Supplementary material

Materials and methods

Bacterial growth conditions

H. pylori cultures were grown microaerobically at 37°C in BHI medium (Oxoid) supplemented with 4% foetal calf serum (FCS) and an antibiotic–fungicide mix (57). Antibiotics were added when appropriate to the final concentration of 20 μg ml^{-1} for kanamycin (Kan) and 5 μg ml^{-1} for chloramphenicol (Cm). Isopropylthiogalactoside (IPTG) was added to the cultures to a final concentration of 1 mM when necessary. Transcription arrest was performed by rifampicin treatment at a final concentration of 100 μg/ml. 10 ml aliquots were taken 0, 2, 5, 10, 15 and 20 min after rifampicin addition and added to 1.25 ml of 5% phenol in ethanol for subsequent total RNA preparation as described below. For sucrose gradient centrifugation, cells were typically harvested at the end of the exponential growth phase (OD_{600} 0.8-0.9) and stored in pellets at -80°C.

Induction of the recombinant proteins in *E. coli* was performed at 30°C during 4 hours at OD_{600} 0.5-0.6 by the addition of 1mM IPTG for BL21-CodonPlus-RIL and of 2% L-arabinose plus 1mM IPTG for BL21-AI strains.

Construction of plasmids and strains

Strains are listed in Table S1, plasmids in Table S2 and primers in Table S3. The procedure described in (25) was used to construct two TOPO® -derived plasmids (Invitrogen), pILL2301 and pPH132, that carry the 514 and 541 bp-long 3'-end sequences of *rnj* and *rhpA,* respectively, in fusion with the TAP-tag sequence followed by a non-polar kanamycin resistance cassette (amplified from plasmid pILL851C, (25)) and 504 and 539 bp of the downstream DNA regions. Each of these plasmids was verified by sequencing and introduced into *H. pylori* strain 26695 by natural transformation. Allelic exchange was selected on kanamycin containing plates and constructs were verified by PCR and sequencing. The resulting *H. pylori* strains UPH298 and UPH691 were expressing chromosomal Hp-RNase J and RhpA, respectively, fused to the TAP tag at the C-terminus, as controlled by Western blotting with peroxidase-coupled anti-peroxidase antibodies (SIGMA).

To construct the *H. pylori* B128 strains that allow the controlled expression of the full length Hp-RNase J or truncated ΔN-Hp-RNase J, *rnj* or ΔN-*rnj* were amplified with primers 910/911 and 948/911 respectively and cloned into *Nde*I/*Kpn*I sites of pPH85, a derivative of the *E. coli*/*H. pylori* shuttle vector pILL2157 (27) from which the *lacZ* sequence was removed. This resulted in plasmids pPH134 and pPH135. They were introduced into *H. pylori*

strain B128 by mobilization, as described in Backert *et al.* (28). To replace the chromosomal copy of *rnj* with a kanamycin cassette (Kan), plasmid pPH138 derived from pCR®8/GW/TOPO®-TA (Invitrogen) was constructed. It carried 462 bp sequence upstream and 517 bp sequence downstream of *rnj* locus amplified with primer pairs 284/285 and 286/287, respectively, interrupted by non-polar kanamycin cassette (amplified with primers 832/918). The plasmid was verified by sequencing and introduced into the *H. pylori* strains carrying plasmids pPH134 or pPH135 by natural transformation. The resulting strains UPH738 and UPH739 in which *rnj* was replaced by *kan* through allelic exchange were selected on Kan-containing plates and verified by PCR.

Chromosomal deletion of *rhpA* (ORF number HPB128_21g22 in B128) and replacement with the Kan cassette was done using plasmid pPH104 derived from pCR®8/GW/TOPO®-TA (Invitrogen). Fragments of 483bp and 548bp corresponding to the upstream and downstream DNA sequences of *rhpA* respectively were amplified with primers 1029/1030 and 1031/1032. They were fused to the Kan cassette and the resulting fragment was cloned into pCR®8/GW/TOPO®-TA resulting in plasmid pPH104. *rhpA* deletion mutant was obtained with pPH104 as described above resulting in the strain UPH740.

Plasmid pGEX-4T-2 (GE Healthcare) was used for cloning and expression of RhpA tagged with glutathione S-transferase (GST) at the N-terminus. Transformation of the obtained plasmid pPH86 into *E. coli* BL21-AI cells resulted in strain UPH597.

Plasmids pPH42, pPH133, pPH123 and pPH121 derived from vector pET28a+ (Novagen) were constructed to express Hp-RNase J, ΔN-Hp-RNase J and RhpA, respectively, each fused at the N-terminus to a hexahistidine sequence. Their transformation to BL21-CodonPlus®-RIL strain resulted in strains UPH600, UPH733, UPH688 and UPH672 respectively. Co-expression of His-tagged Hp-RNase J or ΔN-Hp-RNase J together with GST-RhpA and of His-Hp-RNase J with GST alone was performed in the BL21-AI background (strains UPH574, UPH598 and UPH 780 respectively).

Purification of recombinant *H. pylori* proteins from *E. coli*.

A 1 ml column was packed with Ni^{2+}-nitrilotriacetic (NTA) agarose resin (QIAGEN) and equilibrated with lysis buffer (25 mM Tris-HCl pH 7.8, 500 mM NaCl, 10% glycerol, 0.5% Triton X-100). Cell pellets were resuspended in 10 ml of lysis buffer containing a tablet of Complete® Mini EDTA-free Protease Inhibitor Cocktail (Roche). Bacteria were lysed in a French Press pressure cell at 1,400 bar twice, centrifuged for 20 minutes at 10,000 g and the supernatant was applied to the Ni^{2+}-NTA column. The column was then washed consecutively with 10 ml of the following buffers, wash buffer 1 (25 mM Tris-HCl pH 7.8,

300 mM NaCl, 20 mM imidazole), wash buffer 2 (25 mM Tris-HCl pH 7.8, 1.5 M NaCl, 20 mM imidazole) and wash buffer 3 (25 mM Tris -HCl pH 7.8, 300 mM NaCl, 50 mM imidazole). The His-tagged proteins were eluted in eight fractions of ~1.4 ml each with elution buffer (25 mM Tris -HCl pH 7.8, 300 mM NaCl, 250 mM imidazole). Protein content was monitored with Quick Start Bradford protein reagent (Bio-Rad). Fractions containing recombinant protein were pooled and dialysed overnight in 2 L of 25 mM Tris-HCl pH 7.8, 300 mM NaCl, 10% glycerol and 1 mM DTT. Proteins were concentrated by ultrafiltration when appropriate and stored in aliquots at -80°C.

The Hp-RNase J/RhpA complex was purified from 100 ml of induced cultures of strains UPH574 and UPH598 co-expressing His-Hp-RNase J with GST-RhpA and His-ΔN-Hp-RNase J with GST-RhpA, respectively. Strain UPH780 expressing His-Hp-RNase J and GST alone was used for a control. Cells were pelleted and resuspended in 7 ml of running buffer (PBS pH 7.4, 100 mM NaCl) containing 10% glycerol, 0.5% Triton X-100 and a tablet of Complete®, Mini, EDTA-free Protease Inhibitor Cocktail (Roche). Cells were lysed in the French Press pressure cell at 1,400 bar and centrifuged for 20 min at 10,000g. The supernatant was applied to 1 ml GSTrap™ FF column (GE Healthcare), previously equilibrated with the running buffer. The column was then washed with 10 ml running buffer and GST-tagged protein was eluted in eight fractions of ~1.4 ml each with 50 mM Tris-HCl pH 7.8, 100 mM NaCl containing 10 mM glutathione. Fractions containing recombinant proteins were concentrated and stored in 10 % glycerol at -80°C.

Strains UPH574 and UPH598 were also used for complex purification on Ni^{2+}-NTA column as described above. Salt concentration in the buffers was kept low to maintain complex integrity. Lysis buffer was 25 mM Tris-HCl pH 7.4, 100 mM NaCl, 10 % glycerol, 0.5 % Triton X-100, wash buffer was 25 mM Tris-HCl pH 7.4, 100 mM NaCl and elution buffer was 25 mM Tris-HCl pH 7.4, 100 mM NaCl, 250 mM imidazole.

