Table S1. Primers used in this study

Primer[a]	Oligo sequence (5′ to 3′)[b]	Purpose[c]
Mutagenesis		
Pr1097	gagatctagaaccgtttgatttttaatggataatg	Janus cassette For
Pr1098	gagactcgagcctttccttatgcttttggac	Janus cassette Rev
Pr1836	gagagtcgacaccgtttgatttttaatggataatg	Janus cassette For
Pr1837	gagaccatggcctttccttatgcttttggac	Janus cassette Rev
Pr2841	actacatcacaatggctgcc	*pdxT* upstream For
Pr2842	aaatctagaccgattttcatctattttcc	*pdxT* upstream Rev
Pr2843	aaactcgagttgagattgaatttctcaac	*pdxT* downstream For
Pr2844	accaggttttgctctatgtc	*pdxT* downstream Rev
Pr2845	gaagcctttgaacgtcttgg	*pdxS* upstream For
Pr2846	aaatctagacaccacccttgagcatctg	*pdxS* upstream Rev
Pr2847	aaactcgagtattaatgaaaatgaaatcc	*pdxS* downstream For
Pr2848	tggcctatggttcgacccac	*pdxS* downstream Rev
Pr2919	gcggatgatcttgcaagagggcaat	*pdxR* upstream For
Pr2920	cgcggtcgacacggatagaaggcaggcgactac	*pdxR* upstream Rev
Pr2921	cgcgccatggcacagtcaactgggcttgga	*pdxR* downstream For
Pr2922	aacacttgacaagtaacttggatat	*pdxR* downstream Rev
Complementation		
Pr2967	ttttgtcgacaactacatcacaatggctgc	*pdxS* promoter and ORF For
Pr2838	cggggatccgttctgcaaaggccccttgc	*pdxS* promoter and ORF Rev
Pr2968	ttttgtcgacataaaggccagccatcaagg	*pdxR* promoter and ORF For
Pr2969	cgcggatccgcagagttggatgtcattcc	*pdxR* promoter and ORF Rev
Overexpression		
Pr2862	cgcggctagcatgactgaaaatcgttatga	*pdxS* ORF For
Pr2863	cgcgggatccttgcaaggccaatattcc	*pdxS* ORF Rev
Pr2864	cgcgcatatgaaaatcggaatattggc	*pdxT* ORF For
Pr2865	cgcgggatccaatacatcgctattgttta	*pdxT* ORF Rev
Pr2917	cgcgccatggagaaacaaagcaagtacaaagag	*pdxR* ORF For
Pr2918	cgcgaagctttccaattctgcttttaaatagtttaaaaca	*pdxR* ORF Rev
Heterogeneous expression in *E. coli* mutant		
JY161	cgcaatattaaaagttaacccttcgaccc	*E. coli crp* promoter For
JY170	cgggctagcggtacctctagacatgcgcggttatcctctg	*E. coli crp* promoter Rev
Pr2835	cgggaattcatgaaaatcggaatattggc	*pdxT* ORF For
Pr2854	cgggctagcaagttgagaaattcaatctc	*pdxT* ORF Rev
Pr2855	cggtctagaatgactgaaaatcgttatg	*pdxS* ORF For
Pr2838	cggggatccgttctgcaaaggccccttgc	*pdxS* ORF Rev
Northern blot		
Pr2926	cttagccattactggaatactaac	*pdxS* probe
Pr2782	actttccactctcacactcg	16S rRNA probe

[a] *pdxST* double mutant was generated with *pdxS* upstream primers and *pdxT* downstream primers.

[b] The restriction site is underlined if presents in an oligo.

[c] Two pairs of primers flanking the Janus cassette were used for combination of different restriction sites. For, forward primer; Rev, reverse primer.

FIGURE LEGENDS

Figure S1. Western blot analyses to verify the deletion of *pdxT* and *pdxS*. WT (D39) and its derivatives, including Δ*pdxT* (ST2675), Δ*pdxS* (ST2676) and Δ*pdxST* (ST2677), were initially grown in complete CDM and then incubated for 4 h in depleted CDM. The protein samples were analyzed with the indicated antibodies (right side). Blotting with antibody against SPD_1063 was served as a loading control.

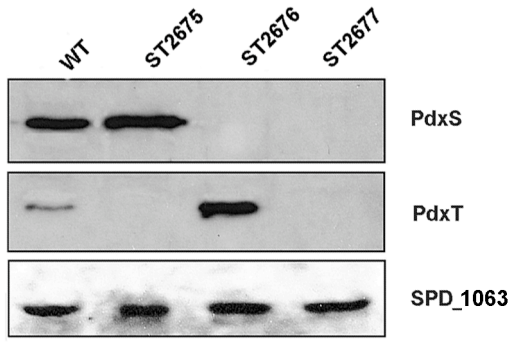
Figure S2. Growth of bacterial strains on M9 plates in the presence or absence of 0.1 mM PLP. ec022 is the *E. coli* WT strain. ec048 and ec053 are *E. coli* Δ*pdxA* and Δ*pdxJ*, respectively. ST2688 and ST2689 express pneumococcal *pdxS* in Δ*pdxA* and Δ*pdxJ*, respectively. Strains ec061 and ec065 contain the control vector in Δ*pdxA* and Δ*pdxJ*, respectively. Images of plates were taken after incubation for 18 h at 37 ºC

Figure S3. (A) Putative GntR family proteins in *S. pneumoniae*. All these proteins have a wHTH motif as a signature of GntR family proteins. Three proteins have an UbiC transcription regulator-associated (UTRA) domain. SPD_1225 has an AAT domain. (B) Verification of deletion and complementation of *pdxS* and *pdxR*, respectively, using PCR. Internal primers of *pdxR* and *pdxS* were used for PCR. Bacterial DNA samples isolated from the indicated strains, including WT (D39), ST2726 (Δ*pdxR*), ST2786 (Δ*pdxR*/*pdxR*+), ST2787 (Δ*pdxR*/vector control), ST2676 (Δ*pdxS*), ST2784 (Δ*pdxS*/*pdxS*+) and ST2785 (Δ*pdxS*/vector control), were used as templates.

