Supplemental Material

Figure Legends

Figure S1. The bile-induced phospholipid changes occur in a variety of environments. When grown in minimal media in the presence of bile, *V. cholerae* produces additional phosphate-containing lipids observed below and above lyso-PE. When grown with bile under microaerophilic conditions, *E. coli* slightly alters its phospholipid content (increase in CL, decrease in PG), whereas the response in *V. cholerae* is akin to aerobic conditions. However, the phospholipid profile of *V. cholerae* grown anaerobically is devoid of PG regardless of the presence of bile, while changes in the other phospholipids resembles other conditions.

Figure S2. The effects of bile on *V. cholerae* phospholipids occurs despite translational inhibition and chemical inhibition of fatty acid biosynthesis. Exposure of *V. cholerae* to bile after chloramphenicol-induced inhibition of protein synthesis still resulted in the altered phospholipid profile associated with bile. Despite chemical inhibition of bacterial fatty acid biosynthesis with cerulenin, *V. cholerae* still undergoes changes in its phospholipid profile.

Figure S3. The bile-induced phospholipid profile is not dependent upon the *V. cholerae* virulence cascade.

A. Deletion of *toxR* had no effect on the phospholipid profile of *V. cholerae* when exposed to bile.

B. Chemical inhibition of ToxT with the small molecule virstatin had no effect on the phospholipid profile of *V. cholerae* when exposed to bile.

Figure S4. Phospholipid profiles of *E. coli* (A) and *V. cholerae* (B) following exposure to the major individual components of bile. *E. coli* exhibits essentially no alteration to its phospholipid profile when grown in LB containing individual major bile acids, lecithin or bilirubin. In *V. cholerae*, chenodeoxycholic acid and deoxycholic acid induce an increase in cardiolipin and decrease in phosphatidylglycerol. None of the individual components account for the stark migratory shift in cardiolipin or the increase in the unknown phospholipid species (denoted by ?). Bile, 0.4%; CDCA, chenodeoxycholic acid; CA, cholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; TCA, taurocholic acid; GCA, glycocholic acid; Lec, lecithin.

Figure S5. Mass spectrometric analysis reveals identical spectra for PG (A) and PE (B) from *E. coli* grown in the presence or absence of bile.

Figure S6. Mass spectrometric analysis of CL extracted from *V. cholerae* grown in the presence and absence of sediment.

A. Negative-ion ESI-MS of $[M-2H]^{2-}$ ions of *V. cholerae* CL isolated from cultures grown in the presence and absence of sediment. Selected peaks (*) found only in the presence of bile were subsequently analyzed by MS/MS.

B. Negative-ion MS/MS analysis of the product-ion spectra of the $[M-2H]^{2-}$ ions at m/z 706.50 and 713.47 detected only in the presence of bile. The $[M-H]^-$ species contained monounsaturated fatty acids, including C17:1, C19:1 and C20:1.

Table S1. Bacterial strains used in this study

Strain	Description	Source or Reference
Escherichia coli		
W3110	Wild type K-12, F-,λ-	*E. coli* Genetic Stock Center (Yale University)
ETEC	Wild type	ATCC
EHEC	Wild type	ATCC
Salmonella typhimurium		
BC155 LT2	Wild type	Brad T. Cookson (University of Washington)
Vibrio cholerae		
O395	Classical biotype, Ogawa	Shelley M. Payne (University of Texas at Austin)
O139	Clinical isolate: 19641/96 O139	Shelley M. Payne (University of Texas at Austin)
E7946	Clinical biotype: El Tor, O1 tox+	Karl E. Klose (University of Texas-San Antonio)
KKV366	E7946 Δ*toxR*	Provenzano et al., 2000
Vibrio alginolyticus Z096	Clinical isolate	ETSU[a] Clinical Microbiology Laboratory
Vibrio fischeri ES114	Wild type	Boettcher and Ruby, 1990
Vibrio parahaemolyticus	Clinical isolate	ETSU[a] Clinical Microbiology Laboratory
Vibrio vulnificus CAP-D-08	Clinical isolate	ETSU[a] Clinical Microbiology Laboratory

