fragment containing *cdrA*; and pTA10 contains *cdrA* disrupted by a 3.13-kb *xylE-kan* fragment inserted at the *Tth*111I site. Arrows above the maps show the direction of transcription. Numbers represent the nucleotide positions starting from the right *Bam*HI site. S, *Sac*I; M, *Mul*I; V, *Eco*RV; B, *Bam*HI; G, *Bgl*II; E, *Eco*RI; T, *Tth*111I; H, *Hin*dIII.

A

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                                                               P1
                                                         ----------->
1551 AGGGAGTTTGGCGTTGGTGAAAGAAGAGTTATTATTAGGCGTGAAGCACACGAAAGGATACTTAATATGGAAACAATTCCTACAAAATCAGAACTGAATT
   1          -35                -10                      SD             M  E  T  I  P  T  K  S  E  L  N  W
                                                                                                   Tth111I
1651 GGGAATTTGTAGAGCCGCTCAATGAAAAGGCGTTGAGTGGGTTAGAATATCAATTAAAAATGGGGTTGAGCGATGCGTTTAAGGACTTTGTCAAGCGATC
  13  E  F  V  E  P  L  N  E  K  A  L  S  G  L  E  Y  Q  L  K  M  G  L  S  D  A  F  K  D  F  V  K  R  S
1751 AAACTATGGTTTTAGCCAATGGCGATCTTTTATGGTGGGCAATAAATCTTACACATTCAAACATGTTTTGAACTTCAATTTAGATGGCAAGGGCTTATTG
  46  N  Y  G  F  S  Q  W  R  S  F  M  V  G  N  K  S  Y  T  F  K  H  V  L  N  F  N  L  D  G  K  G  L  L
1851 ATTGATTTTATGCAGAGTTTAAAAGAATGGTTAGAGCCTGAAGAAATAATCTTTGCTAATGACGGGTATGAGGGGTATTATCTTTTGAATACGGCTAGTG
  79 I  D  F  M  Q  S  L  K  E  W  L  E  P  E  E  I  I  F  A  N  D  G  Y  E  G  Y  Y  L  L  N  T  A  S  D
1951 ATGTGGTGCTGTTTTTAGACACTGATGATGGCTCAAAACATGCACTGTTGCACCTTAAAATGTTTTTAATTAAAAGGGAGTTGAAAGACTTGCAAAGAGA
 113  V  V  L  F  L  D  T  D  D  G  S  K  H  A  L  L  H  L  K  M  F  L  I  K  R  E  L  K  D  L  Q  R  E
                                                                                          P4
                                                                                   ------------>
2051 GCAAGATTTTTGGACCAAAAGCTCTCAGTTTAAAGTGTCTGTGCCGGTGGGGTGGGATATTAACCACAAAGAGGTGTGTTTTGAGATCGGTGAAGCGCAA
 146  Q  D  F  W  T  K  S  S  Q  F  K  V  S  V  P  V  G  W  D  I  N  H  K  E  V  C  F  E  I  G  E  A  Q