Total RNA preparation

Cell growth was stopped by the addition of 1.25 ml of 5% phenol in cold ethanol. After centrifugation, cells were resuspended in 800 µl of cold buffer 20 mM Tris-HCl pH 8, 2 mM EDTA and lysed by the addition of 40 µl of 20 % SDS. Then, 30 µl of 3 M sodium acetate pH 5 were added and the cell extract was mixed with 1 ml of acid-saturated phenol (SIGMA) that was pre-heated to 64°C. Lysates were vortexed vigorously for 1 min and centrifuged at 4°C during 15 min. The upper phase was subjected to two more rounds of cold acid-saturated phenol-chloroform (1:1) extraction, the second time in Phase-Lock Gel™ (5 PRIME) tubes. Finally, RNA was precipitated with isopropanol, washed with 70% ethanol and resuspended

in 40 µl of RNase-DNase free water. RNA concentration was measured with NanoDrop ND-1000 (Labtech) and adjusted to 1 µg/µl.

ATPase activity test

500 µl reaction buffer contained 10 mM HEPES-NaOH pH 7.5, 75 mM KCl, 2 mM ATP, 2 mM $MgCl_2$, 0.5 mM phosphoenolpyruvate (PEP), 0.25 mM NADH, 100 µg/ml lactate dehydrogenase and 100 µg/ml pyruvate kinase. Yeast RNA (Ambion) was added to a final concentration of 100 µg/ml when indicated. Reactions were incubated for 1 h at 37°C in the thermostatic chamber of a Cary 50 Bio spectrophotometer (Varian). NADH oxidation was monitored at 340 nm and ATP hydrolysis rate was calculated from linear NADH oxidation plots assuming NADH extinction coefficient of 6300 M^{-1} cm^{-1}.

Supplemental Tables

Table S1. Strains used in this study

Strain Name	Organism	Strain	Chromosomal construct	Replicative plasmid(s)
UPH531	*E. coli*	One Shot® TOP10		pPH104
UPH574	*E. coli*	BL21-AI		pPH86 + pPH42
UPH598	*E. coli*	BL21-AI		pPH86 + pPH133
UPH597	*E. coli*	BL21-AI		pHP86
UPH600	*E. coli*	B21 Codon+RIL		pPH42
UPH672	*E. coli*	B21 Codon+RIL		pPH121
UPH733	*E. coli*	B21 Codon+RIL		pPH133
UPH737	*E. coli*	One Shot® TOP10		pPH138
UPH780	*E. coli*	B21 Codon+RIL		pGEX4T2+pPH42
UPH298	*H. pylori*	26695	*rnj*-TAP	
UPH691	*H. pylori*	26695	*rhpA*-TAP	
UPH738	*H. pylori*	B128	*rnj::Kan*	pPH134
UPH739	*H. pylori*	B128	*rnj::Kan*	pPH135
UPH740	*H. pylori*	B128	*rhpA::Kan*	

Table S2. Plasmids used in this study

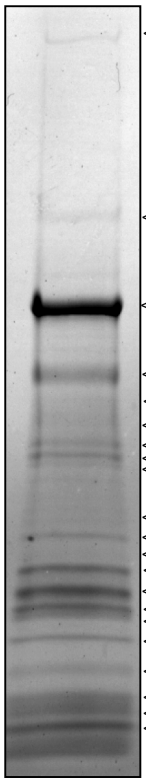
Plasmid	Vector	Construct
pILL2301	pJRD184	*rnj*-TAP
pPH42	pET28a+	His_6-RNJ
pPH85	pILL2157	lacZ replaced with linker
pPH86	pGEX4T2	GST-RhpA
pPH104	pCR8/GW/TOPO-TA	*rhpA::Kan*
pPH121	pET28a+	His_6-RhpA
pPH132	pCR8/GW/TOPO-TA	*rhpA*-TAP
pPH133	pET28a+	His_6-ΔN-RNJ
pPH134	pILL2157	*Pi-rnj*
pPH135	pILL2157	*Pi-ΔN-rnj*
pPH138	pCR8/GW/TOPO-TA	*rnj::Kan*

Table S3. DNA oligonucleotides used in this study

Name	Sequence	Purpose
284	GCAGGGGCTTATTGGTGATGACGCAAAAGG	PCR amplification of *rnj* upstream fragment for *rnj* disruption with Kan cassette, forward
285	GGCATTAGCCTTTTAAAAGGTAATTTAAGAGC	PCR amplification of *rnj* upstream fragment for *rnj* disruption with Kan cassette, reverse
286	ATGCCCATTCTTTTTGATTGTAACGC	PCR amplification of *rnj* downstream fragment for *rnj* disruption with Kan cassette, forward
287	CATCGCCTAACGCTAGAGTTAGGGTGGTAG	PCR amplification of *rnj* downstream fragment for *rnj* disruption with Kan cassette, reverse
H323	AACTGCAGGTTTTTCTTACCCGTGCATGGGGAATATAAC	PCR amplification of *rnj* upstream fragment for TAP tag cloning, *Pst*I site is underlined
H324	CTCTTTTCCATGGATCCAAAAGAATGGGCATGACAAATGAT	PCR amplification of *rnj* upstream fragment for TAP tag cloning
H325	TAGTACCTGGAGGGAATAATGCCCATTCTTTTTGATTGTAACGCTATTG	PCR amplification of *rnj* downstream fragment for TAP tag cloning
H326	CCATCGATCGCTAGAGTTAGGGTGGTAGAAGTCGTTGG	PCR amplification of *rnj* downstream fragment for TAP tag cloning, *Cla*I site is underlined
PH001	AACTGCAGGGCTACAGATGTGGCGAGTCGTGGGCTAGA	PCR amplification of *rhpA* upstream fragment for TAP tag cloning, *Pst*I site is underlined
PH002	CTCTTTTCCATGGATCCACGGCGTTTGGGTTTTTTAGAATA	PCR amplification of *rhpA* upstream fragment for TAP tag cloning
PH003	TAGTACCTGGAGGGAATAATGCCCATTGATTTGAACGAACATTTA	PCR amplification of *rhpA* downstream fragment for TAP tag cloning
PH004	CCATCGATGAGCGCACCACATCGCGCACCACAGGGTTG	PCR amplification of *rhpA* downstream fragment for TAP tag cloning, *Cla*I site is underlined
PH015	ATCGTCATATGGAGAATTTGTATTTTCAGGGTACGGATAACAACCAAAACAATGAAAACC	*rnj* cloning to pET28a+, *Nde*I site is underlined
PH016	ATCGTGAATTCTCAAAAAGAATGGGCATGAC	*rnj* cloning to pET28a+, *Eco*RI site is underlined
H346	CTAATACGACTCACTATAGGGAGAATTTCCCTATCCCTGCACCGACC	PCR amplification for 5S riboprobe, T7 RNA polymerase promoter in underlined
5S-Fw	AGAGAAGAGGAACTACCC	PCR amplification for 5S riboprobe
832	CGGTACCCGGGTGACTAA	PCR amplification of Kan cassette, forward
910	CCACCACATATGATGACGGATAACAACCAAAACAATG	Forward for *rnj* cloning to pILL2157, *Nde*I site is underlined
911	CAACAGGGTACCTCAAAAAGAATGGGCATGACAAATG	Reverse primer for *rnj* cloning to pILL2157, *Kpn*I site is underlined
914	TATGACTAGTGCTCTACCGCCGTAGCAATG	Linker for *lacZ* replacement in pILL2157
915	GATCCATTGCTACCGCCGTAGAGCACTAGTCA	Linker for *lacZ* replacement in pILL2157
918	ACTCTAGAGGATCCCCGGGT	PCR amplification of Kan cassette, reverse
948	CCACCACATATGAACTTAAACTCTAAAGCGAGCG	Forward for ΔN-*rnj* cloning to pILL2157, *Nde*I site is underlined
958	GTTGTCATCACTTATTCTGCAC	PCR amplification for *ureI* riboprobe, reverse
959	GCTCTAATACGACTCACTATAGGGTAGGTGAAACCAAACAATAACCC	PCR amplification for *ureI* riboprobe, T7 RNAP polymerase promoter in underlined
1009	AATCTCCCGGGGAATTGAATCAACCACCACTCCCT	*rhpA* cloning to pGEX4T2, *Sma*I site is underlined
1010	AGCAATCTCGAGACGGCGTTTGGGTTTTTTAGAATAA	*rhpA* cloning to pGEX4T2, *Xho*I site is underlined
1020	CATCAACATATGAACTTAAACTCTAAAGCGAG	*ΔN-rnj* cloning to pET28a+, *Nde*I site is underlined
1021	CACACAGAATTCTCAAAAAGAATGGGCATGACAAATG	*ΔN-rnj* cloning to pET28a+, *Eco*RI site is underlined
1029	GGTAAGGAGGCTGTAATCATCAC	PCR amplification of *rhpA* upstream fragment for *rhpA* replacement with *Kan*, forward
1030	GTTAGTCACCCGGGTACCTTTATAAAAAGCTAATAAAAGGC	PCR amplification of *rhpA* upstream fragment for *rhpA* replacement with *Kan*, reverse