[a]ETSU, East Tennessee State University

Table S2

	No Bile[a]	0.4% Bile[a]
***E. coli* K-12 (W3110)[b]**		
Cardiolipin	7.5 ± 1.2	7.8 ± 1.2
Phosphatidylglycerol	11.2 ± 1.5	10.5 ± 0.8
Phosphatidylethanolamine	81.2 ± 1.2	82.0 ± 1.3
***S. enterica* serovar Typhimurium**		
Cardiolipin	6.7 ± 0.6	7.7 ± 0.6
Phosphatidylglycerol	14.3 ± 1.5	14.0 ± 1.5
Phosphatidylethanolamine	79.0 ± 1.7	78.3 ± 2.0
***V. cholerae* El Tor (E7946)**		
Cardiolipin	4.0 ± 1.2	8.7 ± 2.1
Phosphatidylglycerol	10.1 ± 3.2	5.0 ± 2.2
Phosphatidylethanolamine	83.9 ± 4.2	56.9 ± 5.5
Lyso-phosphatidylethanolamine	1.9 ± 2.3	29.6 ± 6.1

[a]The data shown is the average of seven biological replicates shown in Fig. 2.
[b]Similar data was obtained for ETEC and EHEC.

Table S3

	Percent Bile[b]				
Phospholipid	**No Bile**	**0.01%**	**0.1%**	**0.2%**	**0.4%**
Cardiolipin	4.3 ± 0.6	4.7 ± 0.6	6.3 ± 0.6	7.7 ± 0.6	8.7 ± 0.6
Phosphatidylglycerol	9.7 ± 0.6	11.3 ± 0.6	10.0 ± 2.0	7.3 ± 0.6	4.7 ± 0.6
Phosphatidylethanolamine	85.0 ± 0.0	81.7 ± 1.5	69.7 ± 1.5	62.7 ± 2.5	58.0 ± 1.7
Lyso-phosphatidylethanolamine	1.0 ± 0.0	2.3 ± 0.6	14.0 ± 2.6	22.3 ± 2.5	28.7 ± 1.5

[a]The data shown is the average of three biological replicates including that shown in Fig. 3A.

	Time[b]				
Phospholipid	**0m**	**15m**	**30m**	**1h**	**2h**
Cardiolipin	4.0 ± 0.0	7.0 ± 3.5	7.0 ± 2.6	9.3 ± 1.5	10.0 ± 1.7
Phosphatidylglycerol	15.0 ± 5.0	3.3 ± 2.3	3.3 ± 3.2	3.0 ± 2.6	4.0 ± 2.6
Phosphatidylethanolamine	79.7 ± 5.5	50.7 ± 2.1	51.7 ± 3.2	49.7 ± 11.0	50.0 ± 4.0
Lyso-phosphatidylethanolamine	1.3 ± 0.6	39.0 ± 5.6	36.3 ± 5.5	33.0 ± 5.2	33.7 ± 7.8

[b]The data shown is the average of three biological replicates including that shown in Fig. 3B.

Supplemental References

Boettcher, K. J. & E. G. Ruby, (1990) Depressed light emission by symbiotic *Vibrio fischeri* of the sepiolid squid Euprymna scolopes. *J Bacteriol* **172**: 3701-3706.

Provenzano, D. & K. E. Klose, (2000) Altered expression of the ToxR-regulated porins OmpU and OmpT diminishes *Vibrio cholerae* bile resistance, virulence factor expression, and intestinal colonization. *Proc Natl Acad Sci U S A* **97**: 10220-10224.

