Hfq influences multiple transport systems and virulence in the plant pathogen *Agrobacterium tumefaciens*

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Table S1: Strains and plasmids used in this study.

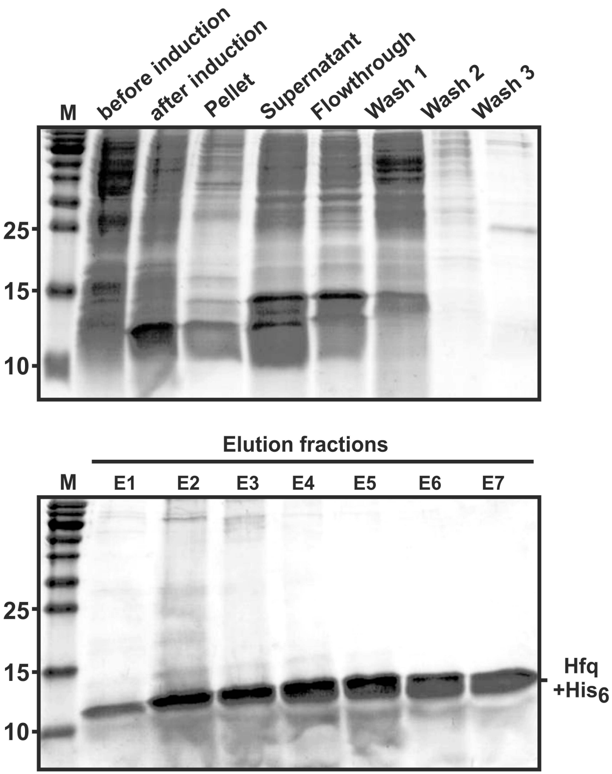
Strain or plasmid		Relevant characteristics	Reference or source
Strains			
Escherichia coli			
DH5α		Cloning host	(2)
BL21 (DE3)		Overexpression strain	Novagen, Madison, USA
Agrobacterium tumefaciens			
C58		Wild-type	C. Baron, Montreal, Canada
C58 *Δhfq*		Derivate of the wild-type with deletion of the *hfq* gene	This study
Plasmids			
pUC18		Amp^r; cloning vector	(4)
pET24b		Km^r; vector for His-tag fusion and overexpression	Novagen, Madison, USA
pBBSyn		Km^r; complementation vector	(1)
pK19*mobsacB*		Km^r; suicide vector	(5)
pK_up/do_*hfq*	(pBo1649)	Km^r; pK19*mobsacB* derivate carrying the up- and downstream regions of *hfq*	This study
runoff _C2A	(pBo1608)	Amp^r; pUC18 derivate carrying AbcR1 with additional T7-promotor	(6)
runoff_*atu2422*	(pBo1649)	Amp^r; pUC18 derivate carrying *atu2422* (100 bp) with additional T7-promotor	(6)
pET24b_*hfq*	(pBo2400)	Km^r; pET24b derivate carrying the *hfq* gene for protein purification	This study
pBBSyn_*hfq*	(pBo2405)	Km^r; pBBSyn derivate carrying the *hfq* gene for complementation	This study
pBISN1	(pBo2317)	Km^r; pBin19 derivate carrying T-DNA borders enclosing the *gusA* intron	(3)

Table S2: Oligonucleotides used in this study.

T7 promoter sequences for generation of *in vitro* transcripts are written in bold letters. Restriction sites are underlined.