1031	TACCTGGAGGGAATAAATATTTAAAAAG GAAATTCATGCCC	PCR amplification of *rhpA* downstream fragment for *rhpA* replacement with *Kan*, forward
1032	GCGCACCACAGGGTTGATG	PCR amplification of *rhpA* downstream fragment for *rhpA* replacement with *Kan*, reverse
1078	GGGAATTCCCCATATGGAATTGAATCA AC	*rhpA* cloning in pET28a+, *Nde*I site is underlined
1079	TATGCGGGATCCTTAACGGCGTTTGGG TTTTTTAG	*rhpA* cloning in pET28a+, *Bam*HI site is underlined
1091	AGCTTTTGAATTTTCACTGCTGTT	PCR amplification for seRNA72
1093	ATGACGGATAACAACCAAAACAATG	PCR amplification for asRNA66
1094	CTAATACGACTCACTATAGGGAGAAAA GTTATGACGGATAACAACCA	PCR amplification for seRNA72, T7 RNA polymerase promoter in underlined
1095	CTAATACGACTCACTATAGGGAGAAGC TTTTGAATTTTCACTGCTGTT	PCR amplification for asRNA66, T7 RNA polymerase promoter in underlined
1108	FAM-TCACCCGTGCGCCACTAATC	FAM-labelled primer for primer extension for 16S rRNA
1109	CTGACTAAATAGAGTGAGGG	For PCR sequencing of 16S end
1110	TCACCCGTGCGCCACTAATC	For PCR sequencing of 16S end
1114	FAM-GAGCAGTATTATCAGCGATGAAG	FAM-labelled primer for primer extension for 5S rRNA
1115	GACTACTACTAATAGAGCGTTTG	For PCR sequencing of 5S
1116	GAGCAGTATTATCAGCGATGAAG	For PCR sequencing of 5S
1117	FAM-CCATTCGGACATCTACGCATC	FAM-labelled primer for primer extension for 23S rRNA
1118	GCAAGTTCTACAAGCTAAAAGC	For PCR sequencing of 23S
1119	CCATTCGGACATCTACGCATC	For PCR sequencing of 23S

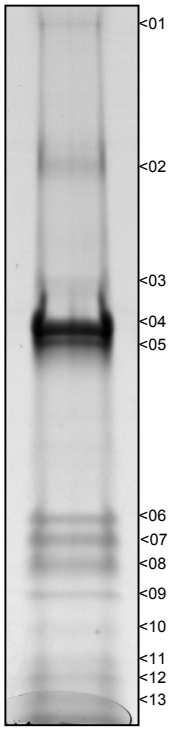
Table S4. Mass-spectrometry data for proteins co-purified with Hp-RNase J by TAP



	Name	Accession number [a]	MW	Peptide count	Mascot Score [b]	CI% [c]	Total Ion Score	CI%	Sequence coverage (%)
Band 01	DNA-dependent RNA polymerase beta-beta prime subunit	HP1198	324397,9	24	142	100	126	100	6
Band 02	ND [d]	ND							
Band 03	RNaseJ	HP1430	77509,1	18	669	100	538	100	40
Band 04	ND [d]	ND							
Band 05	Predicted ATP-dependent RNA helicase	HP0247	55885,4	8	107	100	73	100	18
Band 06	Predicted translation elongation factor Tu	HP1205	43734,4	14	276	100	176	100	39
Band 07	Predicted translation elongation factor Tu	HP1205	43734,4	17	463	100	324	100	51
Band 08	Predicted translation elongation factor Tu	HP1205	43734,4	14	316	100	218	100	35
Band 09	Predicted S-adenosylmethionine synthetase	HP0197	42677,7	4	66	99,957	49	99,993	15
Band 10	Predicted UDP-3-0-(3-hydroxymyristoyl) glucosamine N-acyltransferase	HP0196	36737,3	6	110	100	77	100	15
Band 11	Predicted ribosomal protein S2	HP1554	30839,1	13	158	100	84	100	30
Band 12	Predicted fructose-bisphosphate aldolase	HP0176	33865,4	9	120	100	69	100	28
Band 13	Predicted ribosomal protein L2/peptidyl-transferase	HP1316	30309,1	10	212	100	152	100	38
Band 14	Predicted ribosomal protein S3	HP1313	26420,6	7	161	100	133	100	30
Band 15	Predicted ribosomal protein L4	HP1318	24122,5	8	255	100	206	100	40
Band 16	Predicted ribosomal protein S4	HP1294	23949,8	17	314	100	186	100	53
	Predicted ribosomal protein L4	HP1318	24122,5	5	135	100	109	100	29
Band 17	Predicted ribosomal protein L3	HP1319	21292,2	13	321	100	226	100	48
Band 18	Predicted ribosomal protein L6	HP1304	19474,5	10	319	100	232	100	46
	Predicted ribosomal protein L5	HP1307	20271,9	5	101	100	84	100	21
	Predicted ribosomal protein L10	HP1200	18650,9	6	80	99,998	47	99,987	26
Band 19	Predicted ribosomal protein S7	HP1196	17959,6	6	135	100	110	100	30
	Predicted ribosomal protein L16	HP1312	16092,7	2	82	99,999	75	100	17
Band 20	Predicted ribosomal protein L15	HP1301	14896,2	6	227	100	188	100	35
	Predicted ribosomal protein S8	HP1305	15231,2	7	89	100	33	99,618	42
Band 2	Predicted ribosomal protein S9 involved in 30S ribosome subunit assembly	HP0083	14499	2	80	99,998	70	100	20
	Predicted ribosomal protein S13	HP1296	13566,5	5	55	99,477	22	95,826	40

[a] Genome of strain 26695, annotation from the PyloriGene database http://genolist.pasteur.fr/PyloriGene/.
[b] The presented protein hits have a Mascot score ≥ 45.
[c] The presented protein hits have a GPS Explorer protein confidence index ≥95 %.
[d] ND – not determined