Figure S1

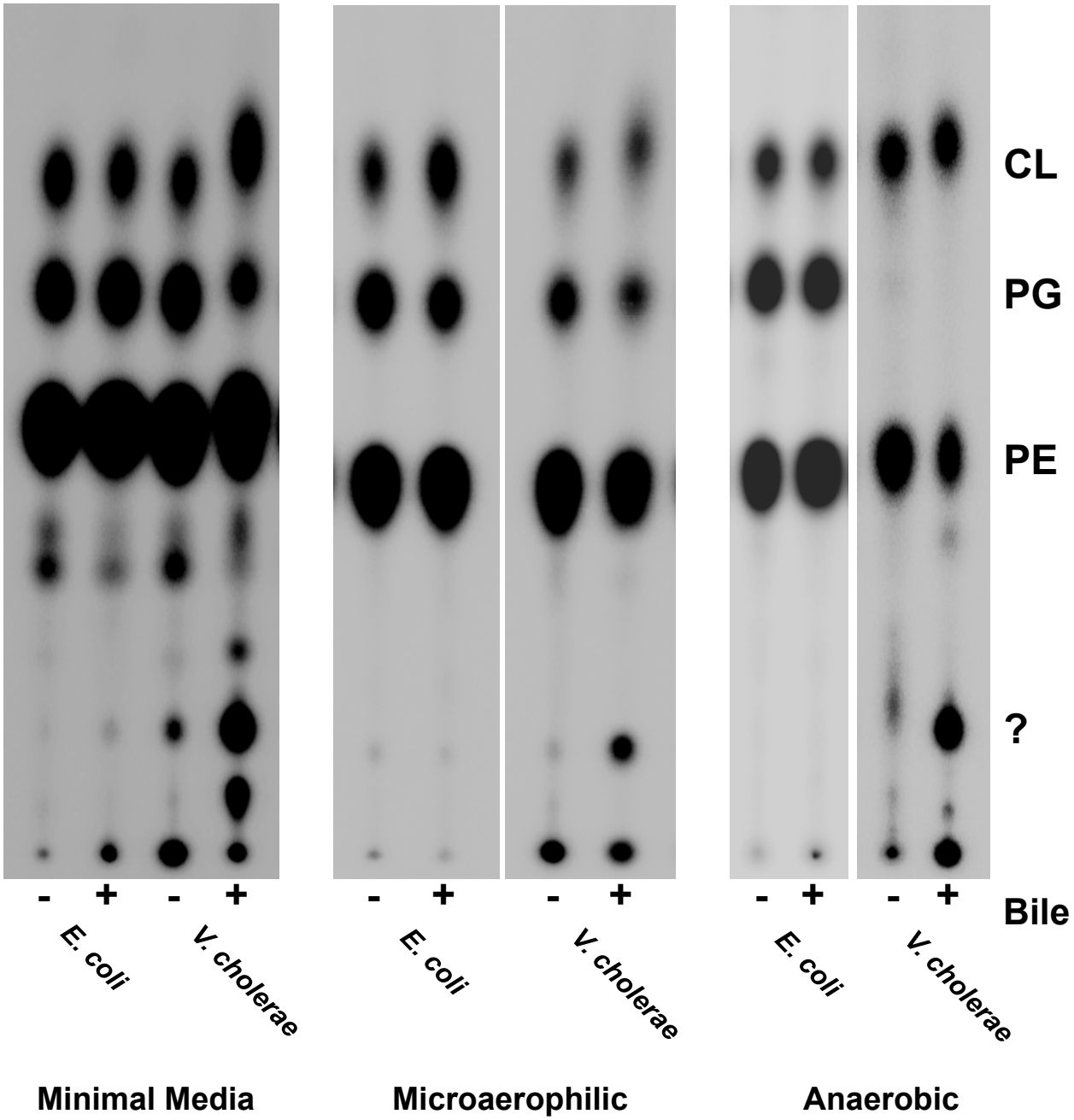

CL
PG
PE
?
- + - +
E. coli
V. cholerae
Bile
Minimal Media
Microaerophilic
Anaerobic

