SUPPLEMENTARY INFORMATION

Supplementary table S1- Primers used in this study

Primer	**Sequence (5'→3')***	**Amplicon size (bp)**	**Application**	**Reference**
icdA-F icdA-R	TGGTATCGGTGTTGATGTCACTC CATCCTGGCCGTAAACCTGTGTG	140	qRT-PCR in bacteria	Modified from [1]
motB1-F motA3-R	TTTATGACGGCGATGATGGC CGGCGTACGAAAATATTCGGC	99	qRT-PCR in bacteria	This work
cheB-F cheB-R	AGTTGGGGGCCATCGATTTT CGCACTTTTTCGGCGATCAT	91	qRT-PCR in bacteria	This work
flgB-F flgB-R	TACCCGATCAGCCTTCTTTG GTTGGCTACCCAGAACGGTA	114	qRT-PCR in bacteria	This work
fliN1-F fliM3-R	TTGAATTCGCTGAATGAGGAAC TCCTGCATAGCACCGCTGACATC	187	qRT-PCR in bacteria	This work
flgM-F flgM-R	CTTTGAAACCCGTTAGCACTGTC GATAGCCGTTTTTAATGCTTCGAC	188	qRT-PCR in bacteria	This work
fliC-F fliC-R	AGATCACCTTAGCTGGCAAAACC CCCCAGAGAAGAACGAACTGC	164	qRT-PCR in bacteria	This work
fliE4-F fliE4-R	AATTCACTCTGGGTGAGCCG CTTGTTGCGCACCTGAATCC	101	qRT-PCR in bacteria	This work
fliF1-F fliF1-R	CACGCAATCCAATACCAGCG CGATAGGCGACAGAATGGCT	110	qRT-PCR in bacteria	This work
tsr1-F tsr1-R	GGGTATCCGCTGGATGATGG TTCACTCTGACGTGGATCGC	148	qRT-PCR in bacteria	This work
fliA3-F fliA3-R	TATCAATTG*GTG*AATTCACTGTATACCGC (MfeI) TATAAGCTTCTATAACTTACCCAGTTTGGTG (HindIII)	738	Cloning in pJF119EH	This work

flgM3-F flgM3-R	TATGAATTC*ATG*AGCATTGACCGTACCTC (EcoRI) TATCTCGAG*TTA*TTTACTCTGTAAGTAGCT CTG (XhoI)	312	Cloning in pGEX4T-1.	This work
fliE2-F fliE2-R	GGGAATTCC*ATG*GCAGCAATACAGGG (EcoRI) AGCAAGCTT*TTA*AACCTGCATAGACATCA CTT (HindIII)	333	Cloning in pBAD22 or pJF119EH.	This work
fliE1-F fliE1-R	AGGGGATTGAAGGGGTTATTAGCCA ACGATCCAGCGCAGCATGTAG	Variable[a]	fliEΔ42 screening and sequencing	This work
mB-actin-F mB-actin-R	GCTTCTTTGCAGCTCCTTCGT CGTCATCCATGGCGAACTG	68	qRT-PCR in cecum	[2]
mCXCL1-F mCXCL1-R	CTTGGTTCAGAAAATTGTCCAAAA ACGGTGCCATCAGAGCAGTCT	84	qRT-PCR in cecum	[2]
mIL17a-F mIL17a-R	CTCCAGAAGGCCCTCAGACTAC GGGTCTTCATTGCGGTGG	69	qRT-PCR in cecum	[2]
mIFNg-F mIFNg-R	TCAGCAACAGCAAGGCGAAA CCGCTTCCTGAGGCTGGAT	143	qRT-PCR in cecum	[3]
mTNFa-F mTNFa-R	CATCTTCTCAAAATTCGAGTGACAA CCTCCACTTGGTGGTTTGCT	63	qRT-PCR in cecum	[2]
mLcn2-F mLcn2-R	CCATCTATGAGCTACAAGAGAACAAT TCTGATCCAGTAGCGACAGC	89	qRT-PCR in cecum	Muñoz N. PhD thesis
mS100A9-F mS100A9-R	CACCCTGAGCAAGAAGGAAT TGTCATTTATGAGGGCTTCATTT	95	qRT-PCR in cecum	Muñoz N. PhD thesis

*: restriction sites are underlined and indicated in parenthesis. Start and stop codons are written in italics. [a]: amplicon variable length depending on presence or absence of *fliEΔ42* deletion, 77bp or 119bp, respectively.

