C. MATERIALS

1. Bacterial strains

Strain	relevant characteristics	reference / source
E. coli		
DH5α	*supE*44, Δ*lac*U169 (Φ80 *lacZ*ΔM15) *hsdR*17, *recA*1, *endA*1, *gyrA*96, *thi*-1, *relA*1.	Hanahan, 1983
BL21(DE3)	*hsdS, gal* (λ*cIts*857 *ind*1 *S*am7 *nin*5 *lac*UV5-T7 gene *1*).	Studier *et al*., 1990
H. pylori		
CCUG17874	clinical isolate, *cagA*$^+$, wild type.	Xiang *et al*., 1995
G27	clinical isolate, *cagA*$^+$, wild type.	Xiang *et al*., 1995
G27(*cagA::km*)	*km*^{r}, *cagA*$^-$, G27 derivative in which the first 2884 bp of *cagA* and 254 bp of the upstream untranslated region have been substituted by a *km*^{r} cassette.	This study
G27(cagWT)	*cm*^{r}, *cagA*$^-$, G27(*cagA::km*) derivative obtained by transformation with plasmid pCagWT.	This study
G27(cag2)	*cm*^{r}, *cagA*$^-$, G27(*cagA::km*) derivative obtained by transformation with plasmid pCag2.	This study
G27(cag3)	*cm*^{r}, *cagA*$^-$, G27(*cagA::km*) derivative obtained by transformation with plasmid pCag3.	This study
G27(cag4)	*cm*^{r}, *cagA*$^-$, G27(*cagA::km*) derivative obtained by transformation with plasmid pCag4.	This study
G27(cagX1)	*cm*^{r}, *cagA*$^-$, G27(*cagA::km*) derivative obtained by transformation with plasmid pCagX1.	This study
G27(cagX2)	*cm*^{r}, *cagA*$^-$, G27(*cagA::km*) derivative obtained by transformation with plasmid pCagX2.	This study
G27(cagX3)	*cm*^{r}, *cagA*$^-$, G27(*cagA::km*) derivative obtained by transformation with plasmid pCagX3.	This study
G27(*flgR::km*)	*km*^{r}, G27 derivative in which bp 49 to 1142 of *flgR* have been substituted by a *km*^{r} cassette.	This study
G27(*hspR::km*)	*km*^{r}, G27 derivative in which bp 8 to 324 of *hspR* have been substituted by a *km*^{r} cassette.	This study

Table 2: Bacterial strains used in this study.

2. Vectors and plasmids

vector/ plasmid	relevant characteristics	reference/ source
vectors		
pGEMT	cloning vector for PCR products, Amp^r.	Promega
pGEM3	cloning vector, Amp^r.	Promega
pSL1190	cloning vector, Amp^r.	Pharmacia

