## Supplementary Material

## **Properties of the HtrA protease from bacterium** *Helicobacter pylori* whose activity is indispensable for growth under stress conditions

Urszula Zarzecka, Anna Modrak-Wójcik, Donata Figaj, Malgorzata Apanowicz, Adam Lesner, Agnieszka Bzowska, Barbara Lipinska, Anna Zawilak-Pawlik, Steffen Backert and Joanna Skorko-Glonek<sup>\*</sup>

\* Correspondence: J.Skorko-Glonek joanna.skorko-glonek@biol.ug.edu.pl

	Orientations	Sequence (5`> 3`)
HphtrA_rev	reverse	ATACTCGAGTTTCACCAAAATGATTCTATAACCTTG
HphtrAJ99_fw	forward	ATACCATGGGCAATATCCAAAATCCAAAACATGCCC
HphtrA26695_fw	forward	ATACCATGGGCAATATCCAAATCCAGAGCATGCCC
Hp_S221A_fw	forward	CCTGGAAATGCCGGCGGCGCTTTAATTGATAGC
Hp_S221A_rev	reverse	GCTATCAATTAAAGCGCCGCCGGCATTTCCAGG

## Supplementary Table S1. PCR primers used in the work.



**Supplementary Figure S1.** Schemes of the *htrA* locus and the flanking *H. pylori* chromosomal regions in the wt,  $\Delta htrA$ , and  $\Delta htrA/htrA$  complemented N6 strains



**Supplementary Figure S2.** Survival of the stress-exposed *H. pylori* cells. The standard error of mean (SEM) was calculated using at least three repetitions. Data were evaluated using Bonferroni test. Statistical significance was defined by  $p \le 0.05$  (\*). nd- no single colonies detected; ns- no significant differences. The representative pictures presenting *H. pylori* growth under the analyzed stress conditions are presented in Figures 1A, B, 2A, and B that correspond to parts A, B, C and D of this figure, respectively.



**Supplementary Figure S3.** Effects of oxidative stress on growth of *H. pylori* N6  $\Delta$ *htrA*. Bacterial cultures of the N6  $\Delta$ *htrA* and the control strains (N6 and *htrA* complementation) were cultured and challenged with H<sub>2</sub>O<sub>2</sub> and cumene hydroperoxide as described in the Materials and Methods section. Plates were incubated for 3 days at (A) 37°C and (B) 39°C. The experiments were performed at least three times. The red dotted line indicates the diameter of the filter disc. Analysis by Bonferroni test showed no significant differences (p < 0.05) between the tested strains for all experimental conditions.