2151 AACCACACGCTCATTTGC[GGGCGCAGCGGGAGCGGGAAATCC]AATTTCTTGCATGTGTTGATCCAAAATCTGGCTTTCTATTACGCGCCTAATGAGGTCC
 179 N  H  T  L  I  C [G  R  S  G  S  G  K  S] N  F  L  H  V  L  I  Q  N  L  A  F  Y  Y  A  P  N  E  V  Q
                                                                  P3
                                                            <-----------
2251 AACTCTTTTTATTAGACTATAAAGAGGGGGTGGAATTTAACGCATATACAAATCCGACGATTTTAGAGCATGCGAGGTTGGTGAGCGTGGCGAGTTCGGT
 213  L  F  L  L  D  Y  K  E  G  V  E  F  N  A  Y  T  N  P  T  I  L  E  H  A  R  L  V  S  V  A  S  S  V
2351 AGGTTTTGGCGTTAGTTTTTTAAGCTGGCTTGATAAAGAAACGAAAAAAAGAGACGAACTGTTCAAGCAGTTTAATGTGAAAGACTTGAGCGATTACCGA
 246  G  F  G  V  S  F  L  S  W  L  D  K  E  T  K  K  R  D  E  L  F  K  Q  F  N  V  K  D  L  S  D  Y  R
2451 AAGCATGGTGAAATGCCCAGACTGATCGTGGTGATTGATGAATTTCAAGTGCTTTTTAGCGACAGCACCACTAAAGAAAAAGAGAGAGTGGAAGCGTATT
 279 K  H  G  E  M  P  R  L  I  V  V  I  D  E  F  Q  V  L  F  S  D  S  T  T  K  E  K  E  R  V  E  A  Y  L
2551 TAACCAACATTCTCAAAAAAGGCCGCAGCTATGGTGTGCATTTAATTTTAGCCACTCAAACCATGCGCGGTGCTGACATCAATAAAAGCCTTATGGCTCA
 313  T  N  I  L  K  K  G  R  S  Y  G  V  H  L  I  L  A  T  Q  T  M  R  G  A  D  I  N  K  S  L  M  A  Q
2651 AATCGCCAACCGCATCGCTTTGCTTCGCTTTGCTTATGGATGCAGAAGACAGCGAGAGTGTTTTGAGTGATGATGTGGCTTGTGAGCTTGTCAGGCCAGA
 346  I  A  N  R  I  A  L  L  R  F  A  Y  G  C  R  R  Q  R  E  C  F  E  *                 ------->       <---
2751 AGGCATTTTC
```

B

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CdrA(Hp)        ----------------------------------------------------------------------------------------------------
HP0066      1   VKPKSMKEKLVKDMMSILNLRIKMIDVNGLLKELDDALDKVVAKKEPESFLKPIISPIEDYQKSVRQIQAQFTDAPKFNEEGAYPQFLSCGLLEIKGKNG

CdrA(Hp)    1   --------------------------------------------------------------------------METIPTKSELNWEFVEPLNEKALSGL
HP0066    101   ASMEFCLPKVYPFPPKSLYIEHEKDGQFLREMLMRLLSSAPLVQLEVILVDALSLGGIFNLARRLLHKDNDFIYQQRILTESKEIEEALKHLYEYLKVNL
                                                                                           . * * *    * .  * *    .*

CdrA(Hp)   27   EYQLKMGLSDAFKDFVKRS----NYGFSQWRSFMVGNKSYTFKHVLNFNLDGKGLLIDFMQSLKEWLEPEEIIFANDGYEGYYLLNTASDVVLFLDTDD-
HP0066    201   QEKLAGYKDFAHYNEEKKDRLPLKALFLSGVDALSQNALYYLEKIMRFGSKNGVLSFVNLESEKNNKSTEDLKRYAECFKDRTSFERLKYLNIEVINDHG
                 . .*    *     *.      *      .  * *   ... *.  .  *    ..* *    *..     . .          . . . .*

CdrA(Hp)  122   ---------GSKHALLHLKMFLIKRELKDLQREQDFWTKSSQFKVSVPVGWDINHKEVCFEIGEAQNHTLIC[GRSGSGKS]NFLHVLIQNLAFYYAPNEVQ
HP0066    301   IQSKHMKDFADKIKAYYEKKKAVKRELKDLQKDEKFWTESSQFKVSVPVGWDINHKEVCFEIGNEQNHTLIC[GRSGSGKS]NFLHVLIQNLAFYYAPNEVQ
                      *      *    .********...  *** *********************** ************************************

CdrA(Hp)  212   LFLLDYKEGVEFNAYTNPTILEHARLVSVASSVGFGVSFLSWLDKETKKRDELFKQFNVKDLSDYRKHGEMPRLIVVIDEFQVLFSDSTTKEKERVEAYL
HP0066    401   LFLLDYKEGVEFNAYTDPNILEHARLVSVASSVGYGMSFLNWICKEMQERANLFKQFNVKDLSDYRKHGEIPRLIVVIDEFQVLFSDNKS--TKAVEGHL
                *************** *.**************.*.*** ** **  .  *   *****************.***************     .     **  *

CdrA(Hp)  312   TNILKKGRSYGVHLILATQTMRGADINKSLMAQIANRIALLRFAYGCR----RQRECFE
HP0066    499   NTLLKKGRSYGVHLILATQTMRGTDINRSIMAQIANRIALSMDAEDSNSILGDDAACEL
                ...******************.***.*.**********    *   .         *
```