vector/ plasmid	relevant characteristics	reference/ source
pMMB208	broad host range vector, Cm^r.	Morales *et al.*, 1991
pCMVβ	vector containing the *lacZ* gene of *E. coli*.	Clontech
pET22b$^+$	expression vector for His-tagged proteins, Amp^r.	Novagen
pTrcHisA	expression vector for His-tagged proteins, Amp^r.	Invitrogen
pBluescript SK	cloning vector, Amp^r.	Stratagene
plasmids		
pILL600	plasmid containing the kanamycin cassette from *Campylobacter coli*.	Labigne-Roussel *et al.*, 1988
pDT2548	plasmid containing the chloramphenicol resistance gene from *C. coli.*	Wang and Taylor, 1990
pGEMT-P_1	derivative of pGEMT carrying a 182 bp *Nco*I/*Sma*I fragment specific for P_1 and obtained by PCR with oligonucleotides ΔCProm1 and CProm-3'.	This study
pGEM3(*cagA::km*)	pGEM3 derivative containing a 1400 bp *Bam*HI fragment carrying the kanamycin cassette from plasmid pILL600 flanked by a 687 bp *Eco*RI/*Bam*HI fragment obtained with oligonucleotides orf-5'/Δorf-3' and comprising *cagB* and the 5'-half of the *cagAB* intergenic region and a 626 bp *Bam*HI/*Pst*I fragment obtained with primers Δcag-5'/cag-3' and comprising a distal part of the *cagA* gene.	This study
pSKA11.1	pBluescriptSK carrying a 2.89 kb *Hin*dIII fragment comprising part of *cagE*, *cagB-D* and the *cagAB* intergenic region.	Censini *et al.*, 1996
pCagWT	pSL1190 derivative carrying in the following order: a 687 bp *Eco*RI/*Nco*I-fragment obtained with oligonucleotides orf-5'/orf-3' and comprising *cagB* and the 5'-half of the *cagAB* intergenic region; a 214 bp *Nco*I/*Sma*I fragment obtained with oligonucleotides CPromWT/CProm-3' and comprising the 3'-half of the *cagAB* intergenic region; a 3400 bp *Sma*I/*Bam*HI fragment carrying a promoterless *lacZ* gene from plasmid pCMVβ; a 800 bp *Bam*HI/*Xba*I fragment containing a chloramphenicol resistance gene from plasmid pDT2548 and a 626 bp *Xba*I/*Pst*I fragment obtained with oligonucleotides cag-5'/cag-3' and comprising a distal part of the *cagA* gene.	This study
pCag2	derivative of pCagWT in which the 214 bp *Nco*I-*Sma*I fragment has been substituted by a 150 bp *Nco*I-*Sma*I PCR fragment obtained with oligonucleotides ΔCProm2/CProm-3'.	This study
pCag3	derivative of pCagWT in which the 214 bp *Nco*I-*Sma*I fragment has been substituted by a 90 bp *Nco*I-*Sma*I PCR fragment obtained with oligonucleotides ΔCProm3/CProm-3'.	This study
pCag4	derivative of pCagWT in which the 214 bp *Nco*I-*Sma*I fragment has been substituted by a 82 bp *Nco*I-*Sma*I PCR fragment obtained with oligonucleotides ΔCProm4/CProm-3'.	This study
pCagX1	derivative of pCagWT in which the 687 bp *Eco*RI/*Nco*I fragment has been substituted by a 783 bp *Eco*RI/*Nco*I fragment obtained with oligonucleotides orf-5'/ΔXProm1 and in which the 214 bp *Nco*I-*Sma*I fragment has been substituted by a 73 bp *Nco*I-*Sma*I fragment obtained with oligonucleotides ΔCProm5/CProm-3'.	This study
pCagX2	derivative of pCagX1 in which the 783 bp *Eco*RI/*Nco*I fragment has been substituted by a 743 bp *Eco*RI/*Nco*I PCR fragment obtained with oligonucleotides orf-5'/ΔXProm2.	This study
pCagX3	derivative of pCagX1 in which the 782 bp *Eco*RI/*Nco*I fragment has been substituted by a 700 bp *Eco*RI/*Nco*I PCR fragment obtained with oligonucleotides orf-5'/ΔXProm3.	This study
pHTT7f1-NHα	Amp^r; ori-pBR322; *ori-f; ϕ10P-rpoA(H6, Nter).*	Tang *et al.*, 1995, 1996
pHTT7f1-NHα(1–235)	Amp^r; ori-pBR322*; ori-f; ϕ10P-rpoA(H6, Nter)(1-235).*	Tang *et al.*, 1995, 1996