Table S5. Mass-spectrometry data for proteins co-purified with RhpA by TAP



	Name	Accession number [a]	MW	Peptide count	Mascot Score [b]	CI%[c]	Total Ion Score	CI%	Sequence coverage (%)
Band 01	Predicted ATP-dependent RNA helicase	HP0247	55885,4	12	233	100	180	100	22
Band 02	Predicted ATP-dependent RNA helicase	HP0247	55885,4	13	265	100	186	100	26
Band 03	Predicted ATP-dependent RNA helicase	HP0247	55885,4	10	227	100	185	100	19
	RNaseJ	HP1430 [d]	77509,1	6	165	100	149	100	11
Band 04	Predicted ATP-dependent RNA helicase	HP0247	55885,4	15	534	100	435	100	34
Band 05	Predicted ATP-dependent RNA helicase	HP0247	55885,4	15	373	100	273	100	32
Band 06	Predicted ribosomal protein L2/peptidyl-transferase	HP1316	30309,1	10	244	100	167	100	41
Band 07	Predicted ribosomal protein L1	HP1201	25250,5	6	186	100	163	100	28
	Predicted ribosomal protein S3	HP1313	26420,6	6	109	100	83	100	24
Band 08	Predicted ribosomal protein S4	HP1294	23949,8	13	447	100	360	100	41
	Predicted ribosomal protein L4	HP1318	24122,5	3	139	100	123	100	22
Band 09	Predicted ribosomal protein L3	HP1319	21292,2	7	227	100	181	100	40
Band 10	Predicted ribosomal protein L6	HP1304	19474,5	5	175	100	144	100	26
Band 11	Predicted ribosomal protein S6	HP1246	16960,7	6	117	100	88	100	37
	Predicted ribosomal protein S7	HP1196	17959,6	5	88	100	66	100	28
	Predicted ribosomal protein L3	HP1319	21292,2	5	66	99,958	48	99,986	28
	Predicted ribosomal protein S12	HP1197 [d]	15210,6	4	63	99,917	33	99,531	14
Band 12	Predicted ribosomal protein S7	HP1196	17959,6	6	194	100	167	100	32
	Predicted ribosomal protein L22	HP1314 [d]	13125,3	3	91	100	82	100	13
	Predicted ribosomal protein S12	HP1197	15210,6	3	64	99,937	54	99,996	14
Band 13	Predicted ribosomal protein L15	HP1301	14896,2	5	272	100	244	100	27
	Predicted ribosomal protein L22	HP1314 [d]	13125,3	3	90	100	81	100	13

[a] Genome of strain 26695, annotation from the PyloriGene database http://genolist.pasteur.fr/PyloriGene/.
[b] The presented protein hits have a Mascot score ≥ 45.
[c] The presented protein hits have a GPS Explorer protein confidence index ≥95 %.
[d] Proteins with percentage of coverage between 11 and 14, for which additional MS/MS analysis (shown below) were performed. MS/MS was performed as in Stingl *et al.* 2008. In each case, the protein identification was confirmed.

Peptide View from Band 03

MS/MS Fragmentation of **VIMSTFSSNIHR**
Found in **HP1430**, (HP1430) 689 aa H. pylori 26695

Match to Query 58: 1406.699224 from(1407.706500,1+)
MaldiWellID: 498323, SpectrumID: 3119376,

Monoisotopic mass of neutral peptide Mr(calc): 1406.70
Fixed modifications: Carbamidomethyl (C)
Variable modifications:
M3 : Oxidation (M)
Ions Score: 57 **Expect:** 4e-006
Matches (Bold Red): 22/178 fragment ions using 23 most intense peaks

#	Immon.	a	a*	a^0	b	b*	b^0	Seq.	v	w	w'	y	y*	y^0	#
1	72.08	72.08			100.08			V							12
2	**86.10**	**185.16**			213.16			I	1250.56	1263.58	1277.59	1308.64	1291.61	1290.63	11
3	120.05	332.20			360.20			M	1103.52	1102.53		1195.55	1178.53	1177.54	10
4	60.04	419.23		401.22	447.23		429.22	S	1016.49	1015.50		1048.52	1031.49	1030.51	9
5	74.06	520.28		502.27	548.27		530.26	T	**915.44**	928.46	930.44	**961.49**	944.46	943.47	8
6	120.08	667.35		649.34	695.34		677.33	F	768.37			**860.44**	843.41	842.43	7
7	60.04	754.38		736.37	782.38		764.36	S	**681.34**	**680.35**		713.37	696.34	695.36	6
8	60.04	841.41		823.40	869.41		851.40	S	594.31	593.32		**626.34**	609.31	608.33	5
9	**87.06**	955.46	938.43	937.44	983.45	966.42	965.44	N	480.27	**479.27**		**539.30**	522.28		4
10	**86.10**	1068.54	1051.51	1050.53	1096.53	1079.51	1078.52	I	**367.18**	**380.20**	394.22	**425.26**	408.24		3
11	**110.07**	1205.60	1188.57	1187.59	1233.59	1216.57	1215.58	H	**230.12**			**312.18**	**295.15**		2
12	129.11							R	74.02	73.03		**175.12**	158.09		1

Peptide View from Band 11

MS/MS Fragmentation of **SPALVECPQRR**
Found in **HP1197**, (HP1197) 135 aa H. pylori 26695

Match to Query 53: 1311.677924 from(1312.685200,1+)
MaldiWellID: 498331, SpectrumID: 3119589,

Monoisotopic mass of neutral peptide Mr(calc): 1311.67
Fixed modifications: Carbamidomethyl (C)
Ions Score: 33 **Expect:** 0.0011
Matches (Bold Red): 19/157 fragment ions using 20 most intense peaks

#	Immon.	a	a*	a^0	b	b*	b^0	d	Seq.	v	w	y	y*	y^0	#
1	60.04	60.04		42.03	88.04		**70.03**		S						11
2	**70.07**	157.10		139.09	**185.09**		167.08		P	1183.60	1182.60	1225.65	1208.62	1207.64	10
3	44.05	228.13		210.12	**256.13**		238.12		A	1112.56		1128.59	1111.57	1110.58	9
4	**86.10**	341.22		323.21	**369.21**		351.20		L	999.48	998.48	1057.56	1040.53	1039.55	8
5	72.08	440.29		422.28	468.28		450.27		V	900.41	913.43	944.47	927.45	926.46	7
6	102.05	569.33		551.32	597.32		579.31		E	771.37	770.37	845.40	828.38	827.39	6
7	133.04	729.36		711.35	757.35		739.34		C	611.34	**610.34**	**716.36**	699.34		5
8	**70.07**	826.41		808.40	854.41		836.40		P	514.28	513.29	**556.33**	539.30		4
9	101.07	954.47	937.44	936.46	982.47	965.44	964.46		Q	386.23	**385.23**	459.28	442.25		3
10	129.11	1110.57	1093.55	1092.56	**1138.57**	1121.54	1120.56	1025.51	R	230.12	229.13	**331.22**	314.19		2
11	129.11								R	74.02	73.03	**175.12**	158.09		1

Peptide View from Band 12

MS/MS Fragmentation of **VDAGPVLR**
Found in **HP1314**, (HP1314) 122 aa H. pylori 26695

Match to Query 3: 825.470524 from(826.477800,1+)
MaldiWellID: 498332, SpectrumID: 3119619,

Monoisotopic mass of neutral peptide Mr(calc): 825.47
Fixed modifications: Carbamidomethyl (C)
Ions Score: 43 **Expect:** 7.3e-005
Matches (Bold Red): 30/90 fragment ions using 32 most intense peaks

#	Immon.	a	a^0	b	b^0	Seq.	v	w	y	y*	y^0	#
1	**72.08**	**72.08**		**100.08**		V						8
2	88.04	187.11	**169.10**	**215.10**	**197.09**	D	667.39	666.39	727.41	710.38	709.40	7
3	44.05	258.14	240.13	**286.14**	268.13	A	596.35		**612.38**	595.36		6
4	30.03	315.17	297.16	**343.16**	325.15	G			541.35	524.32		5
5	**70.07**	412.22	394.21	**440.21**	422.20	P	442.28	441.28	**484.32**	**467.30**		4
6	**72.08**	511.29	493.28	539.28	521.27	V	**343.21**	**356.23**	387.27	370.24		3
7	**86.10**	624.37	606.36	652.37	634.36	L	**230.12**	**229.13**	**288.20**	**271.18**		2
8	129.11					R	74.02	73.03	**175.12**	158.09		1

Peptide View from Band 12

MS/MS Fragmentation of **VDAGPVLRR**
Found in **HP1314**, (HP1314) 122 aa H. pylori 26695

Match to Query 29: 981.575124 from(982.582400,1+)
MaldiWellID: 498332, SpectrumID: 3119612,