C

Central region

```
CdrA(Hp)      157  VSVPVGWDINHKEVCFEIGEAQNHTLIC[GRSGSGKS]NFLHVLIQNLAFYYAPNEVQLFLLDYKEGVEFNAYTNPTILEHARLVSVAS
SpoIIIE(Cb)   408  LTLALGKDIGGHPVIVDLAKMP-HLLVA[GTTGSGKS]VSLNAMLLSLLYKSTPQQLRLILIDPKM-LELSVYEG---IPHL-LTPVVT
SpoIIIE(Bs)   439  VLIGLGRNISGEAVLAELNKMP-HLLVA[GATGSGKS]VCVNGIITSILMRAKPHEVKMMMIDPKM-VELNVYNG---IPHL-LAPVVT
FtsK(Hi)      163  LPIALGKDISGKPVIVDLAKMP-HLLVA[GSTGSGKS]VGVNTMILSLLYRVQPEDVKFIMIDPKV-VELSVYND---IPHL-LTPVVT
FtsK(Ec)      963  LTVVLGKDIAGEPVVADLAKMP-HLLVA[GTTGSGKS]VGVNAMILSMLYKAQPEDVRFIMIDPKM-LELSVYEG---IPHL-LTEVVT
                    . . .*  *    *  ..      * *..* .*****  .. ..   .    *   ..  ..* *  .*   *      . *  *  * .
```

C-terminal region

```
CdrA(Hp)      282  EMPRLIVVIDEFQVLFSDSTTKEKERVEAYLTNILKKGRSYGVHLILATQTMRGADINKSLMAQIANRIA
SpoIIIE(Cb)   554  ELPQLVVIADEFADMMVVVGKK----VETLIVRLAQKARAAGIHLIFATQRPSVDVITGLIKANIPTRVA
SpoIIIE(Bs)   571  ELPYIVVIVDELADLMMVASSD----VEDSITRLSQMARAAGIHLIIATQRPSVDVITGVIKANIPSRIA
FtsK(Hi)      312  KLSYIVVIVDEFADLMMVAGKQ----IEELIARLAQKARAIGIHLILATQRPSVDVITGLIKANIPSRIA
FtsK(Ec)     1112  KEPYIVVLVDEFADLMMTVGKK----VEELIARLAQKARAAGIHLVLATQRPSVDVITGLIKANIPTRIA
                     ..*. **   .          .*  .  .  . *. *.**. ***       *.   . *.*  *.*
```

N-terminal region

```
CdrA(Hp)       86  KEWLEPEEIIFANDGYEGYYLLNTASDVVLFLDTDDGSKHALLHLKMFLIKRELKDLQRE
SpoIIIE(Bs)   233  KQQMEPEPPDEEGDYETVSPLIHSEPIISSFSDRNEEEESPVIEKRAEPVSKPLQDIQPE
                    *. .***      .*      *...   .   * *   .     ..  .   . *.*.* *

CdrA(Hp)       61  SYTFKHVLNFNLDGKGLLIDFMQSLKEWL
SpoIIIE(Cb)    57  SIVVKHVPNLTGEAGAWLSDFTKNMVGYL
                   *   *** *  . .    * **  .   *
```

FIG. 2. (A) Nucleotide sequence of the *cdrA* gene. The deduced amino acid sequence is indicated under the nucleotide sequence, and an asterisk marks the stop codon. Both −35 and −10 promoter consensus sequences and a putative Shine-Dalgarno (SD) sequence are indicated. An inverted repeat at the end of the ORF is indicated below the sequence (boldface arrows). The arrows above the sequence show the PCR primers (P1, P3, and P4). (B) Alignment of amino acid sequences of *H. pylori* CdrA [CdrA(Hp)] and hypothetical ATP-binding protein HP0066 (26). The C-terminal amino acid sequence of HP0066 is abbreviated. (C) Alignment of partial amino acid sequences of *H. pylori* CdrA [CdrA(Hp)], *C. burnetii* FtsK/SpoIIIE [SpoIIIE(Cb)] (22), *B. subtilis* SpoIIIE [SpoIIIE(Bs)] (29), *H. influenzae* FtsK [FtsK(Hi)] (11), and *E. coli* FtsK [FtsK(Ec)] (2). ATP binding motifs are boxed. In panels B and C, identical amino acids and conserved changes are indicated by asterisks and by dots, respectively; dashes indicate gaps included to optimize the alignment.

