Supplementary Information

Global transcriptional regulation by cell-free sup of *S.* Typhimurium peptide transporter mutant leads to inhibition of intraspecies biofilm initiation

Kasturi Chandra[1], Prerana Muralidhara[2], Sathisha Kamanna[3], Utpal S. Tatu[3], Dipshikha Chakravortty[1,4,#]

[1]Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India.

[2]Department of Molecular Biology, Yuvaraja's College, Mysore, Karnataka, India.

[3]Department of Biochemistry, Indian Institute of Science, Bangalore, India.

[4]Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore, India.

 # Corresponding author

Dipshikha Chakravortty

Email: dipa@iisc.ac.in

Telephone: +91 80 22932842

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Figure S1

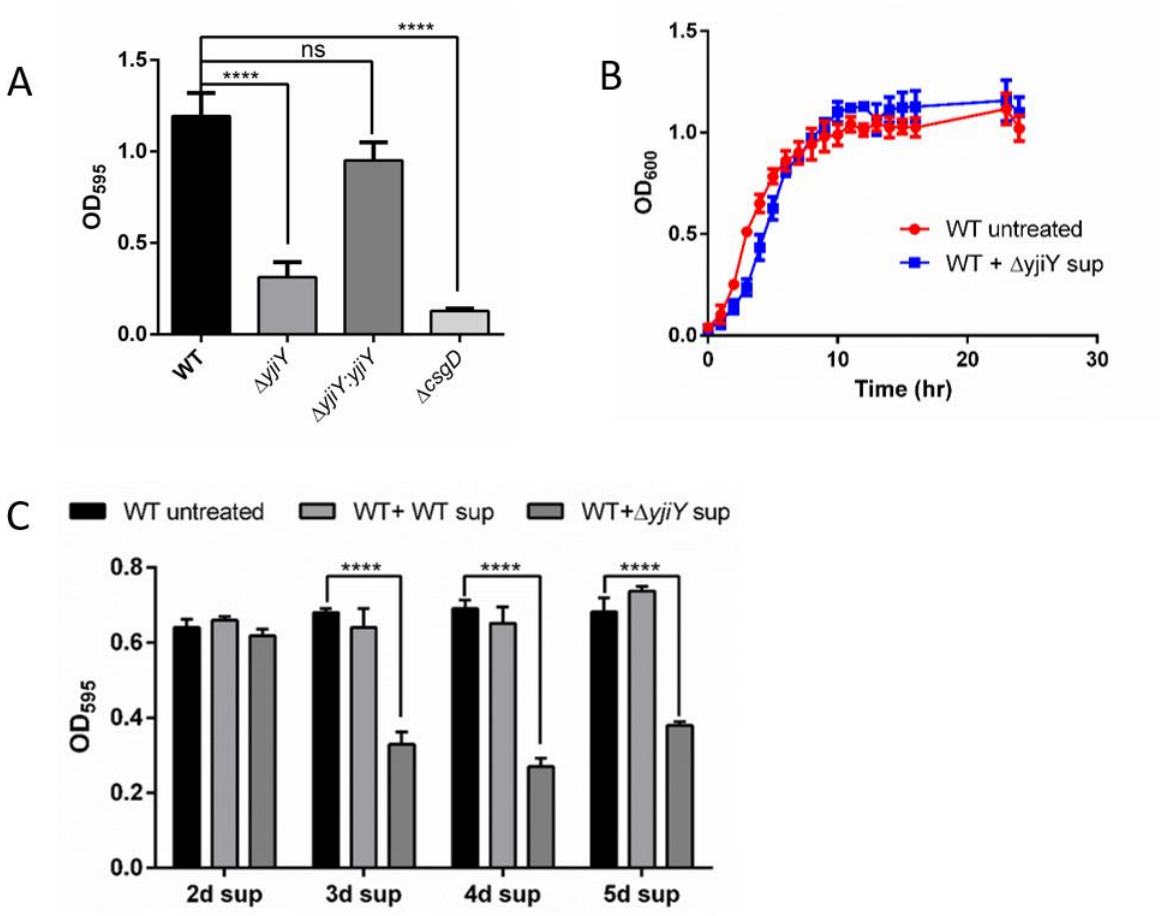

Fig S1. *Salmonella ΔyjiY* supernatant lacks any bactericidal activity, and significant biofilm inhibitory activity is present in 3day old supernatant

A. Biofilm formation ability of the different strains used in this study was checked (Data are presented as mean ± SEM of 5 independent experiments). B. The growth of STM WT bacteria was checked in presence and absence of *ΔyjiY* supernatant (Data are presented as mean ± SEM of 3 independent experiments). C. Biofilm inhibition activity of *ΔyjiY* supernatant, that was collected on different days of biofilm inoculation with pure culture, was checked (Data are presented as mean ± SEM of 5 independent experiments).