DegP (HtrA <sub>Ec</sub> ) DegQ <sub>Ec</sub> HtrA <sub>Cj</sub> HtrA <sub>Hp</sub> 26695 HtrA <sub>Hp</sub> N6 HtrA <sub>Hp</sub> J99 DegP (HtrA <sub>Ec</sub> ) DegQ <sub>Ec</sub> HtrA <sub>Cj</sub> HtrA <sub>Hp</sub> 26695 HtrA <sub>Hp</sub> N6 HtrA <sub>Hp</sub> J99	10 MKKTTLALS- MKK-IFLSL MKKT-LFISL MKKT-LFISL MKKT-FFISL MKKT-FFISL MKKT-FFISL MKKT-FFISL GCGVFND GGGVFND GGGVFND	20 ALALSLGL ALALSVGL SLASALFAAS ALALSLNAGN ALALSLNAGN ALALSLNAGN MFQQFFGD-D EFFKQFFGD-D PFFQQFFGD-D PFFQQFFG-D PFFQQFFG-D PFFQQFFG-D	30 ALSP-LSATA TLSASFQAVA INFNESTTTA IQIQSMPKVK IQIQSMPKVK IQIQNMPKVK IQIQNMPKVK IQIQNMPKVK IQIQNMPKVK IQIQNMPKVF LOGMIPKE LGGMIPKE	40 AETSSATTAQ SIPGQVADQA NRVN-PAAGM ERVSVPSKDD ERISVPSKED ERISVPSKED QSSPFCQGGQ EG RMER RMER	50 QMPSLAPMLE PLPSLAPMLE AVLSYHDSIK TIYSYHDSIK TIYSYHDSIK 120 GGNGGGQQQK 	60 KVMPSVVSIN KVLPAVVSVF DAKKSVVNIS DSIKAVVNIS DSIKAVVNIS DSIKAVVNIS 130 FMALGSGVII LGSGVII ALGSGVII ALGSGVII ALGSGVII	7 VEGSTTVN- VEG-TASQ- STSKTITRSN. TEKKIKNNF TEKKIKNNF TEKKIKNNF MASKGYVTN NASKGYVTN S-KDGYIVTN S-KDGYIVTN S-KDGYIVTN S-KDGYIVTN	) - R I I I		
DegP (HtrA <sub>Ec</sub> ) DegQ <sub>Ec</sub> HtrA <sub>Cj</sub> HtrA <sub>Hp</sub> 26695 HtrA <sub>Hp</sub> N6 HtrA <sub>Hp</sub> J99	150 NHVVDNATVI NHVINQAQKI NHVIDGADKI NHVIDGADKI NHVIDGADKI	160 KVQLS-DGRK SIQLN-DGRE TVNLPGSDTE KVTIPGSNKE KVTIPGSNKE	170 FDAKMVGKDP FDAKLIGSDD YKAKLIGKDP YSATLVGTDS YSATLVGTDS * * *	180 RSDIALIQIQ QSDIALLQIQ KTDLAVIKIE ESDLAVIRIT ESDLAVIRIT ESDLAVIRIT T	190 NPKNLTAIKM NPSKLTQIAI -ANNLSAITF -KDNLPTIKF -KDNLPTIKF -KDNLPTIKF	200 ADSDALRVGD ADSDKLRVGD TNSDDLMEGD SDSNDISVGD SDSNDILVGD ★ ■★★	210 YTVAIGNPFG FAVAVGNPFG UVFAIGNPFG LVFAIGNPFG LVFAIGNPFG	)		
DegP (Htr $A_{Ec}$ ) Deg $Q_{Ec}$ Htr $A_{Cj}$ Htr $A_{Hp}$ 26695 Htr $A_{Hp}$ N6 Htr $A_{Hp}$ J99	220 LGETVTSGIV LGQTATSGIV VGESVTQGIV VGESVTQGIV VGESVTQGIV VGESVTQGIV	230 SALGRSGLNA SALGRSGLNI SALNKSGIGI SALNKSGIGI SALNKSGIGI	240 ENYENFIQTD EGLENFIQTD NSYENFIQTD NSYENFIQTD NSYENFIQTD NSYENFIQTD	250 AAINRGNSGG ASINRGNSGG ASINPGNSGG ASINPGNSGG ASINPGNSGG ASINPGNSGG	260 ALVNLNGELI ALLNLNGELI ALUDSRGGLV ALIDSRGGLV ALIDSRGGLV ALIDSRGGLV	GINTAILAPD GINTAILAPG GINTAILSRG GINTAIISRT GINTAIISKT GINTAIISKT	280 GGNIGIGFAI GGSVGIGFAI GGNNGIGFAI GGNHGIGFAI GGNHGIGFAI GGNHGIGFAI			