Figure S2

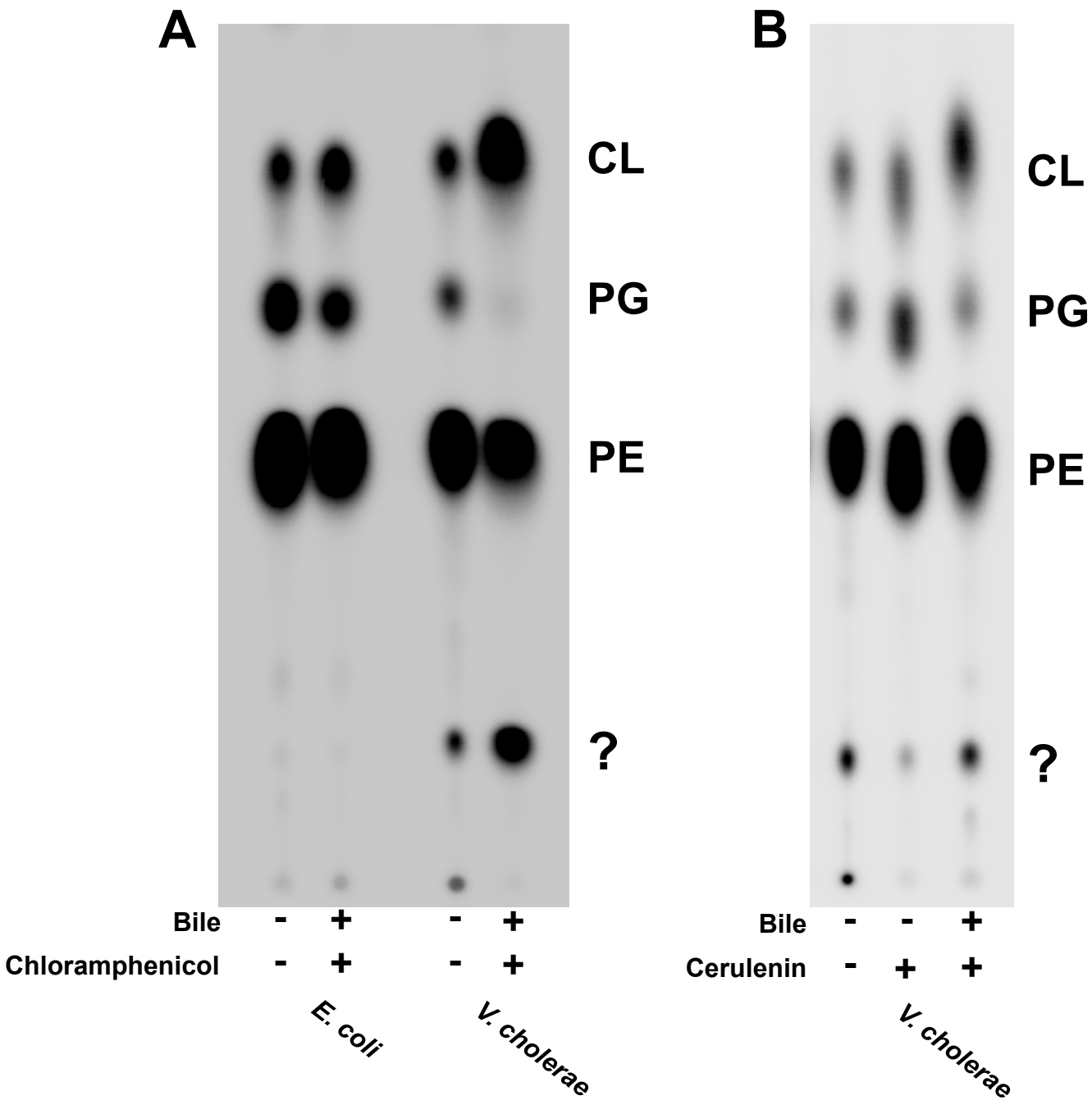

A
CL
PG
PE
?
Bile - + - +
Chloramphenicol - + - +
E. coli
V. cholerae
B
CL
PG
PE
?
Bile - - +
Cerulenin - + +
V. cholerae