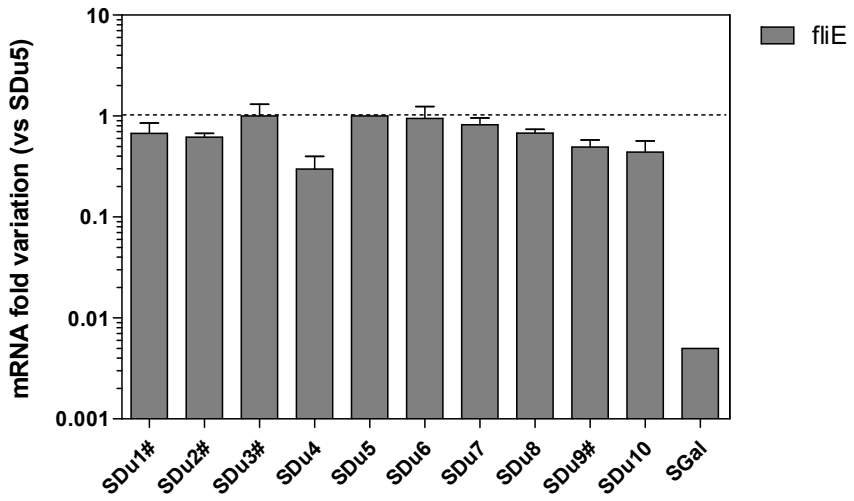


Figure S1- *fliE* mRNA levels quantification in S. Dublin isolates grown in LB to mid-log phase by qRT PCR. Results (means and standard errors) from two independent experiments are shown. The dashed line indicates value 1, arbitrarily assigned to isolate SDu5. SGal is a strain of the aflagellateserovar S. Gallinarum. # indicates non-motile isolates.

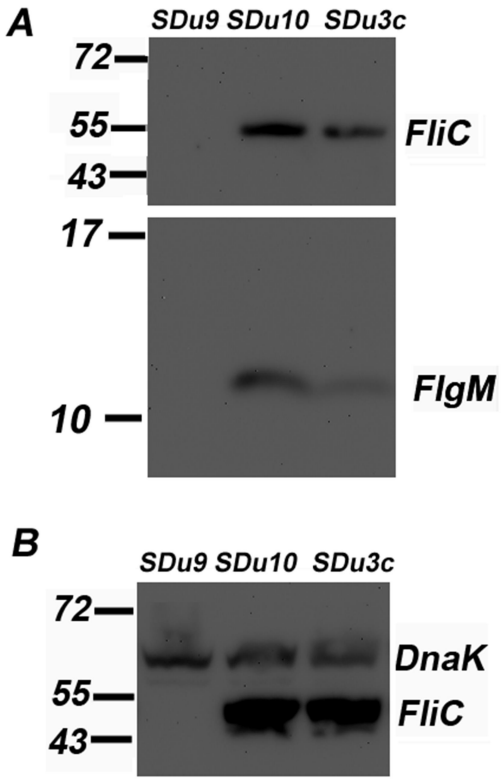


Figure S2- Western blot analysis of secreted (A) or cellular (B) proteins extracts of *S*. Dublin isolates, using anti-FliC and anti-FlgM antisera. SDu9: aflagellate, SDu10: flagellate, SDu3c: SDu3 complemented with *fliE* wild type, grown in presence of inducer. Detection of DnaK (69.1kDa), was used to verify equal loading quantities in cellular fractions (panel B) and the presence of protein in the secreted fraction due to cell lysis (panel A). Sizes of molecular mass markers are indicated in kDa. Sizes of FliC and FlgM are 53kDa and 10.6 kDa, respectively.