DegP (HtrA <sub>Ec</sub> ) DegQ <sub>Ec</sub> HtrA <sub>G</sub> HtrA <sub>Hp</sub> 26695 HtrA <sub>Hp</sub> N6 HtrA <sub>Hp</sub> J99	290 PSNMVKNLTS PSNMARTLAQ PSNMVKDIAK PSNMVKDIVT PSNMVKDIVT	300 QMVEYGQVKR QLIDFGEIKR KLIEKGKIDR QLIKTGKIER QLIKTGKIER	310 GELGIMGTEL GLLGIKGTEM GFLGVTISAL GYLGVGLQDL GYLGVGLQDL ★ ★★	320 NSELAKAMKV SADIAKAFNL QGDTKKAY SGDLQNSY SGDLQNSY SGDLQNSY	330 DAQRGAFVSQ DVQRGAFVSE KNQEGALITD DNKEGAVVIS DNKEGAVVIS X	340 VLPNS SAAKA VLPGS GSAKA VQKGS SADEA VEKDS PAKKA VEKDS PAKKA VEKDS PAKKA	350 GIKAGDVITS GVKAGDIITS GIKRGDLVTF GILVWDLITE GILVWDLITE GLLVWDLITE			
DegP (HtrA <sub>Ec</sub> ) DegQ <sub>Ec</sub> HtrA <sub>Cj</sub> HtrA <sub>Hp</sub> 26695 HtrA <sub>Hp</sub> N6 HtrA <sub>Hp</sub> J99	360 LNGKPISSFA LNGKPLNSFA VNDKVIKSPI VNGKKVKNTN VNGKKVKNTN MGKKVKNTN	PD PD 370 ALRAQVGTMP ELRSRIATTE DLKNYIGTLE ELRNLIGSML ELRNLIGSML	380 VGSKLTLGLL PGTKVKLGLL IGQKISLSYE PNQRVTLKVI PNQRVTLKVI *	390 RDGKQVNVNL RNGKPLEVEV RDGVNKQTSF RDKKERAF RDKKERTF RDKKERTF	400 ELQQSSQNQV TLDTSTSSSA TLK-GEKENP TLTLAERKNP TLTLAERKNP	410 DSSSIFN SAEMITP KGVQS NKKETISAQN NKKETISAQN	420 GIEGAE ALEGAT DLIDGLS GAQGQLNGLQ GAQGQLNGLQ GVQGQLNGLQ			
<b>PDZ1 PDZ2</b> 430 440 450 460 470 480 490 500										
Degr (HtrA <sub>Ec</sub> ) DegQ <sub>Ec</sub> HtrA <sub>Cj</sub> HtrA <sub>Hp</sub> 26695 HtrA <sub>Hp</sub> N6 HtrA <sub>Hp</sub> J99	MSN LSDG LRNLDPRLKD VEDLTQETKR VEDLTQKTKR VEDLTQKTKR	KGKDQG QLKDGGKG RLQIPKDVNG SMRLSDDVQG SMRLSDDVQG SMRLSDDVQG	VVVNNVKTGT IKIDEVVKGS VLVDSVKEKS VLVSQVNENS VLVSQVNENS VLVSQVNENS	PAAQIGLKKG PAAQAGLQKD KGKNSGFQEG PAEQAGFRQG PAEQAGFRQG PAEQAGFRQG PAEQAGFRQG	DVIIGANQQA DVIIGVNRDR DIIIGVGQSE NIITKIEEVE NIITKIEEIE NIITKIEEVE	VKNIAELRKV VNSIAEMRKV IKNLKDLEQA VKSVADFNHA VKSVADFNHA VKSVADFNHA	LDSKPSVLAL LAAKPAIIAL LKQVN-KKEF LEKYKGKPKR LEKYKGKPKR LEKYKGKPKR	NIQRGDST IYLLM QIVRGNES IYLLM TKVWYYRNGF ATLLV FLVLDLNQGY RIILV FLVLDLNQGY RIILV FLVLDLNQGY RIILV	IQ- IR- 7LK 7K- 7K-	

Supplementary Figure S4 Comparison of the amino acid sequences of the selected HtrA homologs: HtrA (DegP) and DegQ E. coli, HtrA C. jejuni and HtrA H. pylori (26695, N6 and J99 strains). The important regulatory loops (LA, LD, L1, L2, L3) as well as domain organisation are marked with dashed lines. PD-stands for the protease domain, T- catalytic triad. Asterisks indicate the conserved residues. The S1 specificity pocket is marked in orange within the L1 and L2 loops. Green squares indicate differences between HtrA<sub>Hp</sub> from *H. pylori* 26695, N6 and J99 strains.



**Supplementary Figure S5.** pH-dependence of the HtrA<sub>*Hp*</sub> (*Hp* 26695) and HtrA<sub>*Ec*</sub> (*Ec*) at 42°C. The error bars represent the standard deviation values from three independent measurements.