Figure S3

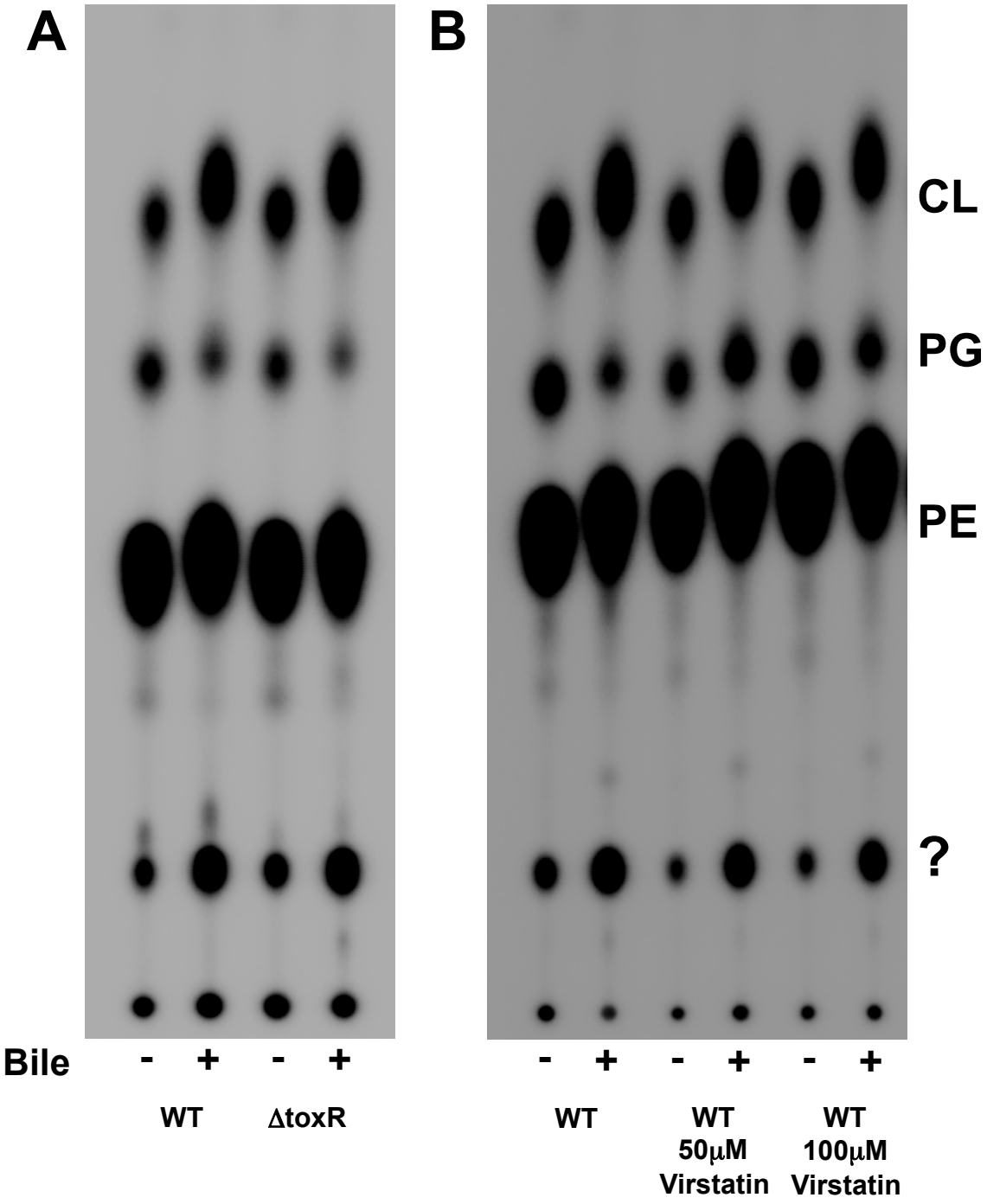

A
B
CL
PG
PE
?
Bile
- + - +
WT
ΔtoxR
- + - + - +
WT
WT
50μM
Virstatin
WT
100μM
Virstatin