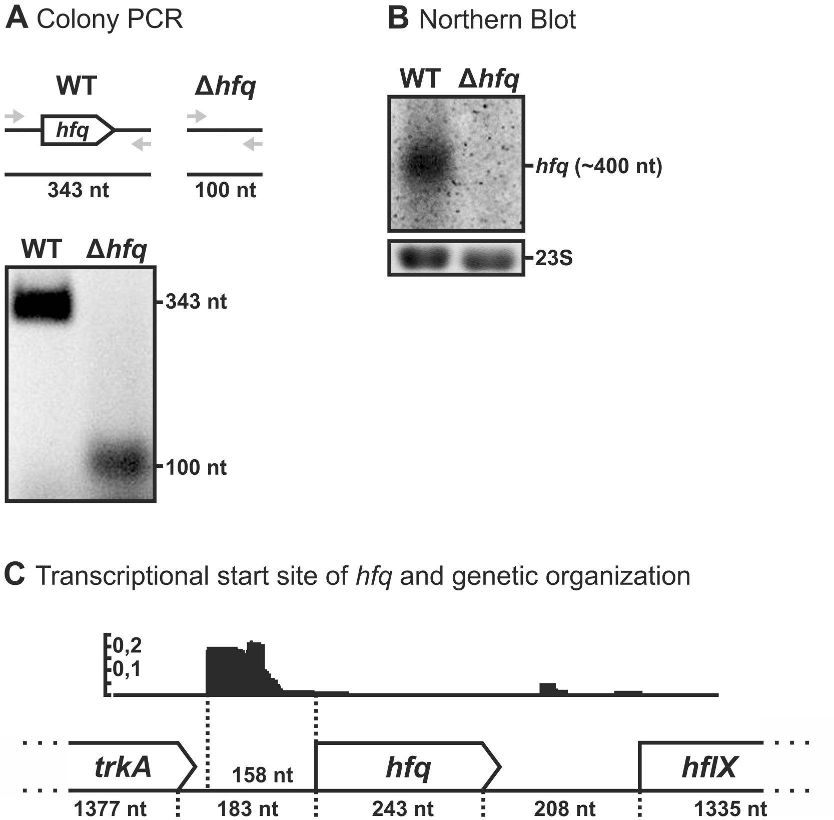
Oligonucleotide	Purpose	Sequence (5' to 3')
Δ*hfq*_up_fw	Δ*hfq* mutant generation, up fragment	AAAAGAATTCGAAGCGCTTGAAACATCGCC
Δ*hfq*_up_rv	Δ*hfq* mutant generation, up fragment	AAAACTGCAGGGCGCCGCTTCTTTCTTTATT
	(Δ*hfq* upstream fragment from 1445724 to 1446123 on the circular chromosome)	
Δ*hfq*_down_fw	Δ*hfq* mutant generation, down fragment	AAAACTGCAGTCAGGCTCCTTTAACGGTATC
Δ*hfq*_down_rv	Δ*hfq* mutant generation, down fragment	AAAAGCATGCAACAGGGTTGCGGGACGCG
	(Δ*hfq* downstream fragment from 1446367 to 1446766 on the circular chromosome)	
Δ*hfq*_check_fw	Δ*hfq* mutant generation, colony PCR	ATTGATTATTTTCCGGTATCCC
Δ*hfq*_check_rv	Δ*hfq* mutant generation, colony PCR	TGTTCCGATTCAGGTCAGTC
RNAprobe_*rpsE*_fw	Northern analysis; RNA-probe for *rpsE*	CCGTGAAGAGCGCGATAGCG
RNAprobe_*rpsE*_rv	Northern analysis; RNA-probe for *rpsE*	**GAAATTAATACGACTCACTATAGGG**CAGCAGAACCTTGCCTGCG
RNAprobe_*4678*_fw	Northern analysis; RNA-probe for *atu4678*	CGTCGATGTCGGCGTTGCGTCG
RNAprobe_*4678*_rv	Northern analysis; RNA-probe for *atu4678*	**GAAATTAATACGACTCACTATAGGG**CGTCGATCTGACCCTGGCTAAG
RNAprobe_*0420*_fw	Northern analysis; RNA-probe for *atu0420*	GCTTCGCGTCCGATCAAGAGC
RNAprobe_*0420*_rv	Northern analysis; RNA-probe for *atu0420*	**GAAATTAATACGACTCACTATAGGG**CGAAGACTTCGCGTGTGCCGT
RNAprobe_*malE*_fw	Northern analysis; RNA-probe for *malE*	CGGTTCCACCGCCCTTGG
RNAprobe_*malE*_rv	Northern analysis; RNA-probe for *malE*	**GAAATTAATACGACTCACTATAGGG**CGCGAACTGCTTCAGCGTGC
RNAprobe_*dppA*_fw	Northern analysis; RNA-probe for *dppA*	CGCCGGAAGGCTTCGATCC
RNAprobe_*dppA*_rv	Northern analysis; RNA-probe for *dppA*	**GAAATTAATACGACTCACTATAGGG**CCACGGGTTCTTGGTATCACCC
RNAprobe_*4259*_fw	Northern analysis; RNA-probe for *atu4259*	CCCAATGACAGCTTGACGGTCG
RNAprobe_*4259*_rv	Northern analysis; RNA-probe for *atu4259*	**GAAATTAATACGACTCACTATAGGG**CCATGGAAAGGGCCTGGC
RNAprobe_*4431*_fw	Northern analysis; RNA-probe for *atu4431*	CCTGAAGACCGGTTATGCCGG
RNAprobe_*4431*_rv	Northern analysis; RNA-probe for *atu4431*	**GAAATTAATACGACTCACTATAGGG**GTTCTCGTTCTTCTGGTTGAGGC
RNAprobe_*2422*_fw	Northern analysis; RNA-probe for *atu2422*	CGGCGTGAAATTCGTTGTC
RNAprobe_*2422*_rv	Northern analysis; RNA-probe for *atu2422*	**GAAATTAATACGACTCACTATAGG**GTCACGCCGGCAGCATTG
DNAprobe_C2A_fw	Northern analysis; DNA-probe for AbcR1	AAAACCTCCAGAGGGGAACAGC
DNAprobe_C2A_rv	Northern analysis; DNA-probe for AbcR1	CCCATATTTTAGTTAGCTGTCAC
runoff_C2A_fw	plasmid generation for AbcR1 runoff transcription	**GAAATTAATACGACTCACTATAGGG**AGTTGATGCACACGGTGGC
runoff_C2A_rv	plasmid generation for AbcR1 runoff transcription	GATATCAAAAAAAGAGGGCCGCCGG
runoff_*atu2422*_fw	plasmid generation for *atu2422* runoff transcription	**GAAATTAATACGACTCACTATAGGG**TCGAACAGGTCCTTGCAAT
runoff_*atu2422*_rv	plasmid generation for *atu2422* runoff transcription	AAAAGATATCAACGCGACCATGGCGGTC
CHis_Hfq_fw	Hfq overexpression plasmid generation	AAAACATATGGCGGAACGTTCTCAAAAC
CHis_Hfq_rv	Hfq overexpression plasmid generation	AAAAGTCGACGGCAGCGCCTTCTTCGCTC
pBBSyn_*hfq*_fw	*hfq* complementation plasmid generation	AAAATCTAGAATGGCGGAACGTTCTCAAAAC
pBBSyn_*hfq*_rv	*hfq* complementation plasmid generation	AAAAGAGCTCTCAGGCAGCGCCTTCTTCG