of HP0066, whereas the N-terminal sequence (residues 1 to 135) exhibited less than 15% identity. In addition, the N-terminal 174-amino-acid sequence of HP0066 had no counterpart in the HPK5 sequence. The nucleotide sequence identities for these strains were 49.4% in the N-terminal region and 88.2% in the central and C-terminal regions, indicating distinct origins for the N-terminal regions of these putative proteins. Since the deduced sequence of the HPK5 protein showed some

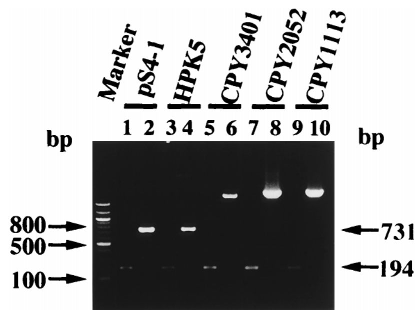

FIG. 3. PCR analysis of the *cdrA* regions of plasmid pS4-1 and *H. pylori* HPK5, CPY3401, CPY2052, and CPY1113. The PCR products obtained with the P3-P4 primer pair (lanes 1, 3, 5, 7, and 9) and with the P1-P3 primer pair (lanes 2, 4, 6, 8, and 10) are shown. The 100-bp ladder markers with 2 kb as the longest are indicated in the left lane.

similarity to the sequences of reported FtsK/SpoIIIE family proteins from *Coxiella burnetii* (778 amino acids) (22), *B. subtilis* (787 amino acids) (29), *Haemophilus influenzae* (529 amino acids) (11), *H. pylori* 26695 (831 amino acids) (26), and *E. coli* (1,329 amino acids) (2), we designated the cloned gene *cdrA*, reflecting its role as a cell division-related gene. The putative gene product, CdrA, though smaller than the other FtsK/SpoIIIE family proteins, had a sequence central to the C-terminal region that was 50 to 57% homologous to the corresponding sequences of this family of proteins (Fig. 2C). In addition, CdrA showed some similarity to SpoIIIE proteins of *B. subtilis* and *C. burnetii* in the N-terminal region. Hydrophobic domains commonly found in the N-terminal region of FtsK/SpoIIIE family proteins, however, were missing in CdrA.

Southern hybridization was performed to identify the *cdrA* gene in the genomic DNAs of *H. pylori* HPK5, CPY3401, and CPY2052. A single hybridization band was observed in each of the *Hin*dIII- or *Bgl*II- and *Bam*HI-digested genomic DNAs. It should be noted that the hybridization bands of the *Hin*dIII-digested DNAs of CPY3401 and CPY2052 were smaller than that of HPK5 (data not shown), suggesting some heterogeneity in the *cdrA* region. To analyze further the polymorphism of the *cdrA* region, PCR was carried out (Fig. 3). By using the P3-P4 primer pair (Fig. 2A), the expected 194-bp product was obtained with all the DNAs tested. On the other hand, the 731-bp product expected with the P1-P3 primer pair (Fig. 2A) was obtained with HPK5 and pS4-1 DNAs, whereas the PCR products with CPY3401, CPY2052, and CPY1113 were longer than 2 kb. In addition, *Tth*111I, which has a single site in *cdrA* of HPK5, did not cut the last three PCR products. Furthermore, we have compared the upstream regions of *cdrA* and the ORF encoding HP0066 and found that the two ORFs encoding HP0065 and HP0064 of *H. pylori* 26695 (26) have no counterpart in HPK5 (data not shown). The above findings indicated that CdrA of *H. pylori* has highly conserved central and C-terminal regions but a heterogeneous N-terminal region and that *cdrA* has a heterogeneous 5′ noncoding region. Such a highly polymorphic region might be useful for genotyping various *H. pylori* strains, a possibility which should be confirmed by further sequence analysis.

