Supplemental Data.

Materials and Methods:

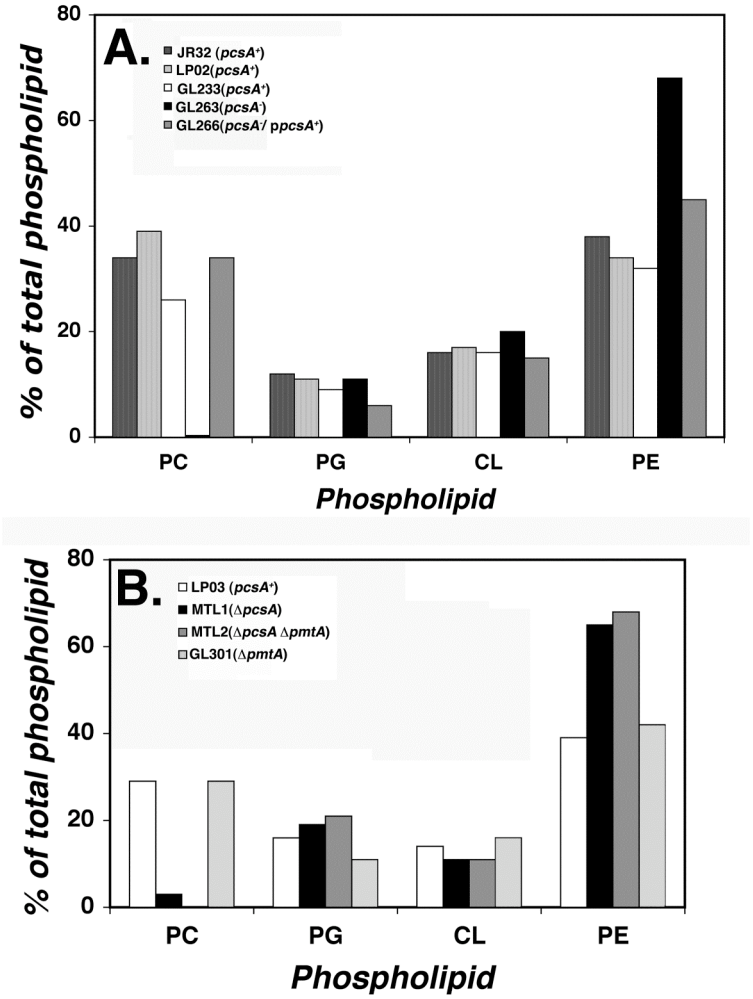
To construct the Δ*pmtA* strain, the 5' fragment 3 was amplified with primers GP219 (TTGCGCGGATCCTACTCCATTTAGAAAGACAAAACG) and GP220 (GTACGCGTCGACCTGTCTGATTTGGATAAATTAAGC). Fragment 5 was a 1209 bp *Sal*I-*Bam*HI fragment spanning the region starting 1191 bp upstream from the ATG and 18 nucleotides downstream from the start codon. The 3' fragment 6 was amplified with GP221 (TTGCGCGGATCCCATCCCCTTTACTTTATGCGGTTG) and GP222 (GAACCCGAGCTCAAAGAGGAAGAGGCTGATTTAA). Fragment 6 yielded a 1011 bp *Bam*HI-*Sac*I product spanning the region starting 12 nucleotides upstream of the stop codon of *pmtA* to position 999 bp downstream from it. To verify the Δ*pmtA* deletion mutant genotype, the primer pairs GP220 and GP222, as well as GP223 (TTTCTAAATGGAGTATTGGTTATG and GP224 (CCTTATCTTTATTTTTGTA AAGCTTCA) were used.

All generated PCR fragments were purified with QIAquick PCR kit (Qiagen, Valencia, CA) before they were digested with the appropriate enzymes. The vector pSR47s was digested overnight with *Sac*I and subsequently purified using the QIAprep spin columns (Qiagen, Valencia, CA), prior to a 4-hour digestion with *Sal*I. The vector and inserts were purified by electrophoresis in low melting temperature agarose (NuSieve GTG agarose; BMA; Rockland, ME) and extracted using QIAquick gel extraction kit (Qiagen, Valencia, CA) before overnight ligation at 16˚C. Ligations were dialyzed in 0.2 μm filters for one hour prior to electroporation into DH5αλ*pir*).

To amplify *pcsA* primer pairs GP174 (GTCAACGAGCTCTGATTAA CTCTAGGATAA) and GP175 (TTTCCCCCCGGGAACTCAATCTTTATTATTTGCCGT)

were used. The complementation plasmid pGC41 *(dotA$^+$ pcsA$^+$)* was introduced into *L. pneumophila* strains by triparental mating, for further experimentation.

FIGURE



Supplemental Fig. 1. Phosphatidylcholine levels are similar in different *L. pneumophila* strain backgrounds. Experiment was performed as in Fig. 1, assaying strains having both wild type levels of DotA (LP02 and JR32) and lacking expression of DotA (LP03). Displayed are relative percentages of phospholipids in various *L. pneumophila* strains having either an *pcsA*$^{+}$ gene or the *pcsA*$^{-}$ mutation. (A). JR32 has levels of PC that are similar to the LP02 *dot*$^{+}$ strain background. (B) The presence of Dot/Icm has no affect on PC content. Shown are derivatives of LP03 (*dotA*$^{-}$) assayed for

phospholipids content as above. PC: phosphatidylcholine. PE: phosphatidylethanolamine. PG: phosphatidylglycerol. CL: cardiolipin.