Figure S4. Growth curve of ST2676 (A) and ST2726 (B) in complete CDM. The bacterial strains tested include WT (D39), ST2676 (Δ*pdxS*), ST2784 (Δ*pdxS*/*pdxS*+) and ST2785 (Δ*pdxS*/vector control), ST2726 (Δ*pdxR*), ST2786 (Δ*pdxR*/*pdxR*+), ST2787 (Δ*pdxR*/vector

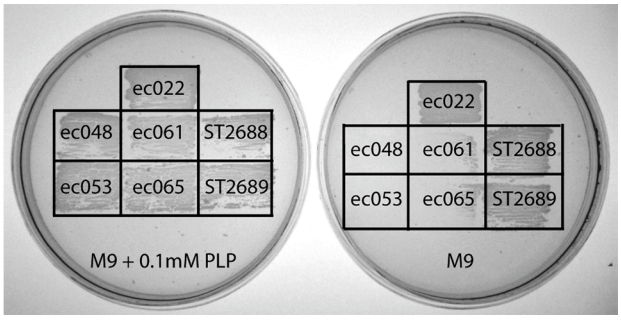
28 control). The bacterial growth was monitored hourly at OD_{620}. Data shown are the means of
29 three repeat experiments. Error bars denote SEM.
30

30 FIG. S1



31

32 FIG. S2



33

34 FIG. S3

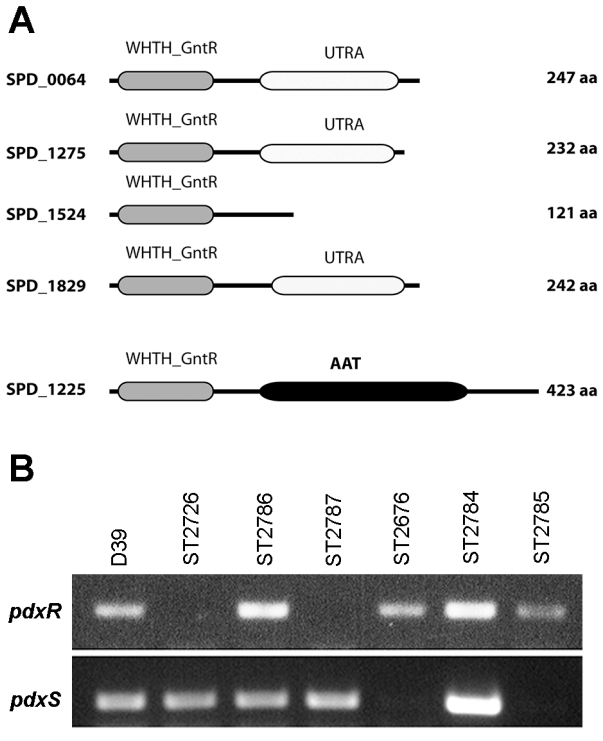


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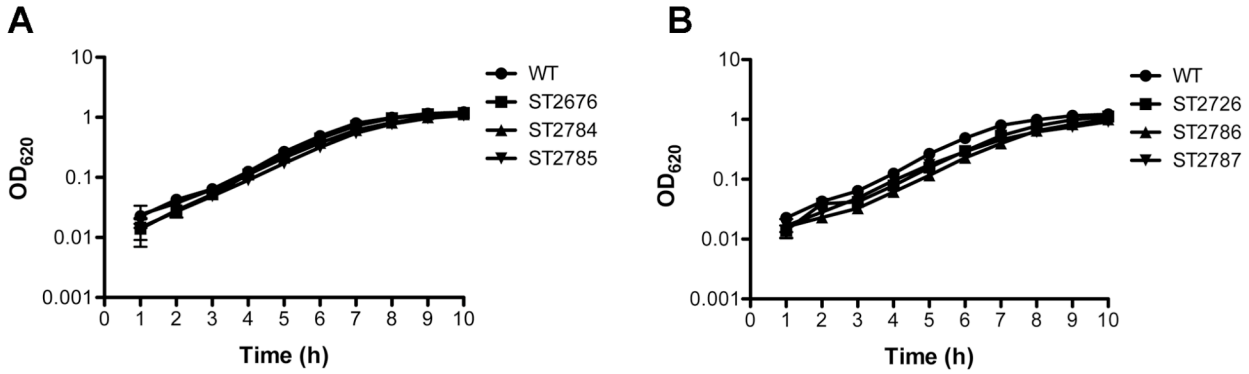
FIG. S4


A
B
WT
ST2676
ST2784
ST2785
ST2726
ST2786
ST2787
OD620
Time (h)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10