To obtain *cdrA*-disrupted mutants, plasmid pMT5074 carrying a 3.13-kb *xylE-kan* cassette was constructed with a 1.88-kb *Kpn*I-*Xho*I fragment containing *xylE* from pTS117 (16, 21) and a 1.25-kb *Sal*I fragment containing a kanamycin resistance gene (*kan*) from pUC4K (20). The *xylE-kan* cassette was then cut out from pMT5074 with *Bam*HI and *Bgl*II and blunt end ligated into the Klenow enzyme-filled *Tth*111I site in *cdrA* in pS4-1 to construct pTA10 (Fig. 1). Purified pTA10 DNA was

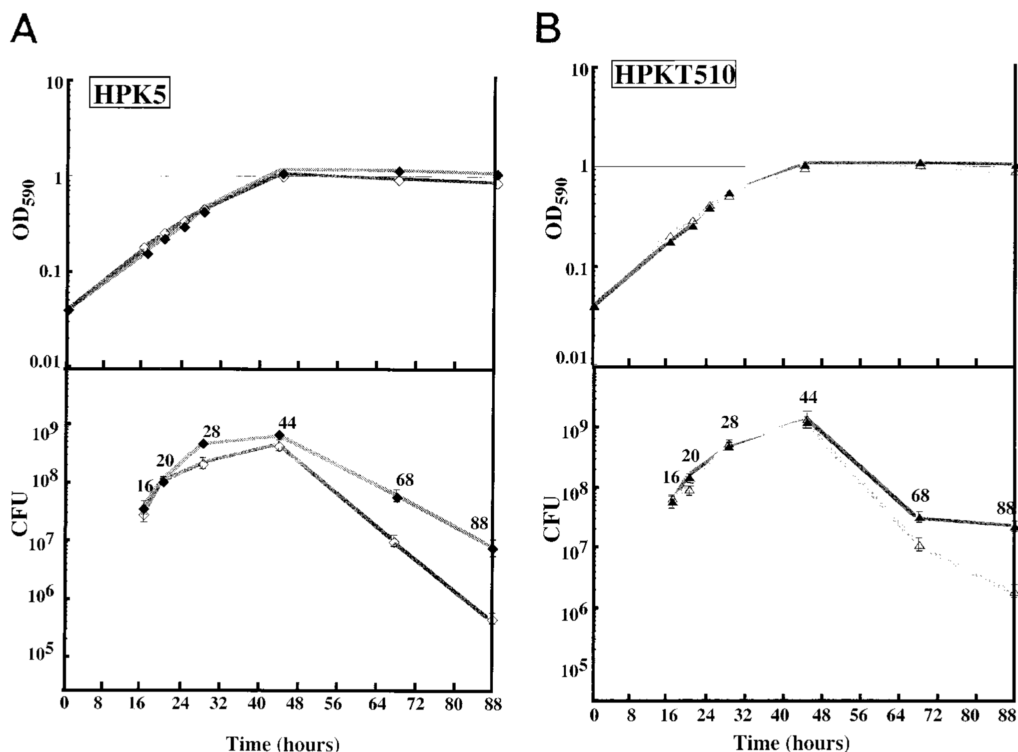

FIG. 4. Growth curves and CFUs of HPK5 (A) and HPKT510 (B). Bacteria were grown in brucella medium at 37°C under microaerobic conditions, and the OD_{590} values and CFUs were measured. ◆, HPK5 in 0.5% NaCl; ◇, HPK5 in 1% NaCl; ▲, HPKT510 in 0.5% NaCl; △, HPKT510 in 1% NaCl.