Figure S2

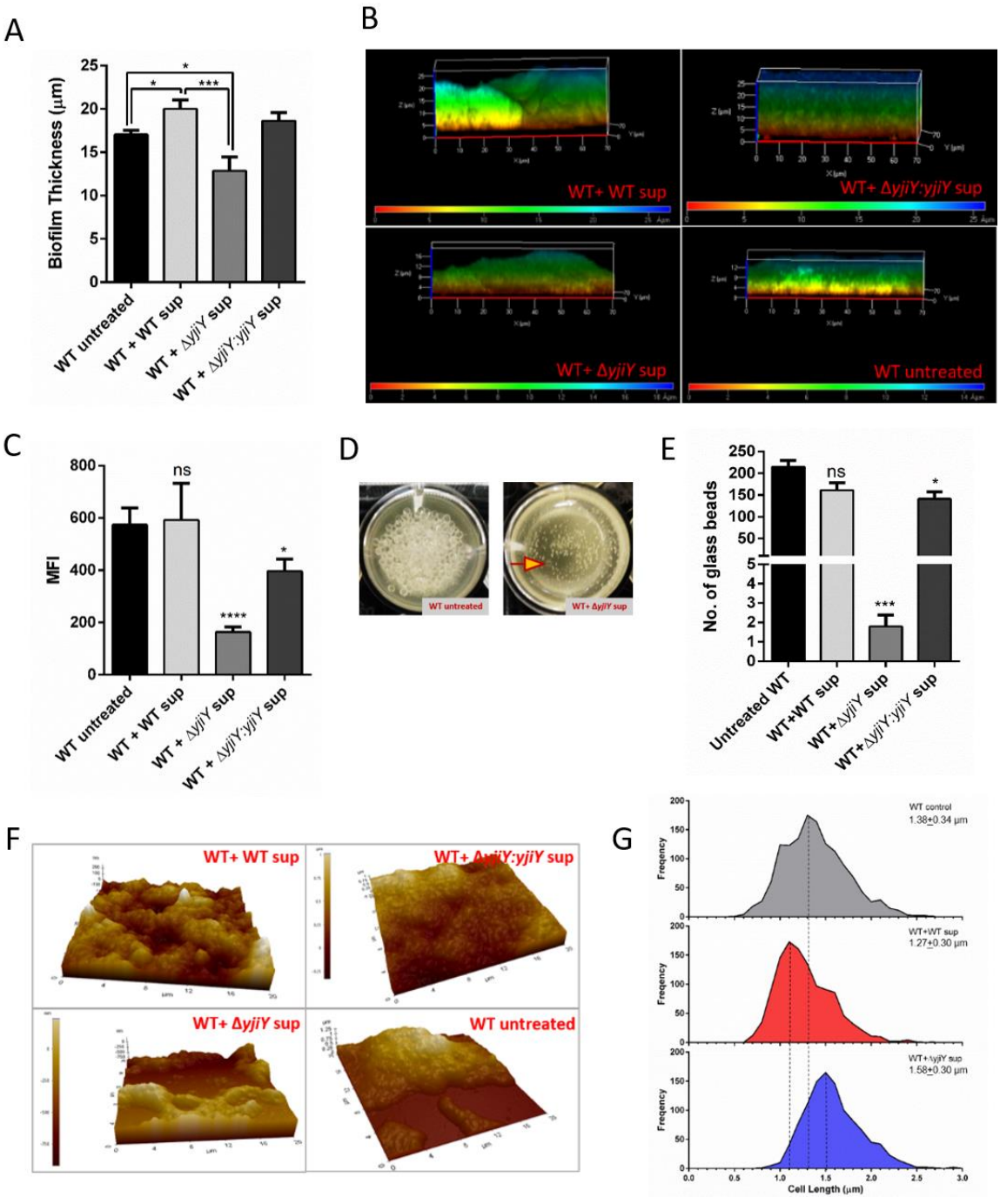

Fig S2. Δ*yjiY* supernatant makes the biofilm thin, fragile as well as it modifies cell length

