SUPPLEMENTAL INFORMATION

1,2-diacylglycerol choline phosphotransferase catalyzes the final step in the unique *Treponema denticola* phosphatidylcholine biosynthesis pathway

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Running title: *CDP-choline pathway for phosphatidylcholine synthesis in bacteria*

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TABLE S1. Strains and plasmids used in this study

Organism	Strain	Relevant features	Source/Reference
T. denticola	35405	parent strain	ATCC (Chan *et al.*, 1993)
T. denticola	LBE3	35405Δ*licCA*	(Kent *et al.*, 2004)
T. denticola	CF819	35405ΔTDE0021	this study
E. coli	DH5α	cloning strain	(Hanahan, 1983)
E. coli	JM109	cloning strain	(Yanisch-Perron *et al.*, 1985)
E. coli	JM110	cloning strain	(Yanisch-Perron *et al.*, 1985)
E. coli	BL21(DE3)/pLysS	expression strain	(Studier, 1991)
S. cerevisiae	HJ091	α *ura3-52 his3-1 leu 2-3, 112 trp1-289 cpt1::LEU2 ept1*	(McMaster *et al.*, 1996, Hjelmstad *et al.*, 1994)

Plasmid	Host	Relevant genes	Source/Reference
pCF737	*E. coli*	TDE0020-21-22	this study
pCF789	*E. coli*	TDE0020-*aphA2*-TDE0022	this study
pET17b-TDE0021	*E. coli*	TDE0021	this study
pCDF-Duet1-LicCA	*E. coli*	*T. denticola licCA*	this study
pYEp352	*S. cerevisiae*	empty vector	(Hill *et al.*, 1986)
pRH150	*S. cerevisiae*	CPT1 in pYEp352	(Hjelmstad & Bell, 1990)
pSP-Gm2	*S. cerevisiae*	empty vector	(Rodríguez-Limas *et al.*, 2011)
pSP-Gm2-Trepo	*S. cerevisiae*	TDE0021 in pSP-Gm2	this study

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Figure S1: TLC of the aqueous phase from the lipid extraction from the yeast complementation experiment (labeling with [^{14}C]choline).

Figure S2: TLCs of the organic and aqueous phases from the lipid extraction from the yeast complementation experiment (labeling with [^{14}C]ethanolamine).

Figure S3: SDS-PAGE of extracts from *E. coli* BL21(DE3).pLysS expressing TDE0021 and/or LicCA.

Figure S4: Positive ion collision-induced dissociation mass spectra of ion m/z 706.5 detected in the lipid extract from *E. coli* BL21(DE3).pLysS expressing LicCA and TDE0021.

Figure S5: pH dependency of CDP-choline phosphotransferase activity of Cpt (TDE0021) from *Treponema denticola*

Figure S1. TLC separation of the aqueous phases from the lipid extraction from the yeast complementation experiment (labeling with [^{14}C]choline). *S.cerevisiae* HJ091 cells harboring pRH150 (yeast CPT1, lane 1), pSP-Gm2-Trepo (TDE0021, lane 2), empty plasmid pSP-GM2 (lane 3), or empty plasmid pYEp352 (lane 4) were labeled for 30 min with [^{14}C]-choline. Aqueous phases obtained during lipid extraction were separated by one-dimensional TLC. Identities of [^{14}C]-choline-containing species are assigned according to the expected Rf values. An unknown compound in the yeast CPT1-complemented strain, possibly glycerophosphocholine, is labeled "?" (Lane 1).