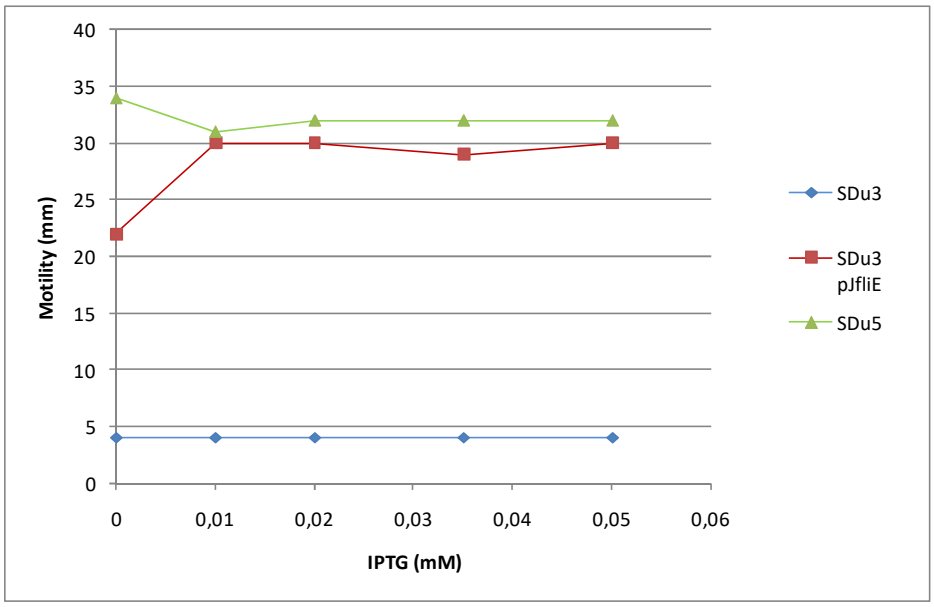
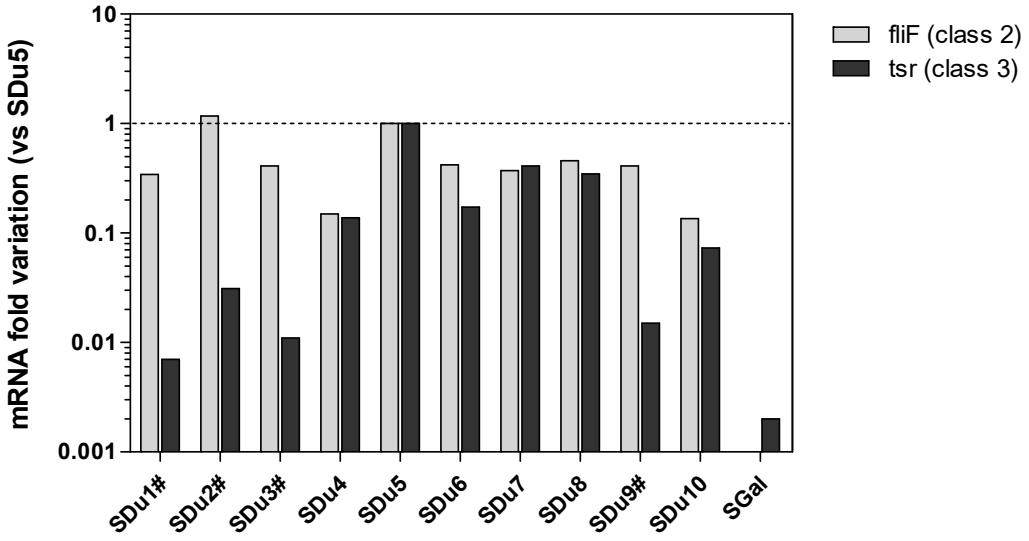


Figure S3- Motility tests of SDu3, SDu5 or SDu3 complemented with pJfliE. Mid-exponential phase cultures grown in LB containing 100ug/ml ampicillin were spotted in LB soft agar containing increasing concentrations of IPTG and incubated at 37°C for 6hs. The diameter of the halo of growth is plotted in the Y axis.