Monoisotopic mass of neutral peptide Mr(calc): 981.57
Fixed modifications: Carbamidomethyl (C)
Ions Score: 38 **Expect:** 0.00019
Matches (Bold Red): 34/113 fragment ions using 41 most intense peaks

#	Immon.	a	a*	a^0	b	b*	b^0	d	Seq.	v	w	y	y*	y^0	#
1	**72.08**	**72.08**			**100.08**				V						9
2	88.04	**187.11**		**169.10**	**215.10**		197.09		D	823.49	822.49	883.51	866.48	865.50	8
3	44.05	258.14		240.13	**286.14**		268.13		A	752.45		**768.48**	**751.46**		7
4	30.03	315.17		**297.16**	**343.16**		325.15		G			697.45	680.42		6
5	**70.07**	412.22		394.21	**440.21**		422.20		P	598.38	597.38	640.43	623.40		5
6	**72.08**	511.29		493.28	539.28		521.27		V	**499.31**	512.33	543.37	526.35		4
7	**86.10**	624.37		606.36	652.37		634.36		L	386.23	**385.23**	444.30	427.28		3
8	**129.11**	780.47	763.45	762.46	**808.47**	791.44	790.46	695.41	R	230.12	**229.13**	331.22	314.19		2
9	**129.11**								R	74.02	73.03	**175.12**	158.09		1

Peptide View from Band 13

MS/MS Fragmentation of **VDAGPVLR**
Found in **HP1314**, (HP1314) 122 aa H. pylori 26695

Match to Query 3: 825.468024 from(826.475300,1+)
MaldiWellID: 498333, SpectrumID: 3119642,

Monoisotopic mass of neutral peptide Mr(calc): 825.47
Fixed modifications: Carbamidomethyl (C)
Ions Score: 56 **Expect:** 4.1e-006
Matches (Bold Red): 33/90 fragment ions using 32 most intense peaks

#	Immon.	a	a^0	b	b^0	Seq.	v	w	y	y*	y^0	#
1	**72.08**	**72.08**		**100.08**		V						8
2	88.04	**187.11**	**169.10**	**215.10**	197.09	D	667.39	666.39	**727.41**	**710.38**	709.40	7
3	44.05	258.14	240.13	**286.14**	268.13	A	596.35		**612.38**	595.36		6
4	30.03	315.17	297.16	**343.16**	325.15	G			**541.35**	**524.32**		5
5	**70.07**	412.22	394.21	**440.21**	422.20	P	442.28	**441.28**	484.32	**467.30**		4
6	**72.08**	**511.29**	493.28	**539.28**	521.27	V	**343.21**	**356.23**	387.27	370.24		3
7	**86.10**	624.37	606.36	652.37	634.36	L	230.12	**229.13**	**288.20**	**271.18**		2
8	129.11					R	74.02	73.03	**175.12**	158.09		1

Table S6. Mass-spectrometry data for Hp-RNase J and RhpA co-purified from *E. coli*

Gel A1 (Fig. 4)

	Name	Accession number	MW	Peptide count	Protein score	CI%	Total Ion Score	CI%	Sequence coverage (%)
Band 01[#]	(HP0247) 492 aa H. pylori 26695	HP0247	55885,4	18	579	100	431	100	40
Band 02	(HP1430) 689 aa H. pylori 26695	HP1430	77509,1	17	680	100	578	100	33

Gel A2 (Fig. 4)

	Name	Accession number	MW	Peptide count	Protein score	CI%	Total Ion Score	CI%	Sequence coverage (%)
Band 01	(HP0247) 492 aa H. pylori 26695	HP0247	55885,4	14	447	100	353	100	30
Band 02	gi|67462334|sp|P0A 6Y8|DNAK_ECOLI Chaperone protein dnaK (Heat shock protein 70)	gi|000154|	69129,5	12	180	100	129	100	24
Band 03	(HP1430) 689 aa H. pylori 26695	HP1430	77509,1	14	480	100	407	100	27
Band 04	gi|62288014|sp|P0A 6F5|CH60_ECOLI 60 kDa chaperonin (Protein Cpn60) (groEL protein)	gi|000155|	57463,8	18	253	100	155	100	34

- Numbers correspond to the bands marked with triangles and asterisks in Fig. 4A-1 and 4A-2 from the top to the bottom.

References

28. Backert S, Kwok T, Konig W (2005) Conjugative plasmid DNA transfer in *Helicobacter pylori* mediated by chromosomally encoded relaxase and TraG-like proteins. *Microbiology* **151**(11)**:** 3493-3503

27. Boneca IG, Ecobichon C, Chaput C, Mathieu A, Guadagnini S, Prevost MC, Colland F, Labigne A, de Reuse H (2008) Development of inducible systems to engineer conditional mutants of essential genes of *Helicobacter pylori*. *Appl Environ Microbiol* **74**(7)**:** 2095-2102

57. Muller C, Bahlawane C, Aubert S, Delay CM, Schauer K, Michaud-Soret I, De Reuse H (2011) Hierarchical regulation of the NikR-mediated nickel response in *Helicobacter pylori*. *Nucleic Acids Res* **39**(17)**:** 7564-7575

25. Stingl K, Schauer K, Ecobichon C, Labigne A, Lenormand P, Rousselle JC, Namane A, de Reuse H (2008) In vivo interactome of *Helicobacter pylori* urease revealed by tandem affinity purification. *Mol Cell Proteomics* **7**(12)**:** 2429-2441

