METHODS

RNA extraction

The D39 WT and the Δ*arcD* mutant were grown in 10 ml THY broth to an OD_{620} of 0.6. Bacteria were harvested by centrifugation, resuspended into 500 µl RLT buffer from the RNeasy Mini kit (Qiagen), and milled with 0.1 mm zirconia beads (Biospec Products) using a mini bead beater (Biospec Products) twice of 100 sec, with 5 min interval of incubation on ice. The lysate was centrifuged, and the supernatant was used to isolate RNA following the instructions of the kit. RNA samples were treated twice with an RNase-free DNase set (Qiagen) as per the manufacturer's instruction. Removal of genomic DNA was verified by PCR using 0.1 µg RNA as template.

cDNA synthesis and reverse transcriptase PCR (RT-PCR)

For each reaction of cDNA synthesis, one microgram of RNA was used as the template for reverse transcription with iScript cDNA Synthesis Kit (Bio-Rad). Subsequently, cDNA was amplified by quantitative PCR with specific primers (Table S1) using iQ SYBR Green Supermix and iQ Single Color Real Time PCR Detection System (Bio-Rad) according to the manufacturer's instruction. Assays were conducted in triplicates of 25 µl reaction volume using 96-well plates as previously described (1). Relative gene expression was analyzed using PFAFFL method (2) and fold changes were normalized to *gyrA*. A two-fold or greater change in mRNA levels of the mutant relative to the D39 WT was considered significant.

References

1. **Thornton J, McDaniel LS.** 2005. THP-1 monocytes up-regulate intercellular adhesion molecule 1 in response to pneumolysin from *Streptococcus pneumoniae*. Infect Immun **73:**6493-8.

2. **Pfaffl MW, Horgan GW, Dempfle L.** 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res **30:**e36.

3. **Krogh A, Larsson B, von Heijne G, Sonnhammer EL.** 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol **305:**567-80.

Table S1. Primers used in this study

Purpose	Primer	Sequence (5′ to 3′)
Mutagenesis		
arcD	Pr1615	AAGGTAGCATCGGTTTCTGGTTG
	Pr1616	ACACTCTAGATTTGTTTTTCCTCCTGATGTCTAA
	Pr1668	ATCGCTCGAGTATTGTAGTGACCATCGCCCTTCT
	Pr1669	TGGAGAGGAGCAAGAATGGTAGC
arcT	Pr1670	TGAATCCATTTGCGACAGG
	Pr1671	ACACTCTAGATCATGGAATCACCTCACTCACTA
	Pr1617	ATCGCTCGAGGCTAGTCAAGGTATCAATGCTGTC
	Pr1618	GATAGACGGCTTCGGCATAA
Janus cassette	Pr1097	GAGATCTAGAACCGTTTGATTTTTAATGGATAATG
	Pr1098	GAGACTCGAGCCTTTCCTTATGCTTTTGGAC
RT-PCR		
cps2A	Pr2809	ATCACGTTCACAGAAAGTGAAGC
	Pr2810	CTAGTAGGACTAACGCAGTTACC
cps2B	Pr2811	TGATGTAGATGACGGTCCCAAGT
	Pr2812	TTCTGCTATCTTCTCTTCCGGAG
gyrA	Pr2783	CGTTACATGCTTGTAGATGG
	Pr2784	TGGCATCATAGTTATCAACG
Other primers		
gyrA promoter	Pr2774	TTTTGTCGACCCAAGCTCTTGTGCAATTCC
	Pr2775	TTTTGGATCCGCATGCGGTACCCATTAATAAATGCCTCATTTC
arcD ORF	Pr2704	ACACGGATCCAGTGAAAAAGCTAAAAAAGGG
	Pr1673	CGGGATCCTCATGGAATCACCTCACTCACTA

Legends of supplemental figures

Fig. S1. Genetic organization and predicted functions of *S. pneumoniae arc* locus. (A) Schematic representation of *arc* locus in *S. pneumoniae*. The putative function of each gene is indicated. The size of each protein (in amino acids) is shown above, and the space of each intergenic region (in base pairs) is also marked between the adjacent genes. (B) Predicted functions of *arcABCD* genes in *S. pneumoniae* based on published homologs. Purple, gray, and yellow colors denote Gram-positive *S. pneumoniae*, capsule, and proteins, respectively. Arg, Cit, and Orn denote arginine, citrulline, and ornithine, respectively.

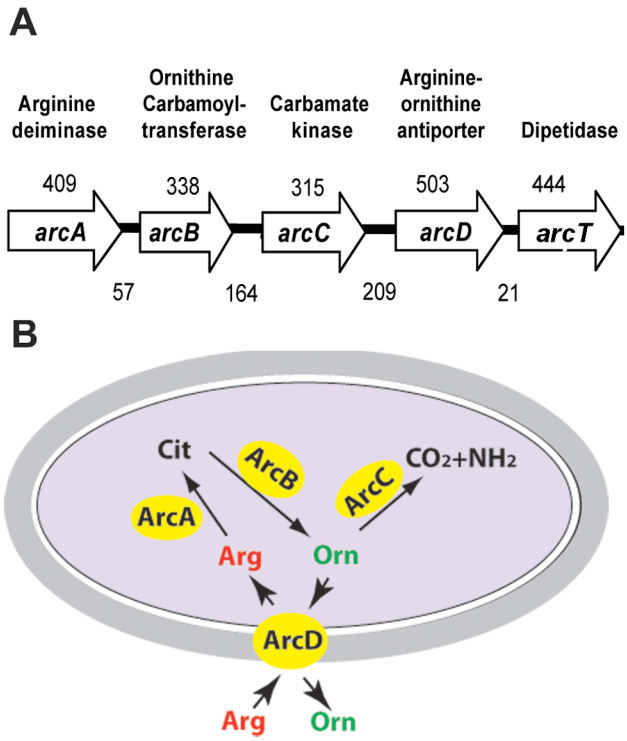
Fig. S2. Infection of A549 cells with the D39 WT and its Δ*arcD* mutant. Gram stain showing bacterial association with A549 cells that were seeded on coverslips and observed under microscope (1000 ×).