Figure S4

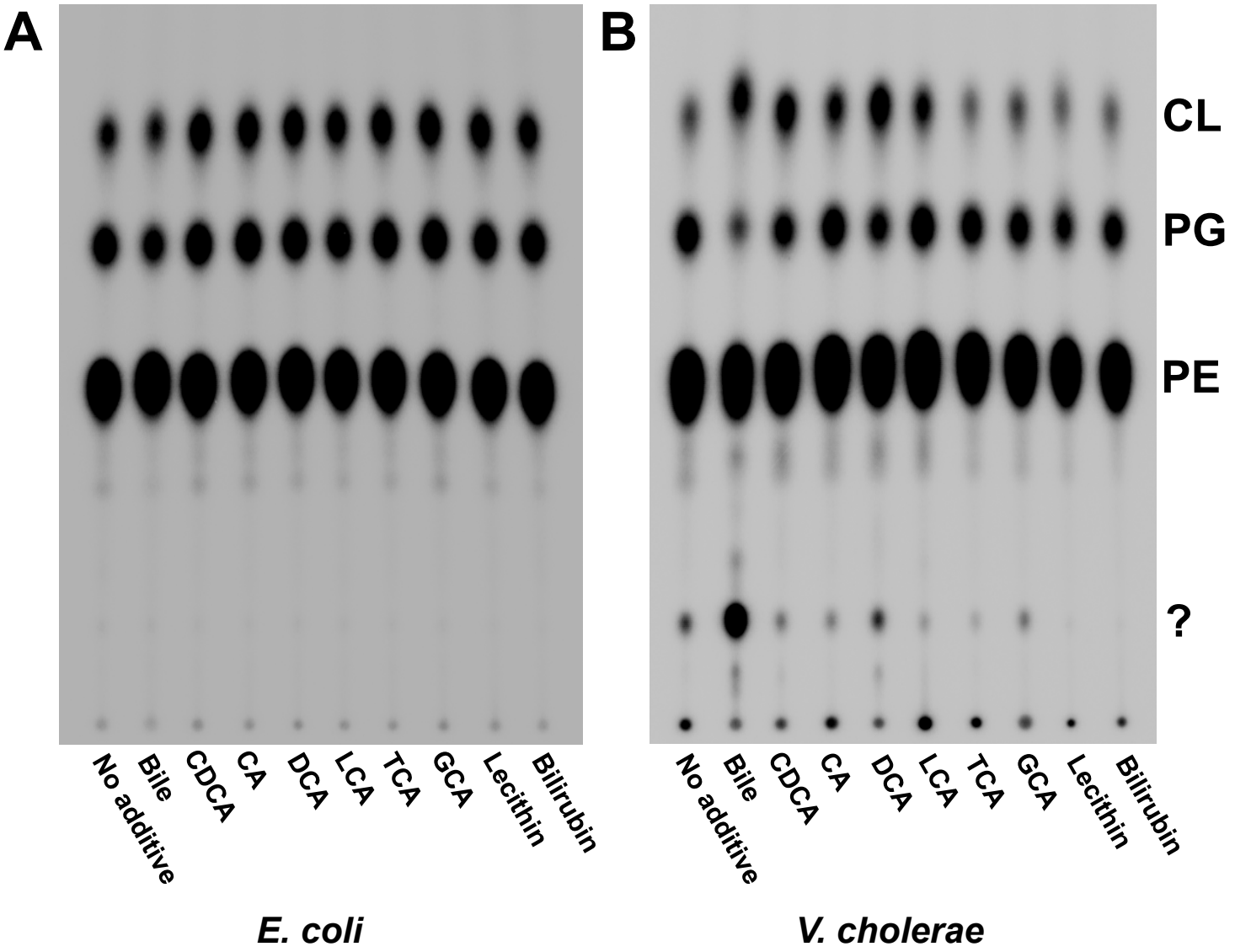

A
B
CL
PG
PE
?
No additive
Bile
CDCA
CA
DCA
LCA
TCA
GCA
Lecithin
Bilirubin
E. coli
V. cholerae