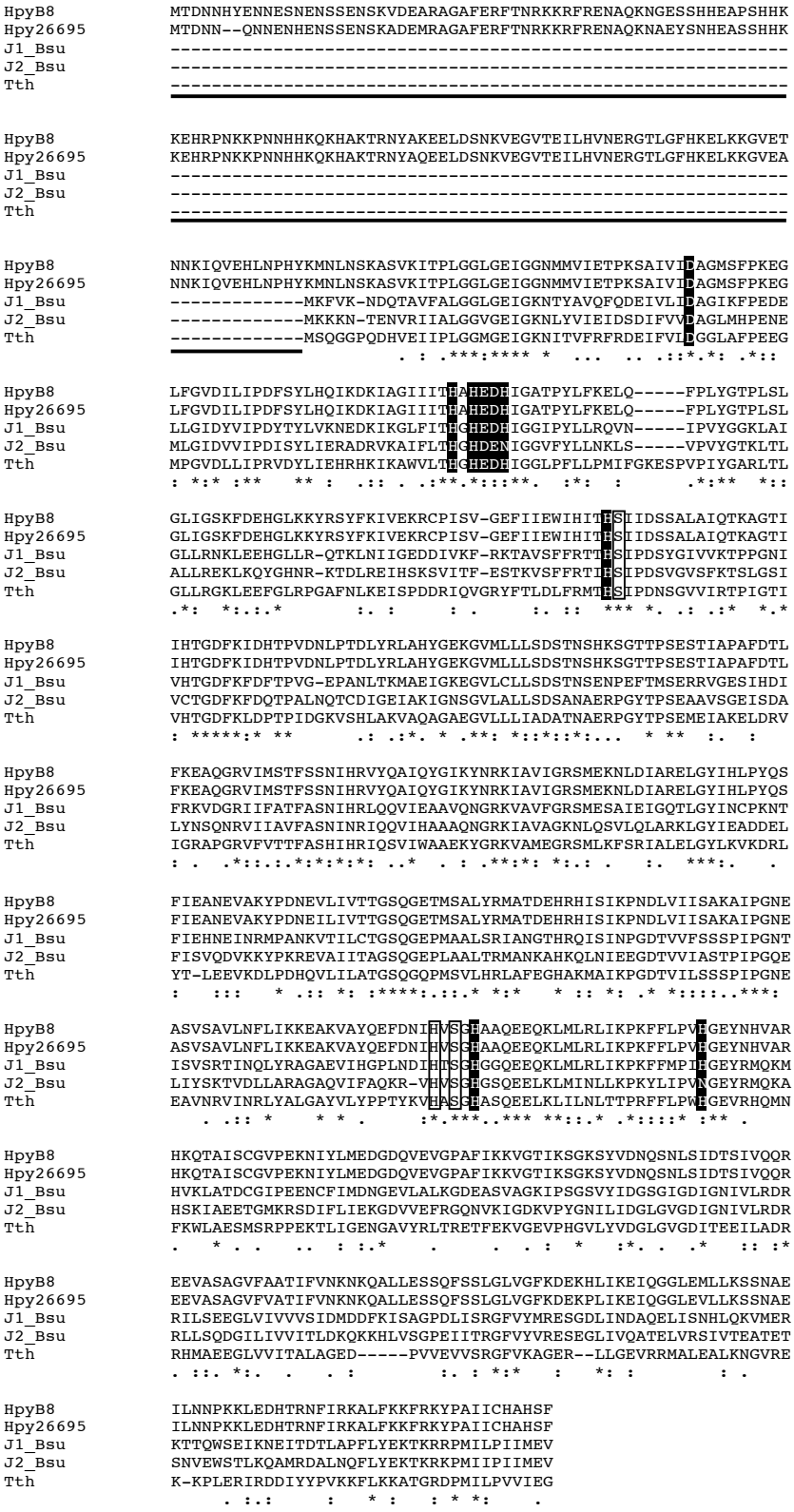

HpyB8 MTDNNHYENNESNENSSENSKVDEARAGAFERFTNRKKRFRENAQKNGESSHHEAPSHHK
Hpy26695 MTDNN--QNNENHENSSENSKADEMRAGAFERFTNRKKRFRENAQKNAEYSNHEASSHHK
J1_Bsu
J2_Bsu
Tth
HpyB8 KEHRPNKKPNNHHKQKHAKTRNYAKEELDSNKVEGVTEILHVNERGTLGFHKELKKGVET
Hpy26695 KEHRPNKKPNNHHKQKHAKTRNYAQEELDSNKVEGVTEILHVNERGTLGFHKELKKGVEA
J1_Bsu
J2_Bsu
Tth
HpyB8 NNKIQVEHLNPHYKMNLNSKASVKITPLGGLGEIGGNMMVIETPKSAIVIDAGMSFPKEG
Hpy26695 NNKIQVEHLNPHYKMNLNSKASVKITPLGGLGEIGGNMMVIETPKSAIVIDAGMSFPKEG
J1_Bsu -------------MKFVK-NDQTAVFALGGLGEIGKNTYAVQFQDEIVLIDAGIKFPEDE
J2_Bsu -------------MKKKN-TENVRIIALGGVGEIGKNLYVIEIDSDIFVVDAGLMHPENE
Tth -------------MSQGGPQDHVEIIPLGGMGEIGKNITVFRFRDEIFVLDGGLAFPEEG
HpyB8 LFGVDILIPDFSYLHQIKDKIAGIIITHAHEDHIGATPYLFKELQ-----FPLYGTPLSL
Hpy26695 LFGVDILIPDFSYLHQIKDKIAGIIITHAHEDHIGATPYLFKELQ-----FPLYGTPLSL
J1_Bsu LLGIDYVIPDYTYLVKNEDKIKGLFITHGHEDHIGGIPYLLRQVN-----IPVYGGKLAI
J2_Bsu MLGIDVVIPDISYLIERADRVKAIFLTHGHDENIGGVFYLLNKLS-----VPVYGTKLTL
Tth MPGVDLLIPRVDYLIEHRHKIKAWVLTHGHEDHIGGLPFLLPMIFGKESPVPIYGARLTL
HpyB8 GLIGSKFDEHGLKKYRSYFKIVEKRCPISV-GEFIIEWIHITHSIIDSSALAIQTKAGTI
Hpy26695 GLIGSKFDEHGLKKYRSYFKIVEKRCPISV-GEFIIEWIHITHSIIDSSALAIQTKAGTI
J1_Bsu GLLRNKLEEHGLLR-QTKLNIIGEDDIVKF-RKTAVSFFRTTHSIPDSYGIVVKTPPGNI
J2_Bsu ALLREKLKQYGHNR-KTDLREIHSKSVITF-ESTKVSFFRTIHSIPDSVGVSFKTSLGSI
Tth GLLRGKLEEFGLRPGAFNLKEISPDDRIQVGRYFTLDLFRMTHSIPDNSGVVIRTPIGTI
HpyB8 IHTGDFKIDHTPVDNLPTDLYRLAHYGEKGVMLLLSDSTNSHKSGTTPSESTIAPAFDTL
Hpy26695 IHTGDFKIDHTPVDNLPTDLYRLAHYGEKGVMLLLSDSTNSHKSGTTPSESTIAPAFDTL
J1_Bsu VHTGDFKFDFTPVG-EPANLTKMAEIGKEGVLCLLSDSTNSENPEFTMSERRVGESIHDI
J2_Bsu VCTGDFKFDQTPALNQTCDIGEIAKIGNSGVLALLSDSANAERPGYTPSEAAVSGEISDA
Tth VHTGDFKLDPTPIDGKVSHLAKVAQAGAEGVLLLIADATNAERPGYTPSEMEIAKELDRV
HpyB8 FKEAQGRVIMSTFSSNIHRVYQAIQYGIKYNRKIAVIGRSMEKNLDIARELGYIHLPYQS
Hpy26695 FKEAQGRVIMSTFSSNIHRVYQAIQYGIKYNRKIAVIGRSMEKNLDIARELGYIHLPYQS
J1_Bsu FRKVDGRIIFATFASNIHRLQQVIEAAVQNGRKVAVFGRSMESAIEIGQTLGYINCPKNT
J2_Bsu LYNSQNRVIIAVFASNINRIQQVIHAAAQNGRKIAVAGKNLQSVLQLARKLGYIEADDEL
Tth IGRAPGRVFVTTFASHIHRIQSVIWAAEKYGRKVAMEGRSMLKFSRIALELGYLKVKDRL
HpyB8 FIEANEVAKYPDNEVLIVTTGSQGETMSALYRMATDEHRHISIKPNDLVIISAKAIPGNE
Hpy26695 FIEANEVAKYPDNEILIVTTGSQGETMSALYRMATDEHRHISIKPNDLVIISAKAIPGNE
J1_Bsu FIEHNEINRMPANKVTILCTGSQGEPMAALSRIANGTHRQISINPGDTVVFSSSPIPGNT
J2_Bsu FISVQDVKKYPKREVAIITAGSQGEPLAALTRMANKAHKQLNIEEGDTVVIASTPIPGQE
Tth YT-LEEVKDLPDHQVLILATGSQGQPMSVLHRLAFEGHAKMAIKPGDTVILSSSPIPGNE
HpyB8 ASVSAVLNFLIKKEAKVAYQEFDNIHMSGHAAQEEQKLMLRLIKPKFFLPVHGEYNHVAR
Hpy26695 ASVSAVLNFLIKKEAKVAYQEFDNIHMSGHAAQEEQKLMLRLIKPKFFLPVHGEYNHVAR
J1_Bsu ISVSRTINQLYRAGAEVIHGPLNDIHISGHGGQEEQKLMLRLIKPKFFMPIHGEYRMQKM
J2_Bsu LIYSKTVDLLARAGAQVIFAQKR-VHMSGHGSQEELKLMINLLKPKYLIPVNGEYRMQKA
Tth EAVNRVINRLYALGAYVLYPPTYKVHMSGHASQEELKLILNLTTPRFFLPWHGEVRHQMN
HpyB8 HKQTAISCGVPEKNIYLMEDGDQVEVGPAFIKKVGTIKSGKSYVDNQSNLSIDTSIVQQR
Hpy26695 HKQTAISCGVPEKNIYLMEDGDQVEVGPAFIKKVGTIKSGKSYVDNQSNLSIDTSIVQQR
J1_Bsu HVKLATDCGIPEENCFIMDNGEVLALKGDEASVAGKIPSGSVYIDGSGIGDIGNIVLRDR
J2_Bsu HSKIAEETGMKRSDIFLIEKGDVVEFRGQNVKIGDKVPYGNILIDGLGVGDIGNIVLRDR
Tth FKWLAESMSRPPEKTLIGENGAVYRLTRETFEKVGEVPHGVLYVDGLGVGDITEEILADR
HpyB8 EEVASAGVFAATIFVNKNKQALLESSQFSSLGLVGFKDEKHLIKEIQGGLEMLLKSSNAE
Hpy26695 EEVASAGVFVATIFVNKNKQALLESSQFSSLGLVGFKDEKPLIKEIQGGLEVLLKSSNAE
J1_Bsu RILSEEGLVIVVVSIDMDDFKISAGPDLISRGFVYMRESGDLINDAQELISNHLQKVMER
J2_Bsu RLLSQDGILIVVITLDKQKKHLVSGPEIITRGFVYVRESEGLIVQATELVRSIVTEATET
Tth RHMAEEGLVVITALAGED-----PVVEVVSRGFVKAGER--LLGEVRRMALEALKNGVRE
HpyB8 ILNNPKKLEDHTRNFIRKALFKKFRKYPAIICHAHSF
Hpy26695 ILNNPKKLEDHTRNFIRKALFKKFRKYPAIICHAHSF
J1_Bsu KTTQWSEIKNEITDTLAPFLYEKTKRRPMILPIIMEV
J2_Bsu SNVEWSTLKQAMRDALNQFLYEKTKRKPMIIPIIMEV
Tth K-KPLERIRDDIYYPVKKFLKKATGRDPMILPVVIEG