A. The thickness of the biofilm formed on glass coverslips was measured using Zen (black edition) and plotted using GraphPad Prism 6 (Data are presented as mean ± SEM of 5

independent experiments). B. Representative CSLM images of the Congo red stained biofilm formed on glass coverslip, depth coding showing a reduced thickness of biofilm after Δ*yjiY* sup treatment (Representative image from 5 independent experiments). C. Median fluorescence intensity (MFI) of the Congo red stained biofilm was measured using ZEN (Black) software and MFI values were plotted with GraphPad Prism 6 (Data are presented as mean ± SEM of 5 independent experiments). D. Representative image of the strong and fragile biofilm formed without or with Δ*yjiY* sup. Yellow arrow shows presence of only one glass bead at the bottom of the well (Representative image from 3 independent experiments). E. Number of glass beads required to sink the biofilm pellicle to the bottom was counted and plotted using GraphPad Prism (Data are presented as mean ± SEM of 3 independent experiments). F. Representative Atomic Force Micrograph images of biofilm on the glass coverslip showing the absence of characteristic dome shaped structure of biofilm with Δ*yjiY* supernatant treatment (Representative image from 2 independent experiments). G. Frequency distribution of the cell length with different supernatant treatment showing a shift towards longer cell length with Δ*yjiY* sup treatment (Data are presented as mean ± SEM of 3 independent experiments, length of approximately 1200-1400 cells from each treatment were measured). One-way ANOVA was used to analyze the data, p values ****<0.0001, ***<0.001, **<0.01, *<0.05.

Figure S3

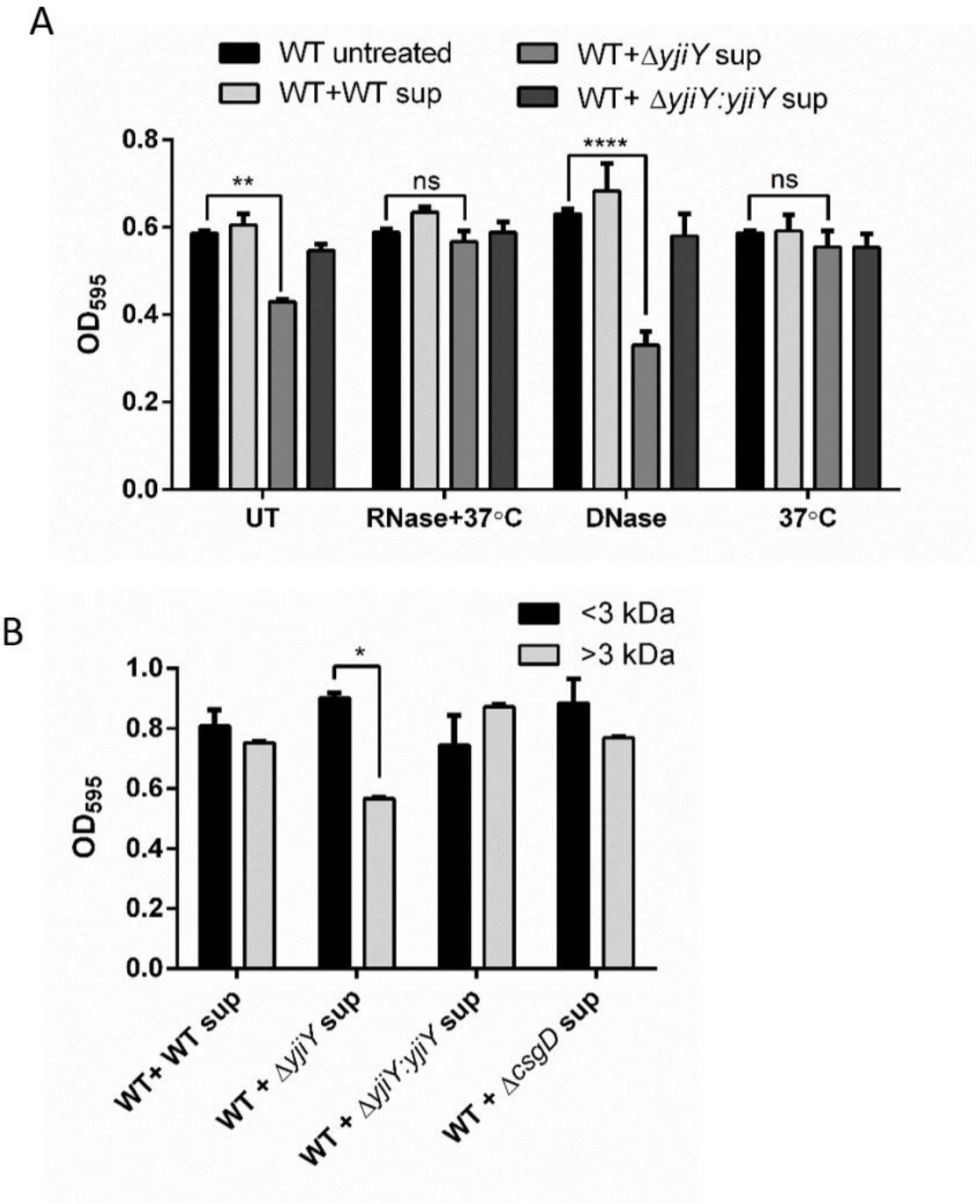

Fig S3. The active component(s) is/are not RNA or DNA and the components are larger than 3kDa in size