Figure S5

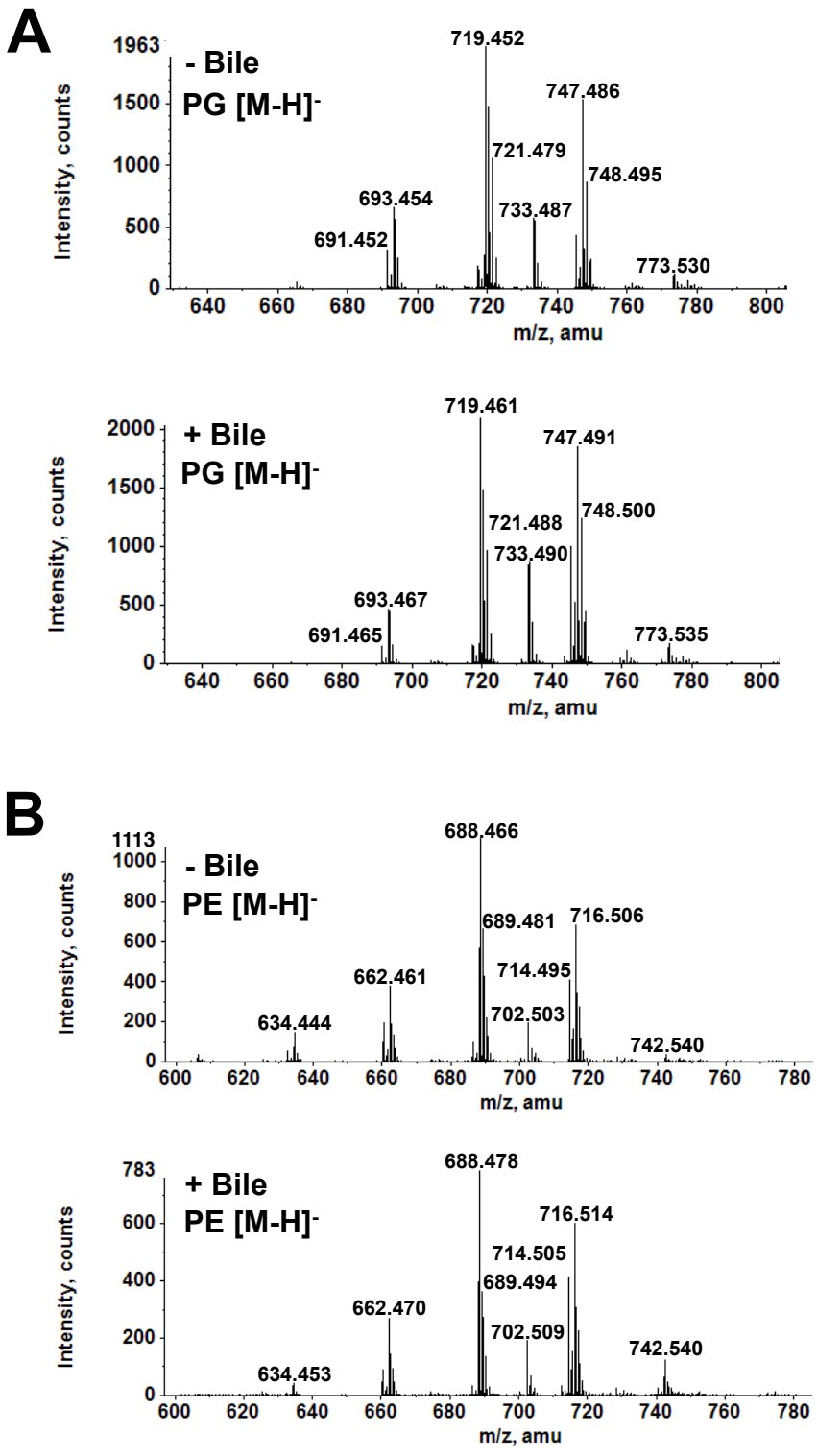
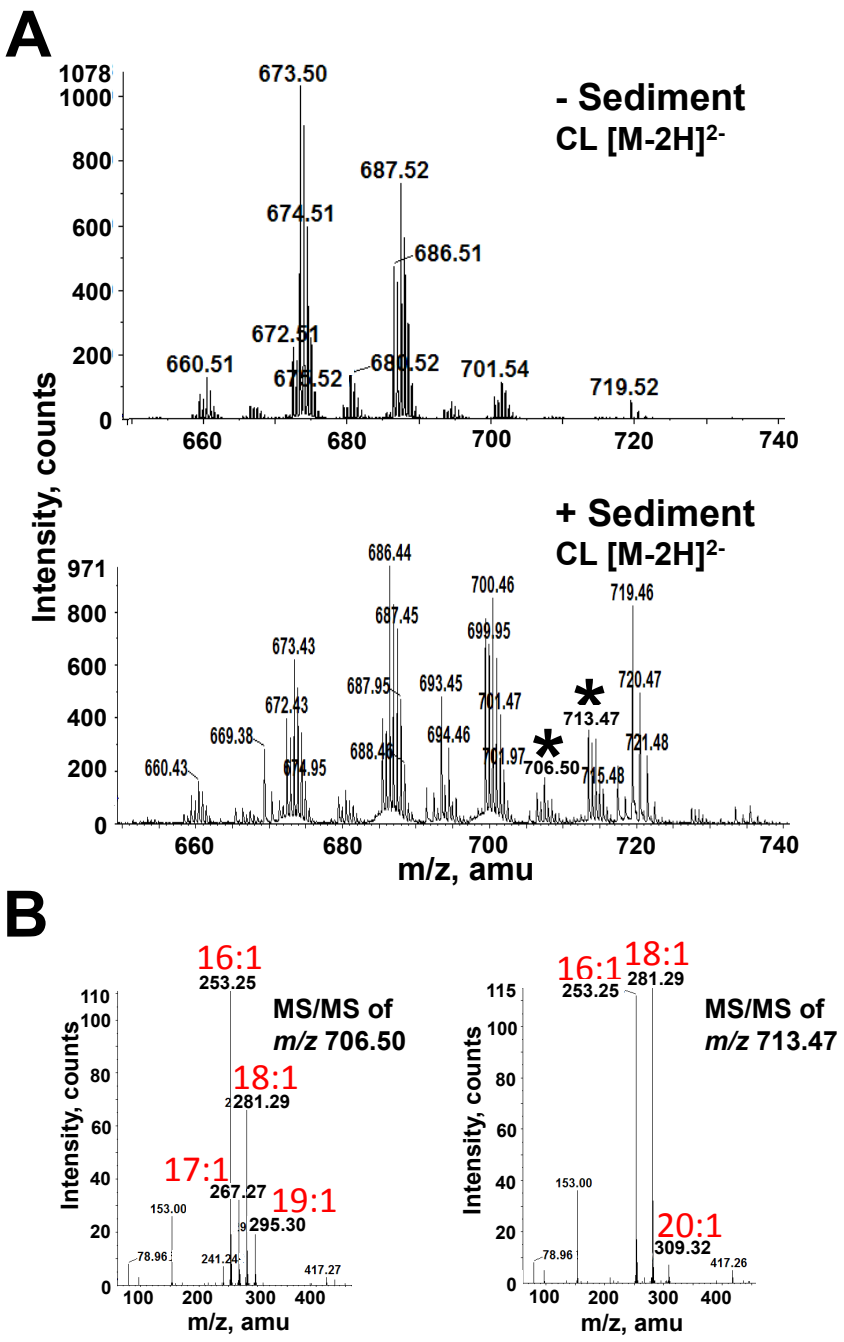

