Supplementary Data S1. Prediction of *Yersinia pestis* Fur consensus

In our previous DNA microarray analysis (3), the mRNA levels from wild-type (WT) *Yersinia pestis* cells treated with the iron chelator 2,2'-dipyridyl (DP) were compared with those supplemented with excessive iron, and then gene expression in the *fur* mutant was compared with that in the WT strain under iron rich condition. The microarray analysis identified genes both directly and indirectly controlled by Fur, leading to the defining of the iron-Fur modulon that was defined as those genes whose transcription was affected by both the DP treatment (the iron starvation) and the *fur* mutation.

All the gene members of this modulon were visually scrutinized according to the transcriptional organization in relation to the surrounding genes, to identify the putative operons (defined as a cluster of adjacent genes that have intergenic regions <100-bp in length and were putatively transcribed in the same orientation). Members of the iron-Fur modulon were combined into 33 putative operons. The 500-bp promoter regions extending upstream from the start codon of every first gene in each indicated operon were retrieved with the *retrieve-seq* program (http://rsat.ulb.ac.be/rsat/).

The *MotifSampler* program (2) were used for detection of the over-represented motifs within the above promoter regions. During this analysis, the program would generate the predicted Fur site in each promoter DNA region (Table A), and the further alignment of these Fur sites would brought the Fur consensus, as shown in Figure A. The Fur consensus could be represented as either a box sequence or a position-scoring matrix [the position frequency matix (PFM) or the position weight matrix (PWM)]. The box sequence was a contiguous oligonucleotide, i.e., a 9-1-9 inverted repeat (5'-AATGATAATNATTATCATT-3'). A major drawback of the box sequence was that it removed much of the information originally present in the set of Fur sites. In contrast, either PFM or PWM retained most of the information and were thus better suited to evaluate new potential sites. In the PFM or PWM, each row represents a position and each column a nucleotide. Representation of consensus patterns with PFM or PWM could give a full description of the uneven composition in each position, i.e., some nucleotides occurred much more frequently than others. Finally, the consensus sequence logo (Figure A) was built by compilation of the potential motif sequences of each genes with the *Logo* algorithm (1).

Table A. The predicted Fur sites

Gene	Potential Fur site	Gene	Potential Fur site
YPO0133D	CATGAAAATAATTCTCAGTA	YPO1911D	AATAATTATTATTATCATAT
YPO0133R	ACTGAGAATTATTTTCATGC	YPO1911R	AATAATAATTATTAACAATT
YPO0205D	AATAAAACTCATTCTTATTT	YPO1912D	AATAATAACCATTATCAATA
YPO0205R	AATGAGTTTTATTTCAATTA	YPO1912R	AATGGTTATTATTCACATTA
YPO0283D	ACTGATAGTCGTTATCATTA	YPO1913D	AATGGTTATTATTCACATTA
YPO0283R	AACGACTATCAGTGTGATAA	YPO1913R	AATAATAACCATTATCAATA
YPO0426D	ATTGGTTATGATTATCATTA	YPO2163D	ATTGAAAATAAATACCGACA
YPO0426R	AATGATAATCATAACCAATA	YPO2193D	AATAATGATAGTTATCAATG
YPO0682D	AATGATAATTACTATCAATC	YPO2193R	AATAGCAATCATGTTCAATA
YPO0682R	ATTGATAGTAATTATCATTA	YPO2386D	AATAATGATTACTGGCAAGA
YPO0988D	AGTGGTTATCATTATCAATT	YPO2386R	AGTAATCATTATTCAACATA
YPO0988R	ATTGATAATGATAACCACTA	YPO2439D	ATTGATAATCATTTTCATTT
YPO0989D	ATTGATAATGATAACCACTA	YPO2439R	AATGATTATCAATACCATTA
YPO0989R	AGTGGTTATCATTATCAATT	YPO2651D	GATGATAATCATAGGCCACC
YPO1011D	AATGATAATCGTTTGCAATT	YPO2958D	AATGATAATCATTTTTATTA
YPO1011R	AACGATTATCATTTTCAAGT	YPO2958R	AATGATTATCATTGTTATCT
YPO1207D	AATGATCGTCAGTTCCCACA	YPO2982D	CTTGATAATTATTCTCATTT
YPO1207R	GTGGGAACTGACGATCATTT	YPO2982R	AATAATTATCAAGCGCATTT
YPO1310D	ATTGATAAGTATTATCATTT	YPO3040D	AATAAGAATTATTATCATTA
YPO1310R	AATGATAATACTTATCAATA	YPO3040R	AATAATTCTTATTTATATTA
YPO1528D	ATTGATAATCACTATCATTC	YPO3339D	ATTAACTGTAATGTTCTACT
YPO1528R	AATGATAGTGATTATCAATA	YPO3340D	AATGATAATTGATATCATTT
YPO1735D	ATTAAGAACCATTTGTATTT	YPO3340R	AATGATATCAATTATCATTA
YPO1735R	AATGGTTCTTAATAGCATTT	YPO3923D	AGTGATTATGATAATCATTA
YPO1783D	AATAGGAATCATTATCTTTA	YPO3923R	AATGATTATCATAATCACTG
YPO1783R	CATGATAATCATTCACATTA	YPO4022D	ATTGGTAATAAAAATCATTA
YPO1854D	AATGATAATCACAATCGTTT	YPO4022R	AATGATTTTTATTACCAATC
YPO1854R	AACGATTGTGATTATCATTC		
YPO1906D	AATAGTTATCATTTTCAATT		
YPO1906R	AATGATAACTATTTCTAATA		