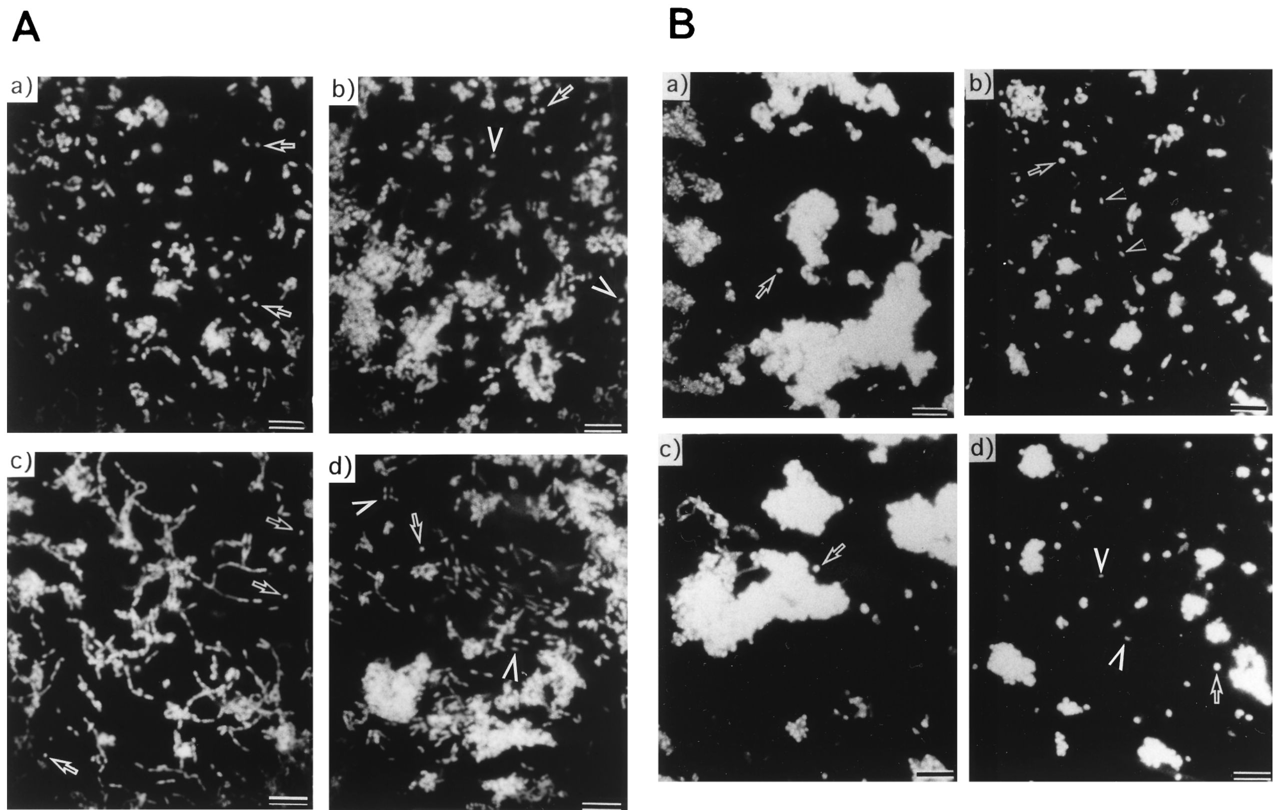

FIG. 5. Morphology of HPK5 and HPKT510 from the cultures represented in Fig. 4 at 44 h (A) and 88 h (B) as determined by DAPI staining. Arrows indicate coccoid forms, and arrowheads indicate short rods. For both panels, segments are as follows: a, HPK5 in 0.5% NaCl; b, HPKT510 in 0.5% NaCl; c, HPK5 in 1% NaCl; d, HPKT510 in 1% NaCl. Bars, 10 μm.

used as the donor DNA to transform HPK5 (10^7 cells). By allelic exchange mutagenesis (27), two transformants were obtained per 0.3 μg of donor DNA when high-salt level brucella medium containing 1% NaCl was used after prolonged incubation. No transformants were obtained when the standard brucella medium containing 0.5% NaCl was used or when the incubation in the high-salt level brucella medium was carried out at 30°C. Southern hybridization analysis confirmed disruption of the *cdrA* gene with the *xylE-kan* cassette (data not shown). One of the transformants, HPKT510, was utilized for further studies.