Figure S1. Alignment of RNase J from *H. pylori* B128 and 26695 strains with RNase J1 and J2 from *Bacillus subtilis* and RNase J from *Thermus thermophilus.*

J1_Bsu - RNase J1; J2_Bsu - RNase J2 of *B. subtilis*; Tth - RNase J of *T. thermophilus*. Amino-acids constituting the active site are highlighted with black boxes (Zn^{2+} coordination) and empty boxes (phosphate coordination). The *H. pylori*-specific N-terminal extension of RNAse J is underlined.

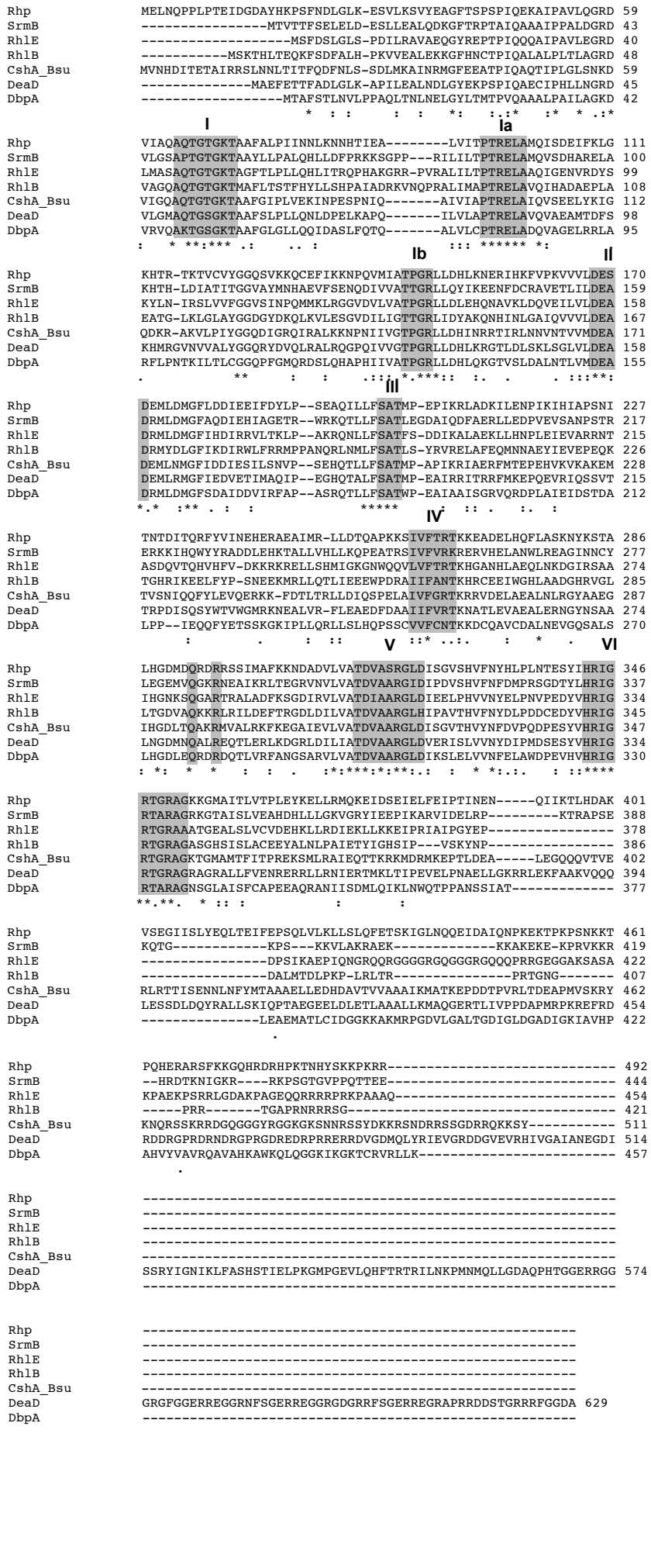

Rhp MELNQPPLPTEIDGDAYHKPSFNDLGLK-ESVLKSVYEAGFTSPSPIQEKAIPAVLQGRD 59
SrmB ----------MTVTTFSELELD-ESLLEALQDKGFTRPTAIQAAAIPPALDGRD 43
RhlE ----------MSFDSLGLS-PDILRAVAEQGYREPTPIQQQAIPAVLEGRD 40
RhlB ----------MSKTHLTEQKFSDFALH-PKVVEALEKKGFHNCTPIQALALPLTLAGRD 48
CshA_Bsu MVNHDITETAIRRSLNNLTITFQDFNLS-SDLMKAINRMGFEEATPIQAQTIPLGLSNKD 59
DeaD ----------MAEFETTFADLGLK-APILEALNDLGYEKPSPIQAECIPHLLNGRD 45
DbpA ----------MTAFSTLNVLPPAQLTNLNELGYLTMTPVQAAALPAILAGKD 42
I Ia
Rhp VIAQAQTGTGKTAAFALPIINNLKNNHTIEA--------LVITPTRELAMQISDEIFKLG 111
SrmB VLGSAPTGTGKTAAYLLPALQHLLDFPRKKSGPP---RILILTPTRELAMQVSDHARELA 100
RhlE LMASAQTGTGKTAGFTLPLLQHLITRQPHAKGRR-PVRALILTPTRELAAQIGENVRDYS 99
RhlB VAGQAQTGTGKTMAFLTSTFHYLLSHPAIADRKVNQPRALIMAPTRELAVQIHADAEPLA 108
CshA_Bsu VIGQAQTGTGKTAAFGIPLVEKINPESPNIQ-------AIVIAPTRELAIQVSEELYKIG 112
DeaD VLGMAQTGSGKTAAFSLPLLQNLDPELKAPQ-------ILVLAPTRELAVQVAEAMTDFS 98
DbpA VRVQAKTGSGKTAAFGLGLLQQIDASLFQTQ-------ALVLCPTRELADQVAGELRRLA 95
Ib II
Rhp KHTR-TKTVCVYGGQSVKKQCEFIKKNPQVMIATPGRLLDHLKNERIHKFVPKVVVLDES 170
SrmB KHTH-LDIATITGGVAYMNHAEVFSENQDIVVATTGRLLQYIKEENFDCRAVETLILDEA 159
RhlE KYLN-IRSLVVFGGVSINPQMMKLRGGVDVLVATPGRLLDLEHQNAVKLDQVEILVLDEA 158
RhlB EATG-LKLGLAYGGDGYDKQLKVLESGVDILIGTTGRLIDYAKQNHINLGAIQVVVLDEA 167
CshA_Bsu QDKR-AKVLPIYGGQDIGRQIRALKKNPNIIVGTPGRLLDHINRRTIRLNNVNTVVMDEA 171
DeaD KHMRGVNVVALYGGQRYDVQLRALRQGPQIVVGTPGRLLDHLKRGTLDLSKLSGLVLDEA 158
DbpA RFLPNTKILTLCGGQPFGMQRDSLQHAPHIIVATPGRLLDHLQKGTVSLDALNTLVMDEA 155
III
Rhp DEMLDMGFLDDIEEIFDYLP--SEAQILLFSATMP-EPIKRLADKILENPIKIHIAPSNI 227
SrmB DRMLDMGFAQDIEHIAGETR--WRKQTLLFSATLEGDAIQDFAERLLEDPVEVSANPSTR 217
RhlE DRMLDMGFIHDIRRVLTKLP--AKRQNLLFSATFS-DDIKALAEKLLHNPLEIEVARRNT 215
RhlB DRMYDLGFIKDIRWLFRRMPPANQRLNMLFSATLS-YRVRELAFEQMNNAEYIEVEPEQK 226
CshA_Bsu DEMLNMGFIDDIESILSNVP--SEHQTLLFSATMP-APIKRIAERFMTEPEHVKVKAKEM 228
DeaD DEMLRMGFIEDVETIMAQIP--EGHQTALFSATMP-EAIRRITRRFMKEPQEVRIQSSVT 215
DbpA DRMLDMGFSDAIDDVIRFAP--ASRQTLLFSATWP-EAIAAISGRVQRDPLAIEIDSTDA 212
IV
Rhp TNTDITQRFYVINEHERAEAIMR-LLDTQAPKKSIVFTRTKKEADELHQFLASKNYKSTA 286
SrmB ERKKIHQWYYRADDLEHKTALLVHLLKQPEATRSIVFVRKRERVHELANWLREAGINNCY 277
RhlE ASDQVTQHVHFV-DKKRKRELLSHMIGKGNWQQVLVFTRTKHGANHLAEQLNKDGIRSAA 274
RhlB TGHRIKEELFYP-SNEEKMRLLQTLIEEEWPDRAIIFANTKHRCEEIWGHLAADGHRVGL 285
CshA_Bsu TVSNIQQFYLEVQERKK-FDTLTRLLDIQSPELAIVFGRTKRRVDELAEALNLRGYAAEG 287
DeaD TRPDISQSYWTVWGMRKNEALVR-FLEAEDFDAAIIFVRTKNATLEVAEALERNGYNSAA 274
DbpA LPP--IEQQFYETSSKGKIPLLQRLLSLHQPSSCVVFCNTKKDCQAVCDALNEVGQSALS 270