A. The supernatants were treated with RNase at 37˚C for 1 hour, as well as with DNase for 1 hour at 65˚C (Data are presented as mean ± SEM of 3 independent experiments). One-way

ANOVA was used to analyze the data, p values ****<0.0001, **<0.01. (UT- Untreated sup treated set). B. The supernatants were concentrated using Amicon ultra filter device 3k MWCO. The flow through (MW <3k) and the concentrated sup (MW >3k) were used separately while inoculating WT biofilm. After 72 hours, crystal violet staining was performed to quantify the biofilm (Data are presented as mean ± SEM of 3 independent experiments). One-way ANOVA was used to analyze the data, p values *<0.05.

Figure S4

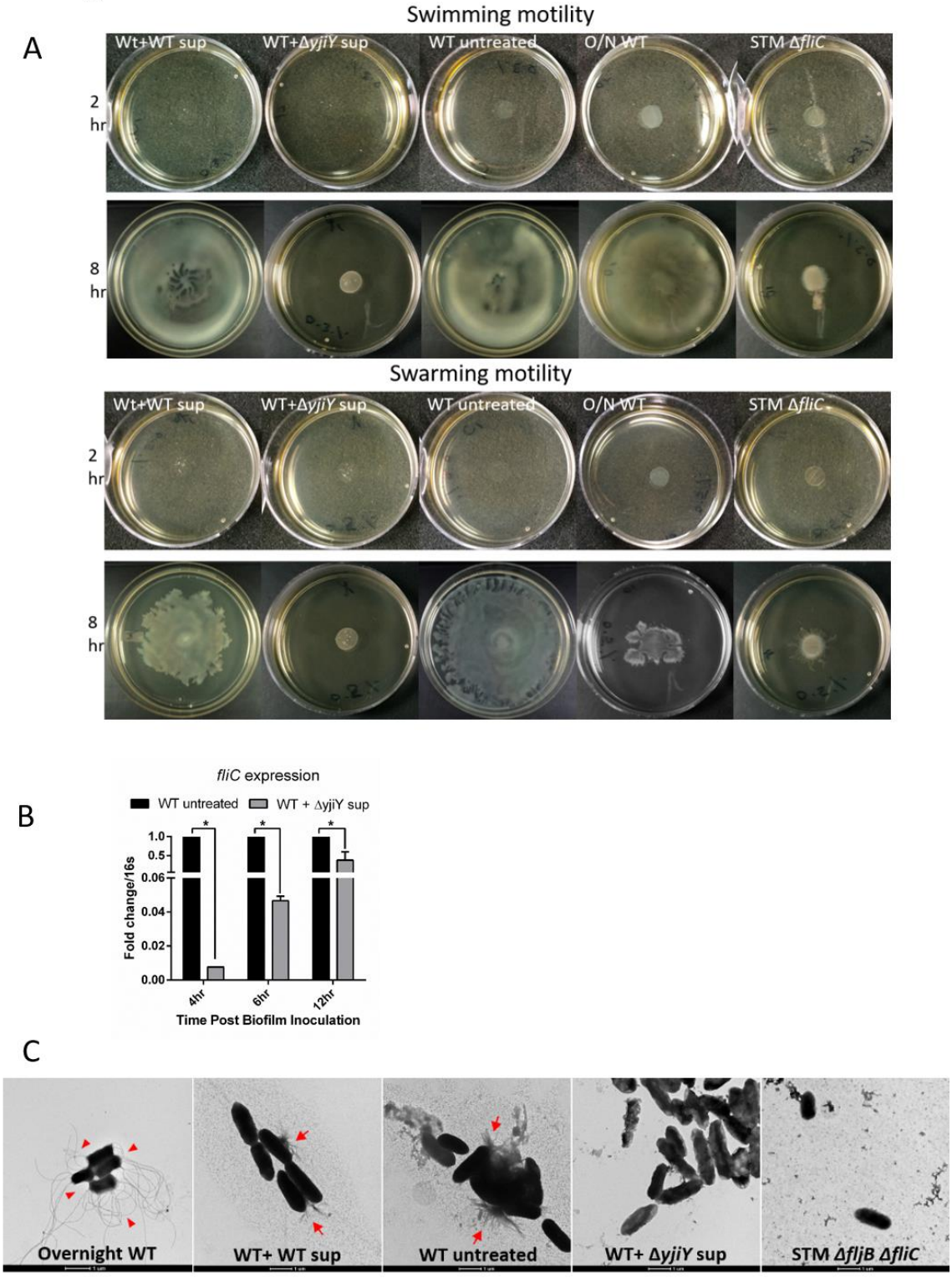

Fig S4. Δ*yjiY* supernatant inhibits flagellar mediated bacterial motility

A. Images of swimming and swarming plates inoculated with treated or untreated WT cells. Δ*fliC* after 2 hours and 8 hours post inoculation (Representative image from 3 independent

experiments). B. *fliC* expression was checked from WT cells after 4 hours, 6 hours and 12 hours of inoculation with Δ*yjiY* supernatant in biofilm media (Data are presented as mean ± SEM of 2 independent experiments). Student's t-test was used to analyze the data, p values *<0.05. C. Representative TEM images of STM WT cells inoculated with or without the supernatants. Cells were stained with uranyl acetate for visualization. Overnight grown STM WT cells and Δ*fljB* Δ*fliC* cultures were used positive and negative controls. Red arrowheads show intact flagella in overnight STM WT culture, red arrows show aggregates of fimbriae and fragmented flagella in untreated WT cells and WT cells treated with WT sup, after 72 hours of inoculation in biofilm media, whereas WT cells treated with Δ*yjiY* supernatant, do not show presence of such aggregates (Representative image from 2 independent experiments, approximately 50-60 cells were imaged from each experiment).