A)



B)

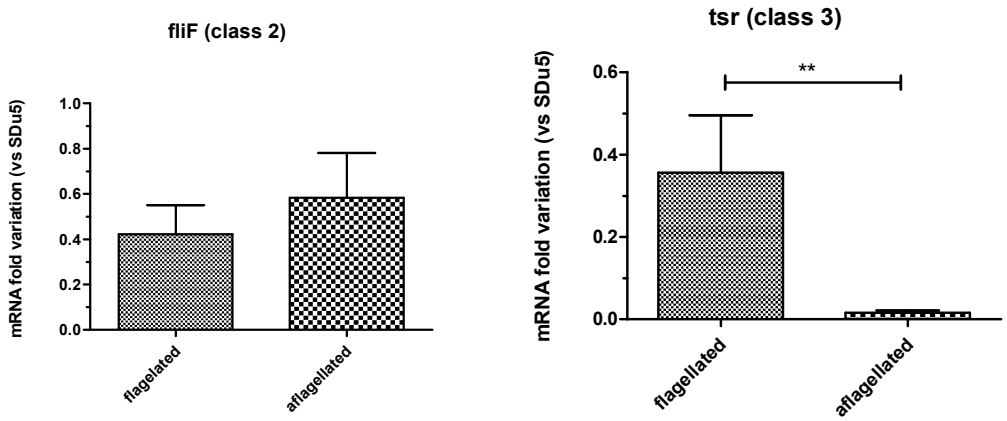
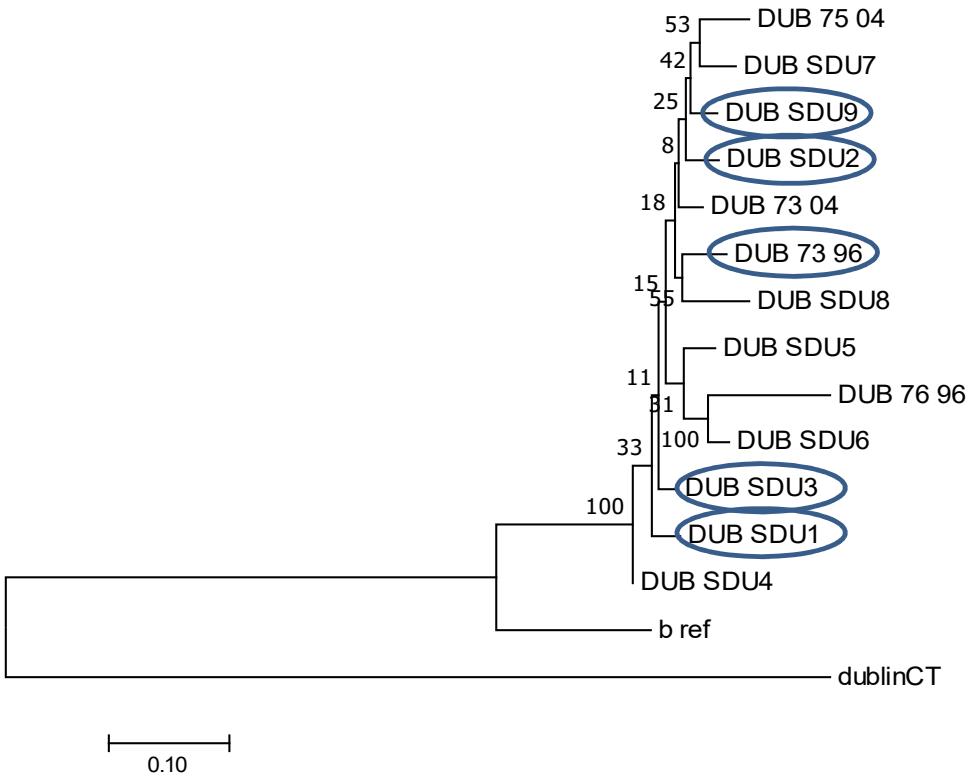