Because of the low frequency of the mutation involving *cdrA* disruption, we carried out some control experiments. First, transformation of HPK5 with plasmid p177L2 containing the same *xylE-kan* cassette in the *Bgl*II site of *ureF* resulted in *ureF* disruption at a frequency of approximately 10^3 mutants per μg of donor DNA. Second, transformation of HPK5 with the genomic DNAs from *cdrA*-disrupted HPKT510 and *ureF*-disrupted mutant HPKT5L2 resulted in *cdrA* and *ureF* disruptions at frequencies of 60 and 8,240 mutants per μg of donor DNA, respectively. Therefore, the low generation frequency of *cdrA*-disrupted mutants might be due to the lethality of the *cdrA* mutation and suggested that HPKT510 might have acquired a secondary mutation, as was observed for the *ftsK* mutation in *E. coli* (2).

To elucidate the growth characteristics of HPKT510, a putative *cdrA* mutant, the effect of salt concentration was studied, since *cdrA* had some similarity to *E. coli ftsK* and since its disruption leads to filamentous cell formation in L broth containing 0.5% NaCl, which was suppressed by 1% NaCl (2). Cells of *H. pylori* from the liquid culture were collected, washed once with sterilized saline, and suspended in an appropriate volume of saline, and then a 10-μl aliquot of the sample was spotted onto two clean slide glasses, followed by Gram staining and by 4′,6-diamino-2-phenylindole (DAPI) staining according to the method of Hiraga et al. (14). When started at an OD_{590} of 0.04, all cultures entered the stationary phase at 44 h and showed an OD_{590} of 1.0 irrespective of strains and NaCl concentrations used (Fig. 4). Intriguingly, however, CFUs of the wild-type HPK5 at 28 h (late log phase) and 44 h (early stationary phase) in the high-salt level medium and at 44 h in the standard medium were significantly lower than those at similar growth phases of the mutant HPKT510. In accordance with this, HPK5 in the high-salt level medium at 44 h formed many multinuclear filamentous cells, whereas HPKT510 remained as short rods (Fig. 5A). It should be noted that the sizes and distribution of bacterial nuclei were apparently normal in the filamentous cells of HPK5, suggesting that the *cdrA* gene might have a repressive role in cell division, possibly after the steps of DNA replication and segregation.

To analyze further the unexpected phenotypes of HPK5 and HPKT510, we constructed plasmid pTA40, which carried the intact *cdrA* gene with the disrupted *ureF* gene, by inserting chloramphenicol resistance gene *cat* (28) from pBSC103. By transforming HPKT510 with pTA40, we obtained a Cm^r transformant, HPKT510R, and confirmed the presence of intact *cdrA* by Southern hybridization (data not shown). When HPKT510R was grown in the high-salt level medium, many multinuclear filamentous cells indistinguishable from those of HPK5 were observed. These results indicated a close associa-

tion of the morphological feature of HPK5 with the presence of *cdrA*. We could not identify, however, the phenotype associated with a presumed secondary mutation in HPKT510, since such a mutation might have been suppressed in HPKT510R.

After HPK5 and HPKT510 cells entered the stationary phase, the CFUs of HPK5 decreased rapidly compared to those of HPKT510 (Fig. 4), resulting in an undetectable level at 136 h. In contrast, the CFUs of HPKT510 were at the level of 10^7 (data not shown). Microscopic observations of DAPI-stained cultures at 88 h (late stationary phase) revealed that most of the HPK5 cells were single or aggregated coccoids, whereas HPKT510 cells were mostly short rods with some coccoids (Fig. 5B). We have repeated the experiments on the long-term growth of strains HPK5 and HPKT510 three times and have obtained essentially the same results. These findings indicated that the wild-type strain carrying intact *cdrA* tended to form coccoids in the stationary phase concomitant with a decrease in CFU, whereas the *cdrA*-disrupted mutant maintained the rod shape and remained viable. It is still a matter of debate whether the coccoid forms of *H. pylori* are viable but not culturable (1, 3, 5–7) or are the morphologic manifestation of bacterial cell death (18), and further studies on the *cdrA* gene, together with characterization of HPKT510, might provide a clue to solve the problem.

Nucleotide sequence accession number. The nucleotide sequence reported in this study has been deposited in GenBank under EMBL accession no. AB003309.

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