Supplementary Table 1. List of proteins only found in *ΔyjiY* supernatant.

UniProt ID	Proteins found in *yjiY* supernatant	Mol. Wt. (kDa)
tr|A0A0F6AXD1	Putative cytoplasmic protein	11.8
tr|A0A0F6AZ15	Putative ABC transporter periplasmic binding protein	56.5
tr|A0A0F6AZ65	Putative lipoprotein	18.8
tr|A0A0F6AZA0	Thioredoxin reductase	34.8
tr|A0A0F6B006	Anti-sigma28 factor **FlgM**	10.5
tr|A0A0F6B054	Transcription-repair-coupling factor **Mfd**	129.9
tr|A0A0F6B0G8	Putative ABC transporter periplasmic binding protein	60.0
tr|A0A0F6B221	Putative cytoplasmic protein	18.6
tr|A0A0F6B2M8	Probable transcriptional regulatory protein **YebC**	26.4
tr|A0A0F6B3Z2	**Ecotin**	18.2
tr|A0A0F6B4D7	Putative cytoplasmic protein	10.2
tr|A0A0F6B9X9	Transcription termination/antitermination protein **NusG**	20.5
tr|A0A0F6BAJ5	Putative outer membrane lipoprotein	12.6
tr|A0A0F6BAL8	Putative arginine-binding periplasmic protein	27.4

Supplementary Table 2. List of common proteins found in both the supernatants.

UniProt ID	Proteins found in both supernatants	Mol. Wt. (kDa)	Relative Abundance (*ΔyjiY*/WT)
sp|A0A0F6B244	DNA-binding protein **H-NS**	15.5	3.35
tr|A0A0F6AYA7	Regulator of nucleoside diphosphate kinase **Rnk**	14.99	3.30
tr|A0A0F6AYC2	Cold shock protein **CspE**	7.4	3.30
tr|A0A0F6B2E5	Cold shock-like protein **CspC**	7.4	5.48
tr|A0A0F6B5B6	Heat shock protein/chaperone **GrpE**	21.8	4.28
tr|A0A0F6B5K2	DNA-binding protein **StpA**	15.4	1.20
tr|A0A0F6B9S5	ATP-dependent protease subunit **HslV**	18.9	1.15
tr|A0A0F6AYJ2	Flavodoxin **FldA**	23.7	0.48
tr|A0A0F6B123	Superoxide dismutase **SodB**	21.3	0.72
tr|A0A0F6B125	Glutaredoxin **YdhD**	12.9	0.53
tr|A0A0F6B1W1	Thiol peroxidase **Tph**	18.0	0.37
tr|A0A0F6B2S0	Ferritin **Ftn**	19.3	0.10
tr|A0A0F6B4P5	Thioredoxin-dependent thiol peroxidase **Bcp**	17.6	0.80
tr|A0A0F6B7N9	Bacterioferritin **Bfr**	18.3	0.06