Figure S4- (A) *fliF* and *tsr* mRNA levels quantification in S. Dublin isolates grown to mid-log phase by qRT PCR. The dashed line indicates value 1, arbitrarily assigned to isolate SDu5. SGal is a strain of the aflagellate serovar S. Gallinarum. # indicates non-motile isolates. (B) mRNA fold variation ratios from panel A were grouped according to phenotype (flagellated vs aflagellated) and Mann Whitney test (GraphPad Prism 4 software) was applied. **: $p<0.01$

A)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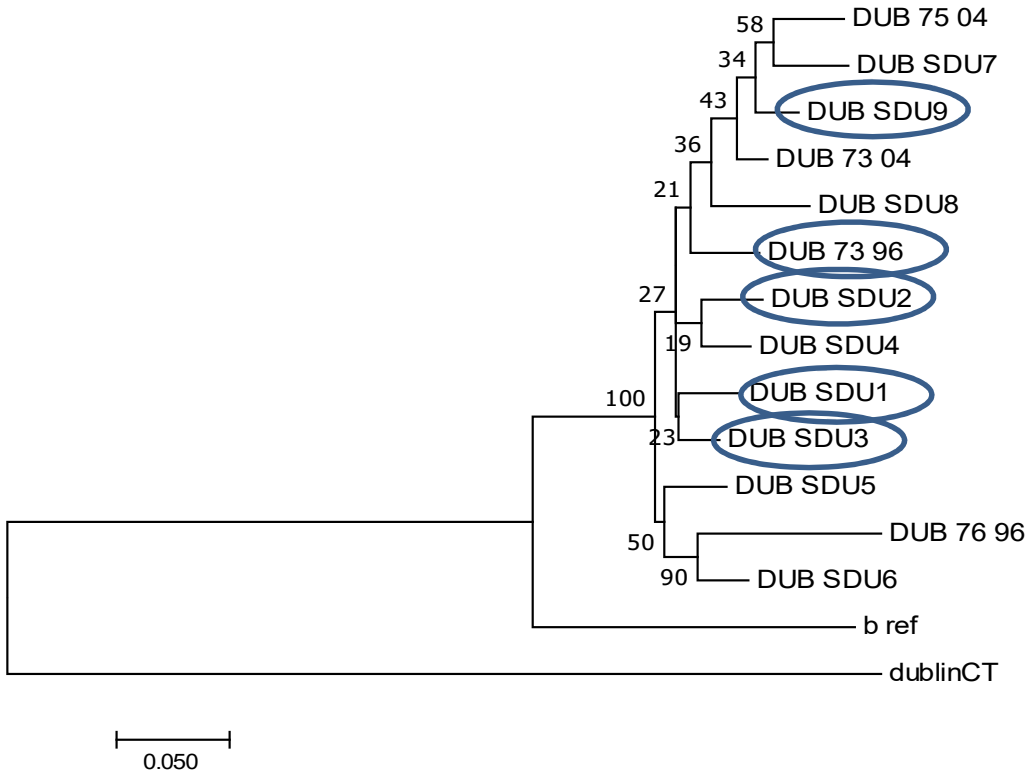
B)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C)