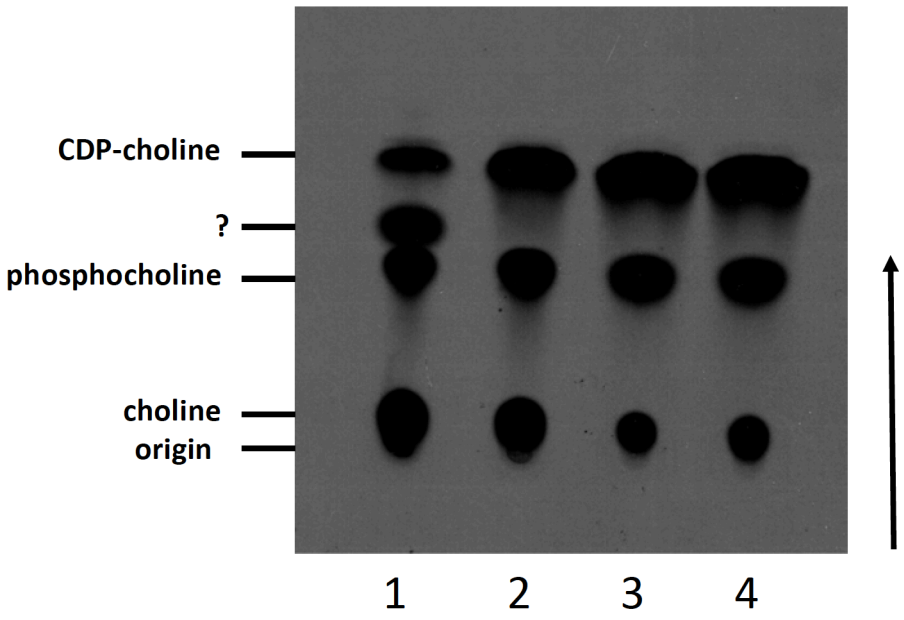

Figure S2. TLCs of the organic and aqueous phases from the yeast complementation experiment (labeling with [14C] ethanolamine). *S. cerevisiae* HJ091 cells harboring empty plasmid pYEp352 (lane 1), empty plasmid pSP-GM2 (lane 2), pSP-Gm2-Trepo (TDE0021, lane 3), or pYEp352-CPT1 (yeast CPT1, lane 4) were labeled for 30 min with [^{14}C]-ethanolamine. Lipids were extracted according to Bligh and Dyer and organic (A) and aqueous (B) phases obtained during lipid extraction were separated by one-dimensional TLC. PE-[^{14}C] phosphatidylethanolamine standard isolated from an *E. coli* strain cultivated in presence of [^{14}C] acetate.

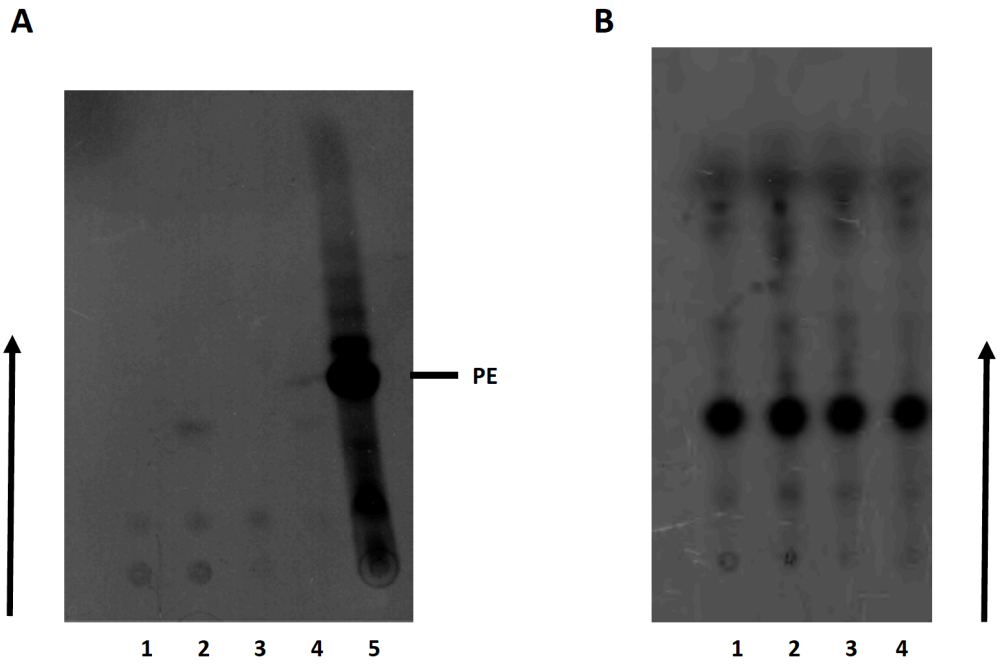

Figure S3. SDS-PAGE of extracts from *E. coli* BL21(DE3).pLysS expressing TDE0021 and/or LicCA. Analysis of cell-free protein extracts and purified membrane fractions from *E.coli* strains. Proteins were separated by 12% SDS-PAGE and stained with Coomassie blue. Cell-free proteins extracts from *E.coli* BL21(DE3).pLysS harboring pCDF-Duet and pET17b (lane1), pCDF-Duet-LicCA and pET17b (lane2) ,pCDF-Duet and pET17b-TDE0021 (lane3), pCDF-DuetLicCA and pET17b-TDE0021 (lane4). Resuspended membrane fractions from *E.coli* BL21(DE3).pLysS harboring pCDF-Duet and pET17b (lane5), pCDF-Duet-LicCA and pET17b (lane6), pCDF-Duet and pET17b-TDE0021 (lane7), pCDF-DuetLicCA and pET17b-TDE0021 (lane8). 5 micrograms of the membrane fractions were run on the gel (lanes 5 to 8). The molecular weight marker used was from Bio-Rad (#0161-0363).