Figure S1



Purification of the *A. tumefaciens* Hfq protein. SDS-PAGE (16%) after Ni-NTA purification of C-terminal His_6-tagged Hfq. The fractions loaded are indicated above. The lower gel shows the elution fractions. The positions of marker proteins (M) are given on the left in kDa.

Figure S2



Verification of the chromosomal *hfq* deletion. (**A**) Colony PCR with wild-type (WT) and Δ*hfq* cells. The 2% agarose gel loaded with PCR samples was stained with ethidiumbromide. Diagrams explaining the PCR product lengths are given above. Primers (Δ*hfq*_check_fw/rv) are indicated as grey arrows. (**B**) Northern blot analysis verifying the absence of *hfq* (~400 nt) in the mutant. Hybridizations were performed with 8 µg of total RNA from *A. tumefaciens* wild-type (WT) and the Δ *hfq* mutant. Primers used for RNA-probe generation are listed in table S3. Ethidiumbromide-stained 23S RNAs were used as loading controls. **(C)** Transcriptional start site of *hfq* revealed by dRNA-seq and genetic organisation with flanking genes *trkA* and *hflX*. The detected band in (B) corresponds to a monocistronic transcript comprised of the 5'-UTR and *hfq* coding region.

Figure S3

Altered tumour formation of Δ*hfq* on plants. Leaves of a *Kalanchoe* plant were inoculated with various *A. tumefaciens* strains. Cultures of the wild-type (WT), Δ*hfq*, the complemented mutant (Δ*hfq*+*hfq*) and control strains harboring the vector (v) (WT+v and Δ*hfq*+v) were diluted to the indicated optical densities and applied on freshly wounded leaves of *Kalanchoe diagremontiana*. Tumour formation was monitored after incubation at room temperature for 4 – 5 weeks and leaves were cut off for evaluation.

(1-6)

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