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1 **Manganese is essential for PlcP metallophosphoesterase activity involved in lipid**
2 **remodelling in abundant marine heterotrophic bacteria**

3

4 Running title: Biochemical characterization of PlcP

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6

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23

24

25

26 **Abstract**

27

28 In vast areas of the ocean, microbes must adapt to the availability of scarce nutrients and a
29 key strategy for reducing the cellular phosphorus (P) quota is to remodel membranes by
30 replacing phospholipids with non-P surrogate lipids. A metallophosphoesterase, PlcP, is
31 essential for lipid remodelling in cosmopolitan marine bacteria of the *Roseobacter* (e.g.
32 *Phaeobacter* sp. MED193) and SAR11 clades (e.g. *Pelagibacter* sp. HTCC7211) and
33 transcription of *plcP* is known to be induced by P limitation. In order to better understand
34 PlcP-mediated lipid remodelling, we sought to characterise PlcP for its metal ion requirement
35 and to determine its selectivity for native bacterial phospholipids. Here, we report the
36 occurrence of a highly conserved binuclear ion centre in PlcPs from MED193 and
37 HTCC7211 and show that manganese is the preferred metal for metallophosphoesterase
38 activity. PlcP displayed high activity towards the major bacterial phospholipids, *e.g.*
39 phosphatidylglycerol but also phosphatidic acid, a key intermediate in phospholipid
40 biosynthesis. In contrast, phosphatidylserine and phosphatidylinositol, both of which are rare
41 lipids in bacteria, are not preferred substrates. This data suggests that PlcP undertakes a
42 generic lipid remodelling role during the cellular response of marine bacteria to P deficiency
43 and that manganese availability may play a key role in regulating the lipid remodelling
44 process.

45

46

47 **Importance**

48

49 Membrane lipids form the structural basis of all cells. In the marine environment, it is well
50 established that phosphorus availability significantly affects lipid composition in cosmopolitan
51 marine bacteria, whereby non-phosphorus lipids are used to substitute phospholipids in
52 response to phosphorus stress. Central to this lipid remodelling pathway is a newly identified
53 phospholipase C type metallophosphoesterase (PlcP). However, little is known about how

54 PlcP activity is regulated. Here, we determined the role of metal ions in regulating PlcP
55 activity and compared PlcP substrate specificities in PlcP enzymes from two model marine
56 bacteria, the marine Roseobacter clade and the SAR11 clade. Our data provides new
57 insights into the regulation of lipid remodelling in these marine bacteria.

58

59

60 Keywords: PlcP, lipid remodelling, Roseobacter, SAR11

Introduction

Large expanses of the ocean, particularly surface waters, contain submicromolar concentrations of essential nutrients required for the growth of phytoplankton and heterotrophic bacteria (2, 18), including macronutrients (*e.g.* P) as well as micronutrients (*e.g.* iron, manganese). Marine bacteria inhabiting these oligotrophic surface waters have developed sophisticated strategies to meet cellular demands for these essential elements (16). For example, many marine microbes express high affinity membrane transporters *e.g.* the ABC transporter PstSCAB for phosphate or SitABCD for manganese uptake in order to acquire specific nutrients present at low concentration (9, 24). Other marine microbes such as the diazotrophic marine cyanobacterium *Crocosphaera watsonii* can degrade iron-rich metalloproteins to release and recycle iron during limitation (23).

Another important mechanism for adapting to nutrient deficiency is to reduce the cellular requirement for key elements (16). This strategy is now well established in marine phytoplankton and heterotrophic bacteria, whereby membrane phospholipids are replaced by non-P containing surrogate lipids in response to P deficiency (3, 26, 28). In marine phytoplankton, substitution of phospholipids by the sulfur-containing glycolipid, sulfoquinovosyl diacylglycerol (SQDG) significantly reduced the cellular quota for P (28). Our previous work has shown that lipid remodelling is also important in cosmopolitan marine heterotrophic bacteria (26). Members of the marine *Roseobacter* clade and SAR11 clade bacteria can substitute phospholipids, primarily phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) using non-P surrogate lipids such as the betaine lipid diacylglyceryl trimethylhomoserine (DGTS), the glycolipids monoglycosyl diacylglycerol (MGDG) and glucuronic acid diacylglycerol (GADG) and ornithine lipids. Central to this lipid remodelling process in these marine heterotrophic bacteria is a phospholipase C - type phospholipase, designated PlcP, which was first described in the soil bacterium *Sinorhizobium meliloti* (30). It is believed that during lipid remodelling phospholipids are

89 degraded by PlcP to diacylglycerol (DAG) which then acts as the precursor for the
90 biosynthesis of surrogate lipids in response to P limitation. Indeed, *plcP* deletion mutants no
91 longer synthesize surrogate non-phospholipids, supporting the essential role of PlcP in lipid
92 remodelling (26, 30).