vector/ plasmid	relevant characteristics	reference/ source
pMMB208cagWT	pMMB208 derivative containing a *Eco*RI/*Bam*HI fragment from pCagWT which comprises *cagB*, the wild type intergenic region between *cagA* and *cagB* and the *lacZ* gene.	This study
pLAW2	plasmid carrying the *rpoA* gene from *E. coli.*	Zou *et al*., 1992
pLAW2Δ256	plasmid carrying a modified *rpoA* gene that results in a truncated α subunit lacking the 73 C-terminal amino acids.	S. Busby
pTE22b-hspR	pTE22b derivative containing a 372 bp *Nde*I/*Xho*I fragment obtained by PCR with oligonucleotides hspRN/hspRC that comprises the entire coding sequence of *hspR.*	This study
pTrcA-flgR	pTrcHisA derivative containing a 1165 bp *Nhe*I/*Bam*HI fragment obtained with oligonucleotides flgRN/flgRC that comprises the entire coding region of *flgR.*	This study
pFlaB	pGEM3 derivative containing a 320 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides fla1/fla2 that comprises the intergenic region between *flaB* and *topA* and 52 bp and 102 bp of the respective coding regions.	This study
pFlgE	pGEM3 derivative containing a 230 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides flgE5/flgE6 that comprises 36 bp of the coding region of *flgE* and 194 bp of its 5' untranslated region.	This study
pFlgD	pGEM3 derivative containing a 233 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides flgE3/flgE4 that comprises 64 bp of the coding region of *orf906* and 169 bp of its 5' untranslated region.	This study
pFlgB	pGEM3 derivative containing a 218 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides flgB1/flgB2 that comprises 97 bp of the coding region of *flgB* and 121 bp of its 5' untranslated region.	This study
pFlgK	pGEM3 derivative containing a 356 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides flgK1/flgK2 that comprises 158 bp of the coding region of *orf1120* and 198 bp of its 5' untranslated region.	This study
pGyrA	pGEM3 derivative containing a 480 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides gyr1/gyr2 that comprises *orf697* and 237 bp of its 5' untranslated region.	This study
pGEM3(*flgR::km*)	pGEM3 derivative containing a 1400 bp *Bam*HI fragment carrying the kanamycin cassette from plasmid pILL600 flanked by a 403 bp *Eco*RI/*Bam*HI fragment obtained with oligonucleotides ntr1/ntr2 and comprising part of *orf702* and a 412 bp *Bam*HI/*Pst*I fragment obtained with oligonucleotides ntr8/ntr9 and comprising a distal part of the *flgR* gene.	This study
pGEM3(*hspR::km*)	pGEM3 derivative containing a 1400 bp *Bam*HI fragment carrying the kanamycin cassette from plasmid pILL600 flanked by a 1069 bp *Eco*RI/*Bam*HI fragment obtained with oligonucleotides hsp1/hsp2 and comprising *cbpA* and a 716 bp *Bam*HI/*Pst*I fragment obtained with oligonucleotides hsp3/hsp4 and comprising the 5' half of *orf1026*.	This study
pCbp12	pBluescript SK derivative containing a 674 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides cbp1/cbp2 that comprises the intergenic region between *cbpA* and *orf1023* and parts of the respective coding regions.	This study
pCbp34	pGEM3 derivative containing a 272 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides cbp3/cbp4 that comprises the intergenic region between *cbpA* and *orf1023.*	This study
pHrcA	pGEM3 derivative containing a 293 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides hrc1/hrc2 that comprises the intergenic region between *orf111* and *orf112.*	This study
pGroE	pGEM3 derivative containing a 427 bp *Eco*RI/*Bam*HI fragment obtained by PCR with oligonucleotides gro1/gro2 that comprises the intergenic region between *groES* and *dnaG.*	This study

Table 3: Vectors and plasmids used in this study.