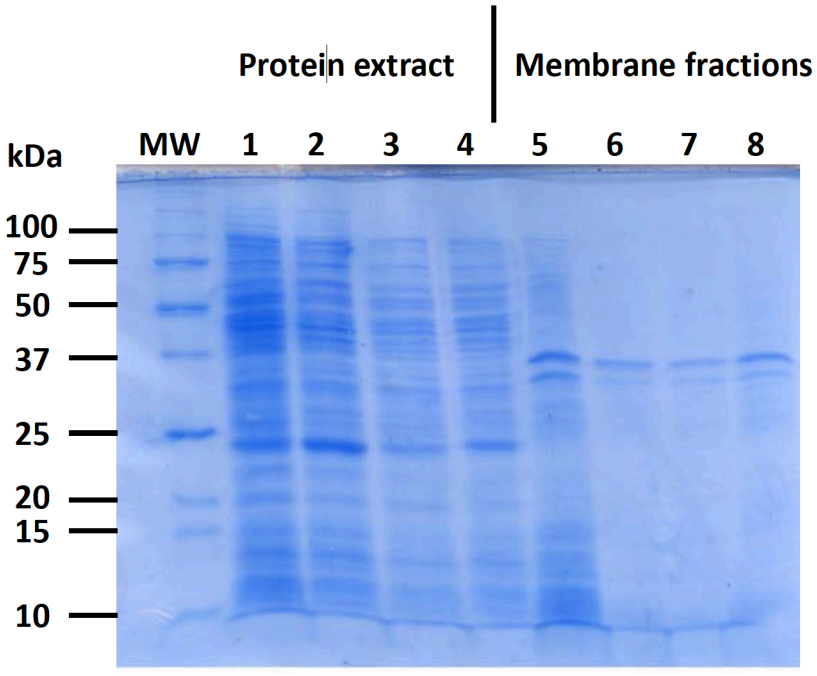

Figure S4. Positive ion collision-induced dissociation mass spectra of ion m/z 706.5 detected in the lipid extract from *E. coli* BL21(DE3).pLysS expressing LicCA and TDE0021.The presence of m/z 184.073 confirms the identity of phosphatidylcholine.

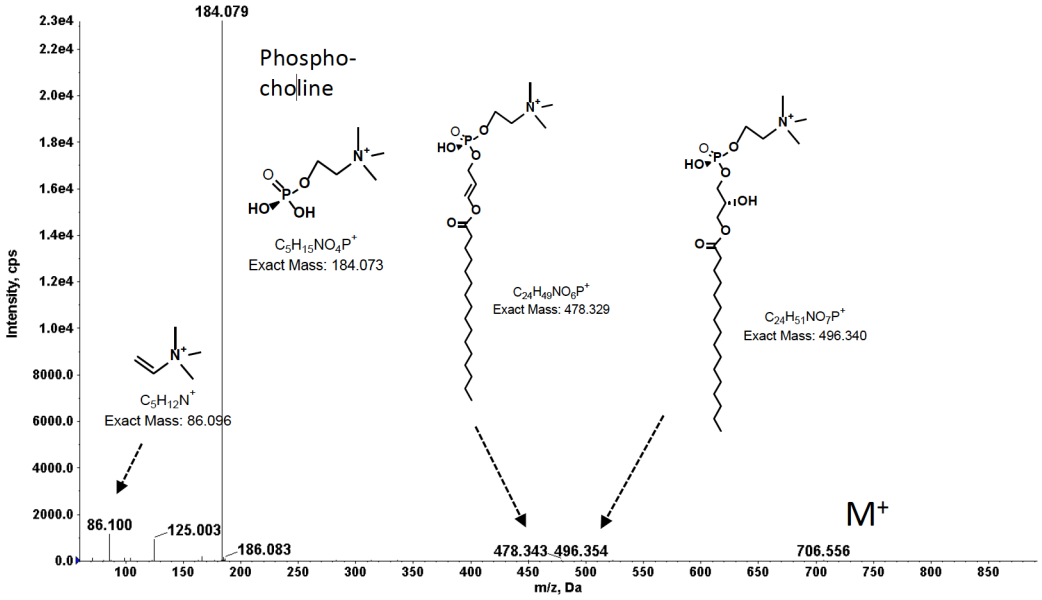

Figure S5. pH dependency of *Treponema denticola* CDP-choline phosphotransferase (TDE0021). TDE0021 activity was assayed at the indicated pH values with 50 mM Bis-Tris/HCl or Tris/HCl. Otherwise, conditions of the standard assay were used. The data points shown are the average of two independent experiments.