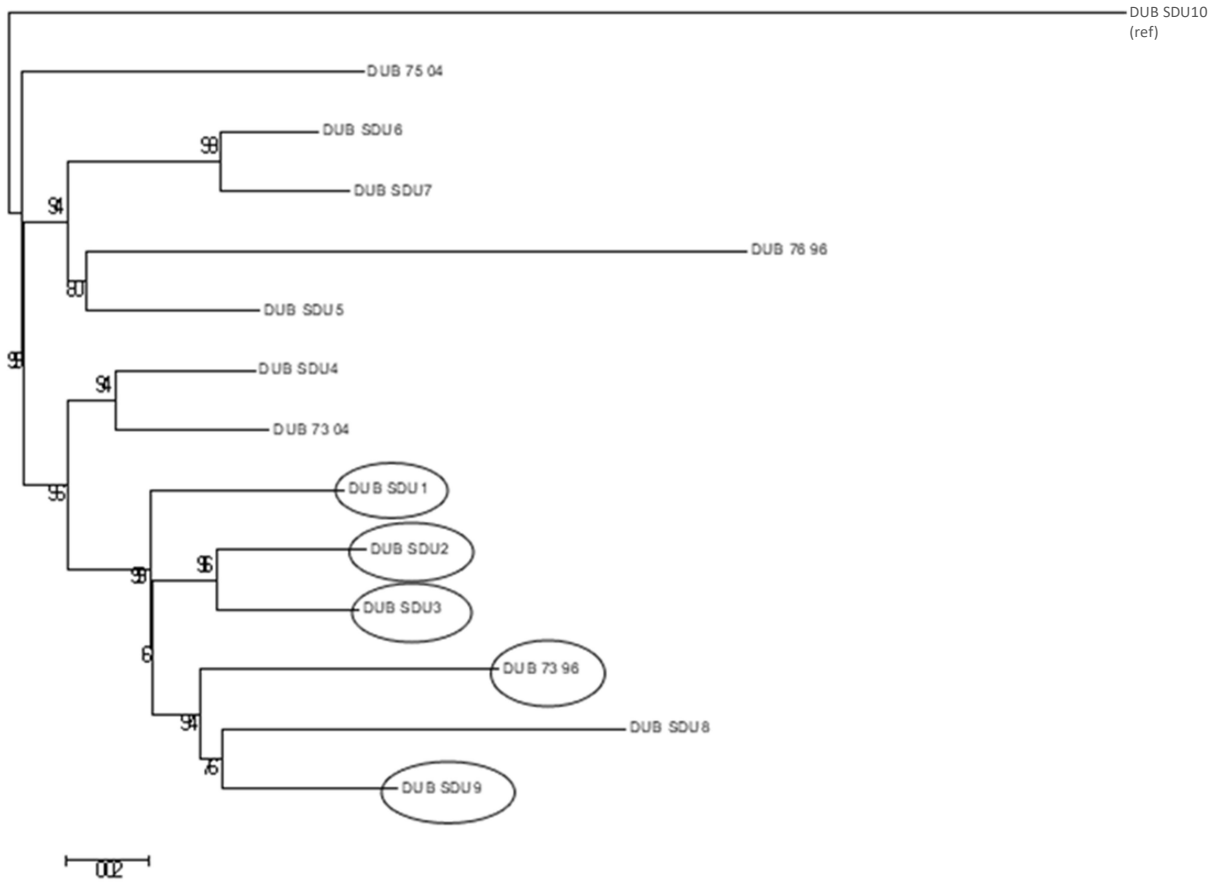

Figure S5- Evolutionary relationships of taxa. Three different methods were applied to obtain phylogenetic trees of sequenced isolates used in this study, based on SNPs of homologous genomic regions. (A) The evolutionary relationship among strains was inferred using the Neighbor-Joining method [4]. Statistical support based on bootstrap test (100 replicates) is shown next to the nodes [5]. The evolutionary distances were computed using the Maximum Composite Likelihood method [6]. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). There were a total of 1767 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [7]. *fliEΔ42*-harboring strains are indicated by an ellipse. Isolate SDu10 (b ref) was used as reference. *S*. Dublin strain CT _02021853 (NCBI reference sequence: NC_011205.1) (dublinCT) was added as outgroup. (B) Molecular Phylogenetic analysis by Maximum Likelihood method. The phylogenetic tree was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model [8]. Bootstrap statistical support based on 100 replicates is shown next to nodes. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join. A discrete Gamma

distribution was used to model evolutionary rate differences among sites (8 categories +G). The analysis involved a total of 1767 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [7]. fliEΔ42-harboring strains are indicated by an ellipse. Isolate SDu10 (b ref) was used as reference. *S*. Dublin strain CT _02021853 (NCBI reference sequence: NC_011205.1) (dublinCT) was added as outgroup. (C) Phylogeny of 14 sequenced *S*. Dublin Uruguayan strains, using CSI Phylogeny-1.4 (Center for Genomic Epidemiology, cge.cbs.dtu.dk/services/CSIPhylogeny/). SDu10 was used as reference. *fliEΔ42*-harboring strains are indicated by an ellipse.

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