Fig. S3. qRT-PCR analysis of *cps2A* and *cps2B* genes compared between the D39 WT and its Δ*arcD* mutant. Data shown are the means of two independent experiments. Error bars denote the SEM.

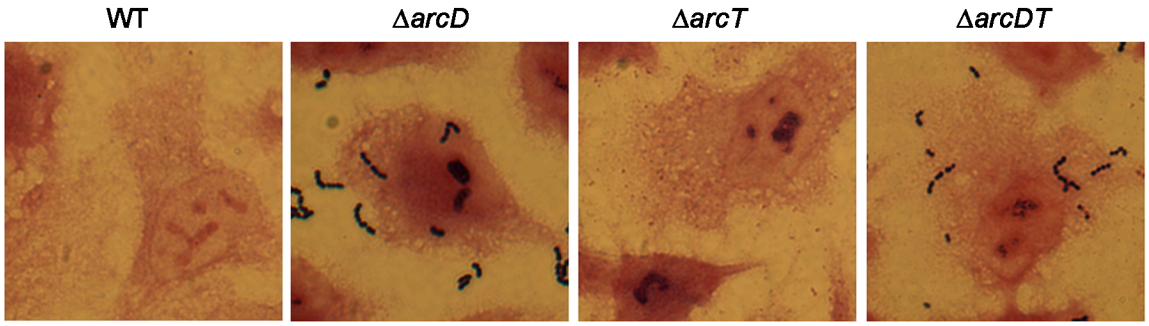
Fig. S4. Topological model showing the predicted structure of ArcD protein in the cytoplasmic membrane. This model was generated using TMHMM program (http://www.cbs.dtu.dk/services/TMHMM) based on a hidden Markov model (3).

Fig. S5. Growth of pneumococci in complete CDM (JRH Bioscience). The D39 WT and its Δ*arcD*, Δ*arcT*, and Δ*arcDT* mutants were inoculated in 10 ml of CDM broth and were monitored hourly at OD_{620} for 15 h. Data shown are the means of three repeat experiments. Error bars denote the SEM.

Fig. S1

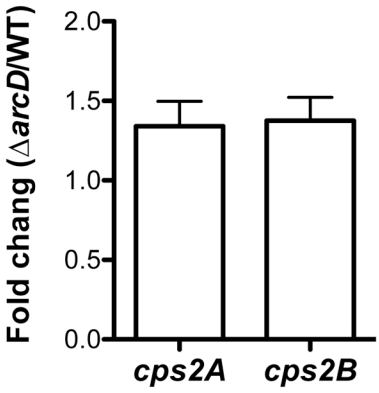

A
Arginine deiminase
Ornithine Carbamoyl-transferase
Carbamate kinase
Arginine-ornithine antiporter
Dipetidase
409
338
315
503
444
arcA
arcB
arcC
arcD
arcT
57
164
209
21
B
Cit
ArcB
ArcC
CO2+NH2
ArcA
Arg
Orn
ArcD
Arg
Orn

57 Fig. S2



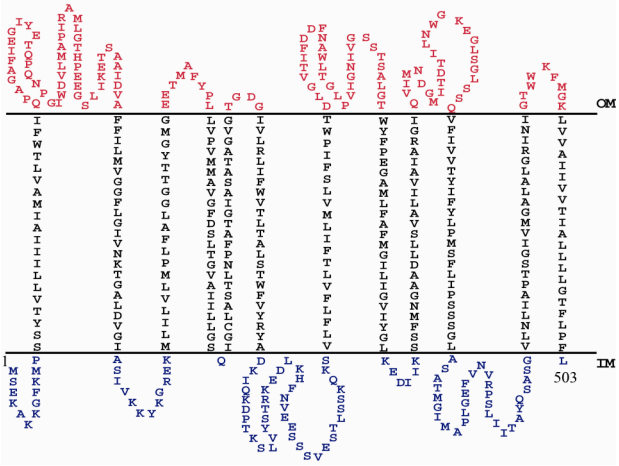
58

59 Fig. S3

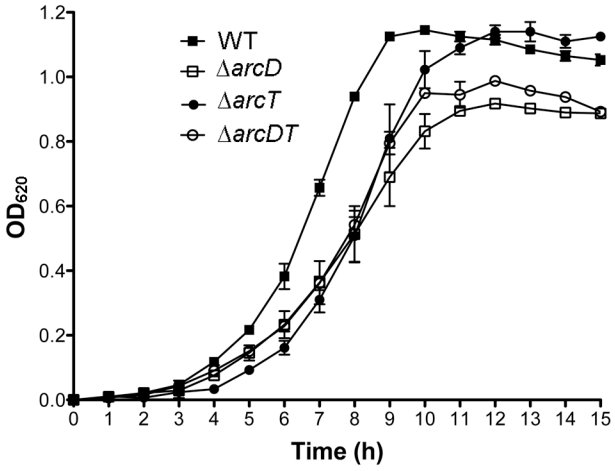


60

Fig. S4



63 Fig. S5


WT
ΔarcD
ΔarcT
ΔarcDT
OD620
Time (h)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

64