93

94 Although PlcP-mediated lipid remodelling appears widespread amongst marine bacteria,
95 since the *plcP* gene has been found in diverse groups of marine heterotrophs including
96 Alphaproteobacteria, Gammaproteobacteria, Flavobacteria and Verrucomicrobia (26), little is
97 known about how PlcP activity is regulated. At the transcriptional level, in the marine
98 bacterium *Phaeobacter* sp. MED193 and the terrestrial bacterium *Sinorhizobium meliloti*, the
99 *plcP* gene is controlled by the two-component system PhoBR, with a Pho box, to which
100 PhoB binds, found upstream of these *plcP* genes (26, 30). The PlcP protein is annotated as
101 a member of the metallophosphoesterase superfamily (Pfam family PF00149, 15).
102 Sequence analyses have shown that PlcP (also known as LpxH2) has moderate sequence
103 similarity to a well-characterized member of PF00149, LpxH, an enzyme catalysing a key
104 step in lipid A biosynthesis in some bacteria (20, 30). Interestingly, recent structural analyses
105 of LpxH have uncovered a conserved binuclear manganese (Mn^{2+}) centre, suggesting that
106 manganese may be important for PlcP activity (5, 19).

107

108 In this study, we present a detailed characterization of the PlcP proteins from
109 representatives of the marine *Roseobacter* and SAR11 clades, *Phaeobacter* sp. MED193
110 and *Pelagibacter* sp. HTCC7211, respectively, both of which are known to employ PlcP for
111 lipid remodelling in response to P deficiency (3, 26). Specifically, we set out to determine the
112 role of metal ions in regulating PlcP activity and to compare PlcP substrate specificities in
113 PlcP enzymes from these two clades.

114 **Results and discussion**

115

116 **PlcP is a member of the metallophosphoesterase family**

117 Functional domain analysis of PlcP proteins from *Phaeobacter* sp. MED193 and
118 *Pelagibacter* sp. HTCC7211 revealed the presence of a highly conserved
119 histidine/aspartate/asparagine cage (**Figure 1A**), a signature sequence motif found in the
120 metallophosphoesterase family of proteins (PFam 00149). Subsequent phylogenetic
121 analysis (**Figure 1B**), comprising representative sequences from key members of the
122 metallophosphoesterase family including phosphodiesterases and pyrophosphatases,
123 showed the most closely related protein to PlcP in this family is LpxH, an enzyme catalysing
124 the formation of key intermediates in lipopolysaccharide biosynthesis. PlcP from
125 *Phaeobacter* sp. MED193 and *Pelagibacter* sp. HTCC7211 has 25% and 22% identity,
126 respectively, to LpxH from *Pseudomonas aeruginosa*. Crystal structures of LpxH from
127 *Pseudomonas aeruginosa* and *Haemophilus influenzae* have been solved recently, both of
128 which showed the presence of a binuclear Mn^{2+} centre in the active site (5, 19).

129

130 We then employed homology modelling to predict metal-binding sites in $PlcP_{193}$ (**Figure 2)**.
131 Modelling predicted a highly conserved Mn^{2+} coordination centre that was superimposable
132 onto that of the LpxH (PDB 5K8K) structure. Using the PDBeFold server we compared the
133 crystal structure of LpxH to our model. This returned a Q-score of 0.91, with 1 being
134 identical, and a root-mean-square deviation (RMSD, a measure of the average distance
135 between atoms) of 0.25, indicating some small differences between the structures in 222 of
136 the 227 residues compared. With a highly comparable structure, key metal-binding residues
137 of H12, D40, N81, H82, H117, D119, H201 and H203 are shown to be orientated similarly in
138 the two proteins. Likewise, potentially key differences are observed (**Figure 1 and SI video**)
139 in the ligand recognition/binding site between the LpxH crystal structure and the $PlcP_{193}$
140 model including His134/Asp136, Typ126/Ala128 and Arg158/Tyr160 respectively, which
141 could play an important role in substrate recognition. Although our modelling procedure is

142 limited to positioning amino acid residues and not metal-ion co-factors, given the highly
143 similar overlap of amino acid residues around the metal coordination centre we hypothesised
144 that Mn^{2+} metal cofactors would likely be coordinated similarly in $PlcP_{193}$ and so we have
145 shown these ions superimposed as such (**Figure 2B**).
146
147 To validate whether Mn^{2+} is indeed required for PlcP activity, we over-expressed and purified
148 the PlcP proteins from *Phaeobacter* sp. MED193 and *Pelagibacter* sp. HTCC7211 in *E. coli*
149 (**Figure 3A**). The isolated $PlcP_{193}$ and $PlcP_{7211}$ enzymes from recombinant *E. coli* had no
150 activity. However, when divalent metals were included in the enzyme assay buffer,
151 phosphoesterase activity was immediately restored (**Figure 3B**). Of all the metals tested in
152 this experiment at a range of concentrations (**Figure S1**), Mn^{2+} gives the highest activity,
153 followed by Zn^{2+} and Fe^{3+}. Our data agrees well with known metal requirements for other
154 characterized proteins within this family. For example, a binuclear Mn^{2+}-Mn^{2+} centre is
155 common in Mre11 and LpxH group enzymes, which are evolutionarily more closely related to
156 PlcP than others members of the family (**Figure 1B**). Zn and Fe, on the other hand, have
157 also been found in several proteins of this family, notably GpdQ glycerophosphodiesterase
158 (25). Moreover, our data supports the homology modelling prediction and shows that PlcP
159 from these marine bacteria is a Mn^{2+}-dependent phosphoesterase. Interestingly, both $PlcP_{193}$
160 and $PlcP_{7211}$ proteins had phosphodiesterase and phosphomonoesterase activities as
161 assessed using a general substrate containing either a phosphate monoester (PNPP) or a
162 phosphodiester bond (NPCC) (**Figure 3C**).
163
164 **Site-directed mutants and PlcP activity profile**
165 In order to identify key amino acid residues in the PlcP protein required for phosphoesterase
166 activity, and to further validate the predicted homology model (**Figure 2**), we constructed
167 site-directed mutants of $PlcP_{193}$ and compared their activities with wild type $PlcP_{193}$. The data
168 presented in **Figure 4** confirms the key role of the histidine/aspartate/arginine motifs in PlcP
169 activity. Site-directed mutants of H12, D40/D43, H117/H119, H201/H203 (highlighted in box