A
- Bile
PG [M-H]-
719.452
747.486
721.479
748.495
693.454
733.487
691.452
773.530
Intensity, counts
m/z, amu
+ Bile
PG [M-H]-
719.461
747.491
721.488
748.500
733.490
693.467
691.465
773.535
B
- Bile
PE [M-H]-
688.466
689.481
716.506
714.495
662.461
702.503
634.444
742.540
+ Bile
PE [M-H]-
688.478
716.514
714.505
689.494
662.470
702.509
634.453
742.540

Figure S6


A
- Sediment
CL [M-2H]2-
673.50
687.52
674.51
686.51
672.51
660.51
675.52
680.52
701.54
719.52
+ Sediment
CL [M-2H]2-
686.44
700.46
719.46
687.45
699.95
673.43
687.95
693.45
701.47
720.47
713.47
672.43
669.38
694.46
721.48
660.43
674.95
688.48
701.97
706.50
715.48
Intensity, counts
m/z, amu
B
16:1
253.25
MS/MS of
m/z 706.50
18:1
281.29
17:1
267.27
19:1
295.30
153.00
78.96
241.24
417.27
16:1
253.25
18:1
281.29
MS/MS of
m/z 713.47
153.00
20:1
309.32
78.96
417.26
Intensity, counts
m/z, amu