V VI
Rhp LHGDMDQRDRRSSIMAFKKNDADVLVATDVASRGLDISGVSHVFNYHLPLNTESYIHRIG 346
SrmB LEGEMVQGKRNEAIKRLTEGRVNVLVATDVAARGIDIPDVSHVFNFDMPRSGDTYLHRIG 337
RhlE IHGNKSQGARTRALADFKSGDIRVLVATDIAARGLDIEELPHVVNYELPNVPEDYVHRIG 334
RhlB LTGDVAQKKRLRILDEFTRGDLDILVATDVAARGLHIPAVTHVFNYDLPDDCEDYVHRIG 345
CshA_Bsu IHGDLTQAKRMVALRKFKEGAIEVLVATDVAARGLDISGVTHVYNFDVPQDPESYVHRIG 347
DeaD LNGDMNQALREQTLERLKDGRLDILIATDVAARGLDVERISLVVNYDIPMDSESYVHRIG 334
DbpA LHGDLEQRDRDQTLVRFANGSARVLVATDVAARGLDIKSLELVVNFELAWDPEVHVHRIG 330
Rhp RTGRAGKKGMAITLVTPLEYKELLRMQKEIDSEIELFEIPTINEN-----QIIKTLHDAK 401
SrmB RTARAGRKGTAISLVEAHDHLLLGKVGRYIEEPIKARVIDELRP---------KTRAPSE 388
RhlE RTGRAAATGEALSLVCVDEHKLLRDIEKLLKKEIPRIAIPGYEP---------------- 378
RhlB RTGRAGASGHSISLACEEYALNLPAIETYIGHSIP---VSKYNP---------------- 386
CshA_Bsu RTGRAGKTGMAMTFITPREKSMLRAIEQTTKRKMDRMKEPTLDEA-----LEGQQQVTVE 402
DeaD RTGRAGRAGRALLFVENRERRLLRNIERTMKLTIPEVELPNAELLGKRRLEKFAAKVQQQ 394
DbpA RTARAGNSGLAISFCAPEEAQRANIISDMLQIKLNWQTPPANSSIAT---------- 377
Rhp VSEGIISLYEQLTEIFEPSQLVLKLLSLQFETSKIGLNQQEIDAIQNPKEKTPKPSNKKT 461
SrmB KQTG----------KPS---KKVLAKRAEK-------------KKAKEKE-KPRVKKR 419
RhlE ----------DPSIKAEPIQNGRQQRGGGGRGQGGGRGQQQPRRGEGGAKSASA 422
RhlB ----------DALMTDLPKP-LRLTR---------------PRTGNG------- 407
CshA_Bsu RLRTTISENNLNFYMTAAAELLEDHDAVTVVAAAIKMATKEPDDTPVRLTDEAPMVSKRY 462
DeaD LESSDLDQYRALLSKIQPTAEGEELDLETLAAALLKMAQGERTLIVPPDAPMRPKREFRD 454
DbpA ----------LEAEMATLCIDGGKKAKMRPGDVLGALTGDIGLDGADIGKIAVHP 422
Rhp PQHERARSFKKGQHRDRHPKTNHYSKKPKRR---------- 492
SrmB --HRDTKNIGKR----RKPSGTGVPPQTTEE---------- 444
RhlE KPAEKPSRRLGDAKPAGEQQRRRRPRKPAAAQ---------- 454
RhlB -----PRR-------TGAPRNRRRSG---------- 421
CshA_Bsu KNQRSSKRRDGQGGGYRGGKGKSNNRSSYDKKRSNDRRSSGDRRQKKSY---------- 511
DeaD RDDRGPRDRNDRGPRGDREDRPRRERRDVGDMQLYRIEVGRDDGVEVRHIVGAIANEGDI 514
DbpA AHVYVAVRQAVAHKAWKQLQGGKIKGKTCRVRLLK---------- 457
Rhp SSRYIGNIKLFASHSTIELPKGMPGEVLQHFTRTRILNKPMNMQLLGDAQPHTGGERRGG 574
DeaD GRGFGGERREGGRNFSGERREGGRGDGRRFSGERREGRAPRRDDSTGRRRFGGDA 629

Figure S2. Alignment of RhpA, the DExD box RNA helicase of *H. pylori* with its ortholgues of *E. coli* and *B. subtilis*.
Conserved RNA and ATP binding motifs are numbered from I to VI and highlighted with grey boxes.

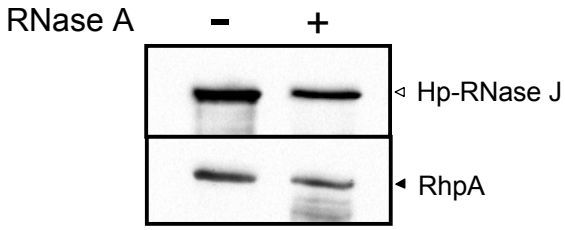


Figure S3. Co-purification of Hp-RNase J and RhpA after RNase A treatment of cell lysates.

His_6-Hp-RNase J and GST-RhpA were co-expressed in *E. coli* as described in Materials and Methods. RNase A (Roche) was added to the cell suspension to final concentration 5 μg/ml before lysis with French Press. The complex was purified as described, on glutathione column (GSTrap™ FF, GE Healthcare) and pooled purified fractions analysed by Western blotting using Hp-RNase J or GST-specific antibodies.

Upper panel - Western blot showing Hp-RNase J in the pooled fractions of purified complex; lower panel - Western blot showing RhpA in the same fractions.