(a) Position frequency matrix (PFM)

Nucleotide	Position 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	52	38	0	16	46	4	33	44	0	8	52	6	8	32	6	2	50	22	2
T	0	13	53	0	0	45	20	3	51	17	0	44	45	13	36	6	2	31	47
C	3	2	3	0	0	3	2	4	5	25	1	5	0	8	9	48	3	3	5
G	2	4	1	41	11	5	2	6	1	7	4	2	4	4	6	1	2	1	3

(b) Position weight matrix (PWM)

A	1.15	0.64	-0.02	0.03	0.92	-0.09	0.48	0.85	-0.02	-0.08	1.15	-0.09	-0.08	0.44	-0.09	-0.07	1.08	0.16	-0.07
T	-0.02	-0.02	1.19	-0.02	-0.02	0.89	0.12	-0.08	1.12	0.05	-0.02	0.85	0.89	-0.02	0.57	-0.09	-0.07	0.41	0.96
C	-0.08	-0.07	-0.08	-0.02	-0.02	-0.08	-0.07	-0.09	-0.09	0.24	-0.05	-0.09	-0.02	-0.08	-0.07	1.00	-0.08	-0.08	-0.09
G	-0.07	-0.09	-0.05	0.74	-0.05	-0.09	-0.07	-0.09	-0.05	-0.09	-0.09	-0.07	-0.09	-0.09	-0.09	-0.05	-0.07	-0.05	-0.08

(c) Sequence-Logo representation

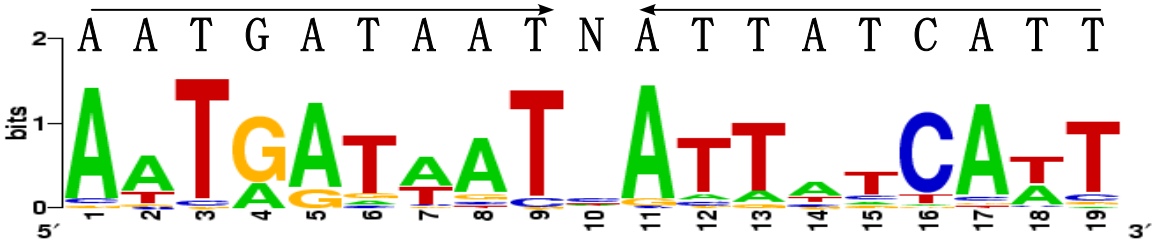

Figure A. Graphic representation of the predicted Fur consensus in *Y. pestis*

The PFM contained $f_{b,i}$ that denoted the frequency of nucleotide b at position i. The PWM was derived from the frequency matrix in (a) using the following formula:

$$p(b,i) = \frac{f_{b,i} + s}{N + 4s}$$

$$W_{b,i} = \log \frac{p(b,i)}{p(b)}$$

where $p(b.i)$ indicated the probability of nucleotide b at position i, s was the pseudocount used to replace zeros to avoid log(0), $W_{b,i}$ wass the resulting weight and $p(b)$ was the background-probability of nucleotide b.

References

1. Schneider, T. D., and R. M. Stephens. 1990. Sequence logos: a new way to display consensus sequences. Nucleic Acids Res 18:6097-100.
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3. Zhou, D., L. Qin, Y. Han, J. Qiu, Z. Chen, B. Li, Y. Song, J. Wang, Z. Guo, J. Zhai, Z. Du, X. Wang, and R. Yang. 2006. Global analysis of iron assimilation and fur regulation in Yersinia pestis. FEMS Microbiol Lett 258:9-17.