3. Oligonucleotides

name	sequence (5' to 3')[a)]	Stran	site[b)]	position[c)]	position[d)]
orf-5'	AAACCTgaattcGCAGTGACGCCTTCTGTAGGA	+	*Eco*RI	18015-18047	
orf-3'	AAAATGccatggCTTTAATAAGAACAAGAAATAAGAAAT	-	*Nco*I	18719-18680	
Δorf-3'	AAAATGggatccCTTTAATAAGAACAAGAAATAAGAAAT	-	*Bam*HI	18719-18680	
cag-5'	TGGCAAtctagaGGATTTCAGCAAGGTAACGCAAGC	+	*Xba*I	21215-21250	
Δcag-5'	TGGCAAggatccGGATTTCAGCAAGGTAACGCAAGC	+	*Bam*HI	21215-21250	
cag-3'	GAGCCActgcagGATTCCTTGAAAGCCCTACCTTAC	-	*Pst*I	21858-21823	
CProm-	TATCGGcccgggGTTAGTGTCAAAGACTGCTAAAAATC	-	*Sma*I	18931-18894	
CPromwt	TTAAAGccatggCATTTTAGCAAATTTTTGTTAATTGTGG	+	*Nco*I	18702-18741	
ΔCProm1	AATTGTccatggAAATGTGAATCGTCCTAGCCTTTAG	+	*Nco*I	18734-18770	
ΔCProm2	TTTAGAccatggCAACGATCGGGCTTTTTTCAATATTA	+	*Nco*I	18766-18803	
ΔCProm3	AAAAAAccatggAATGCTTGATATTGTTGTATAATGAGA	+	*Nco*I	18817-18856	
ΔCProm4	AAAAATccatggTATTGTTGTATAATGAGAATGTTCAAAG	+	*Nco*I	18826-18865	
ΔCProm5	TTGATAccatggTATAATGAGAATGTTCAAAGACATG	+	*Nco*I	18834-18870	
ΔXProm1	CATTAATccatggTAATATTGAAAAAAGCCCGATCG	-	*Nco*I	18816-18781	
ΔXProm2	GGCGTCccatggCTAGGACGATTCACATTTTTACCCAC	-	*Nco*I	18775-18738	
ΔXProm3	ACAAAAccatggTAAAATGAAAGAACTTTAATAAGAAC	-	*Nco*I	18732-18695	
lac[e)]	CTTGTTGGTCAAAGTAAACGACAT	-			
cagN	GTCAATGGTTTCGTTAGTC	-		18983-18965	
orfX	GCAACTCCATAGACCACTAAAG	+		18440-18461	
gyrN	CCAATAACCACCATCCAAG	-			751122-751104
flaB	GCATGAGAAGTTAAAGCGGC	+			124446-124465
flgE	GACACCAGACCATAAAGACC	+			922547-922566
flgE2	GGATTAATGGGAGATGGCATG	-			956504-956484
flgB	AAGACCGATAATCCAACGCC	+			1641322-1641341
flgK	GATGTCTCTTATATCGCGCTCGG	+			1186790-1186812
flaA	CGCATTGATATTTGTATTGACCTG	+			637268-637291
ureA	CATAGTGGAGCATCAAC	+			77911-77927
ntrY	GAATGAAAAGAAACGCATCACTC	-			755012-754990
gyrA	CTGAATTATCTTGCATGTGTC	-			752527-752507
ntrN	ATCATCTTCTACAATGGCG	-			755491-755473
ntr1	aatcgtgaattcTAGAGTGATGGGCGAAG	+	*Eco*RI		755102-755130
ntr2	aaaaaaggatccAGGCTTTTACGCATG	-	*Bam*HI		755523-755497
ntr8	tgtccgggatccCAGAGCATCTATTAGAAAGCGAG	+	*Bam*HI		756042-756076
ntr9	tatgcactgcagCCCAAATGATACGCATCGCACAC	-	*Pst*I		756437-756403
flgE3	attataggatccGCTATTCAAAGCGTTTGCGTTGG	-	*Bam*HI		956559-956525
flgE4	attattgaattcTGTTCTCATTAAGCGCGAATAACG	+	*Eco*RI		956298-956333
flgE5	GCGATTTGGTGGGCTTggatccATTGACACCAGAC	+	*Bam*HI		922521-922556
flgE6	CTAAAGCGAGTTgaattcTTAAGCTTGAGCGATAAC	-	*Eco*RI		922784-922749
flgB1	AAAGGGggatccACATTAGCGATGTTAGAAG	+	*Bam*HI		1641273-1641303
flgB2	AATGGCTCTgaattcGCTTATCGCTCAAGC	-	*Eco*RI		1641514-1641485
flgK1	attattgaattcAAAAGCTTGAATCGCTAGCTG	+	*Eco*RI		1186713-1186745

name	sequence (5' to 3')[a)]	Stran	site[b)]	position[c)]	position[d)]
flgK2	attattggatccCAAGCGGGGAATGCGATGAGC	-	*Bam*HI		1187086-1187054
fla1	attataggatccGCATGAGAAGTTAAAGCGGCG	+	*Bam*HI		124434-122466
fla2	attatagaattcCCTAACATGCCCTTTAGAGGC	-	*Eco*RI		124771-124739
gyr1	tatttaggatccCCAATAACCACCATCCAAGACATG	-	*Bam*HI		751134-751099
gyr2	attattgaattcGATTGGCTAGGCATACAGCCCCCAG	+	*Eco*RI		750711-750746
flgRN	Atcgatgctagccatatg AAAATCGCCATTGTAGAAGATG	+	*Nhe*I, *Nde*I		755450-755489
flgRC	gagtatggatccCCCTAACTCCCTACCTTTCC	-	*Bam*HI		756632-756601
hsp1	TAGTTAgaattcCTTTTAATTGCGCTGAAACGGG	+	*Eco*RI		1087499-1087532
hsp2	atatatggatccGGGTGCACGCCTAAGATTTTAGCC	-	*Bam*HI		1088526-1088491
hsp3	AAAAATggatccACCCCTACGAATTTTTACGAATTG	+	*Bam*HI		1088827-1088862
hsp4	AAGGTTctgcagCGTATCATCGCTATAAGATCCATC	-	*Pst*I		1089560-1089525
hspRN	atatatcatatgTGCGATTATGATGAACCGC	+	*Nde*I		1088500-1088530
hspRC	atatatctcgagTTTTTTAAATAAAATCAGTTCATA	-	*Xho*I		1088914-1088879
groS	GACCCTTTCTCCTAATGGCTG	+			9595-9615
gro1	attattgaattcAGGGATGATGATGCCTGAACTGG	+	*Bam*HI		9529-9563
gro2	attaatgaattcTACAATGTCTATCGTTTGCAAAAGGC	-	*Eco*RI		9973-9936
hrcA	CAAACGCATCTAACAAACTCTC	+			119560-119581
cbp1	attattggatccACCCCAAGACGCGCTAAAGCCC	+	*Bam*HI		1087269-1087302
cbp2	attattgaattcTTTGCGAAAAGCCTCCTTTCCC	-	*Eco*RI		1087960-1087927
cbp3	AAAAAAggatccCTAACGCTAAATAATAATATC	+	*Bam*HI		1087408-1087440
cbp4	attattgaattcATCTTGGCTGGCGTTTTCGCTC	-	*Eco*RI		1087698-1087665
hrc1	attattgaattcTTGGGTTAGGGGGATTTTAAGGG	-	*Eco*RI		119640-119674
hrc2	attattggatccATTCTTGATGAAAGAACCCTCGC	+	*Bam*HI		119958-119924
cheY	GCTATCATCTACTACCAGTAGTTTC	-			1126294-1126270

Table 4: Oligonucleotides used in this study.

a) Capital letters indicate *H. pylori* derived sequences (except for [e)]), small letters sequences added for cloning purposes, small underlined letters restriction recognition sites.
b) Restriction recognition sites.
c) Nucleotide positions refer to the *cag* sequence deposited in the GenBank data base under accession number U60176 (Censini *et al*., 1996).
d) Nucleotide positions refer to the genome sequence published by Tomb *et al*. (1997).
e) The sequence of this oligonucleotide is complementary to the first 24 nucleotides of the coding sequence of the β-galactosidase gene from *E. coli.*

4. Media

LB medium
10 g NaCl
10 g Tryptone
5 g yeast extract
H_2O to 1 l, adjust to pH 7.0

modified Brucella Broth	28 g Brucella broth
	H_2O to 950 ml, autoclave, cool to 45-50 °C, supplement with:
	50 ml fetal calf serum
	5 mg/l vancomycin
	10 mg/l trimetoprim
	6 mg/l cefsulodine
	8 mg/l amphotericine B
	100 mg/l cycloheximide

5. Agar plates

LB-agarplates	10 g NaCl
	10 g Tryptone
	5 g yeast extract
	15 g Noble agar
	H_2O to 1.0 l, adjust to pH 7.6 and autoclave
Columbia-blood agar plates	44 g Columbia agar
	H_2O to 950 ml, autoclave, cool to 45-50 °C, supplement with:
	50 ml horse blood
	0.2% cyclodextrin
	5 mg/l vancomycin
	10 mg/l trimetoprim
	6 mg/l cefsulodine
	8 mg/l amphotericine B
	100 mg/l cycloheximide
H. pylori motility plates	28 g Brucella broth
	3 g Noble agar
	H_2O to 900 ml, autoclave, cool to 45-50 °C, supplement with:
	100 ml fetal calf serum
	5 mg/l vancomycin
	10 mg/l trimetoprim
	6 mg/l cefsulodine
	8 mg/l amphotericine B
	100 mg/l cycloheximide

Antibiotic supplements

ampicillin	100 µg/ml
chloramphenicol	25 µg/ml
kanamycin	20 µg/ml
novobiocin	100 µg/ml

Antibiotic stock solutions

ampicillin	100 mg/ml ampicillin sodium salt in H_2O
chloramphenicol	30 mg/ml chloramphenicol in ethanol
kanamycin	25 mg/ml kanamycin in H_2O
novobiocin	100 mg/ml in H_2O
vancomycin	10 mg/ml in H_2O
trimetoprim	10 mg/ml in N-N-dimethylformamide
cefsulodine	10 mg/ml in H_2O
amphotericine B	10 mg/ml in dimethylsulfoxide
cycloheximide	100 mg/ml in acetone

E. coli strains are grown in LB medium supplemented with the appropriate antibiotic. *H. pylori* cells are recovered from frozen stocks on Columbia-blood agar plates and grown in jars under microaerophilic conditions (Oxoid) for 2-3 days. After passage on fresh plates bacteria are cultured in a 5% CO_2 / 95% air atmosphere. Liquid cultures of *H. pylori* are grown in modified Brucella Broth.

6. Chemicals and enzymes

Chemicals used in this study were supplied by Aldrich, Amersham, BDH, Biorad, Boehringer, Carlo Erba, Difco, Fluka, Life Technologies, Merck, Oxoid, Riedel - de Haen, Roth, Qiagen, Serva, and Sigma. Restriction and DNA-modification enzymes were purchased by Boehringer, Life Technologies, New England Biolabs, Pharmacia, and Promega. Radioactively labeled nucleotides were supplied by Amersham.

7. Instruments

autoclave	DeLama
centrifuges	Beckman TJ-6, J2-21, J2-21ME, J-25
	Heraeus Sepatech Biofuge 13/Biofuge 13R/5417R
computer software	Microsoft-Windows 95, Microsoft Word 7.0
	Freehand 7.0
DNA synthesiser	Expedite™
DNA sequencing apparatus	Applied Biosystems 373 automated DNA sequencer
DNA electrophorese chambers	Elettrofor Rovigo
films for autoradiography	Kodak
Geiger counter	NTS New Technology System
gel dryer	Hoefer Scientific Instruments
incubators	KW Officine Meccaniche Badesse (SI)
	Hotpack
jars	Oxoid
magnetic stirrer	Heidolph MR 2002
microwave oven	Daewoo
PCR machine	Perkin Elmer Gene Amp PCR System 2400
pH-meter	Metrohm 620
phosphorimager	Molecular Dynamics
power supplies	Pharmacia LKB EPS 500/400 and LPS 3000/150
	LKB Bromma 2297 macrodrive 5 and 2197 power supply
protein electrophorese chambers	Hoefer Scientific Instruments
scales	Mettler PE 3600 / AK160
shakers	PBI innova 2300
	New Brunswick Scientific Inc. Co.
sonicator	Branson 450
spectrophotometer	Perkin Elmer Lambda Bio
speedvac	Savant
ultracentrifuges	Beckman L8 70M
UV transilluminator	Pharmacia Biotech Image Master VDS
vortex	Janke & Kunkel IKA-Werk
water bath incubators	PBI international, Gebr. Haake Berlin, Lab-line international instruments, Inc., KW Officine Meccaniche Badesse (SI)
water bath shaker	New Brunswick Scientific Co. Inc.