170 1, 2, 4 and 5 respectively in Figure 1A) to alanine almost completely abolished PlcP activity
171 (**Figure 4E**) supporting our assumption from the homology modelling that these residues are
172 integral to the metal binding site. Similarly, a histidine-to-alanine (H82A, box 3 in Figure 1A)
173 mutation completely abolished PlcP activity. Interestingly, the activity of H82N (53% of wild-
174 type $PlcP_{193}$) and H82R (82% of wild-type $PlcP_{193}$) mutants is largely retained but statistically
175 different to that of the wild type PlcP ($p<0.01$; $p<0.05$, respectively, student *t*-test),
176 suggesting that the presence of a protonated amine group in this position is important in
177 maintaining PlcP activity. The GNXD motif highlighted in box 3 (**Figure 1A**) includes an
178 interesting sequence variation in several enzymes of this family, including LpxH (arginine,
179 R), YfcE (cysteine, C) and MJ0936 (asparagine, N). Structural determination of the LpxH-
180 substrate complex revealed that the arginine residue is required for binding of the phosphate
181 group of lipid X (19). In YfcE, the residue in this position is thought to be critical for the
182 enzyme to switch between a phosphomonoesterase and a cyclic nucleotide
183 phosphodiesterase (11). Steady-state kinetic measurements of the H82N and H82R mutants
184 of $PlcP_{193}$ showed higher affinity (K_m=154.8 ± 4.8 μM, 182.5 ± 5.3 μM, respectively) towards
185 the artificial substrate NPPC compared to the wild-type (K_m=234.2 ± 0.6 μM) $PlcP_{193}$ (**Table
186 1**). This enhanced substrate affinity in these mutants may be attributable to the reduced size
187 of the side chain, from an imidazole ring of histidine to an aliphatic side chain of asparagine
188 and arginine (**Figure 4B-D**).
189

190 **The substrate profiles of PlcP on phospholipids**

191 Although the use of artificial substrates provides an overview of PlcP phosphodiesterase and
192 phosphomonoesterase activity and how these activities are regulated by metal ions, the
193 native substrates of PlcP are believed to be native bacterial phospholipids in the membrane
194 (3, 26). Phospholipids are degraded by PlcP to generate diacylglycerol (DAG) for the
195 biosynthesis of non-P surrogate lipids in response to P limitation and a *plcP* deletion mutant
196 can no long synthesise such surrogate lipids (26). In *Phaeobacter* sp. MED193 and
197 *Pelagibacter* sp. HTCC7211, the native phospholipids are PG and PE. We therefore

198 hypothesized that PG and PE are the preferred native substrates for PlcP. To test this
199 hypothesis, we used a range of phospholipids in addition to PG and PE. These included
200 phosphatidylcholine (PC), a relative uncommon phospholipid in bacteria and absent in the
201 aforementioned marine bacteria, phosphatidic acid (PA), an intermediate in the biosynthesis
202 of phospholipids, and phosphatidylserine (PS) and phosphatidylinositol (PI) both of which
203 are primarily associated with eukaryotes (14, 27). To rule out any potential effect of fatty acid
204 chain length on PlcP activity across different phospholipids species, all lipid substrates
205 contained sn-1 C16:0/ sn-2 C16:0 palmitic acid. The data presented in **Figure 5** shows that
206 PG is the preferred substrate whereas the enzyme is least active towards PI and PS.
207 Interestingly, PlcP from both marine bacteria showed comparable, if not higher, activity
208 towards PA (containing a phosphate monoester bond), an essential intermediate in
209 phospholipid biosynthesis. Our data therefore suggests that PlcP in these marine bacteria
210 have a relatively broad substrate specificity and are able to divert primary bacterial lipids (or
211 the precursor, PA) through the conversion to DAG for the synthesis of non-P lipids in
212 response to P limitation.
213 To conclude, our data shows that the activity of purified PlcP from two model marine
214 heterotrophic bacteria is dependent on metal ions particularly manganese, opening up the
215 possibility that manganese availability may play a role in regulating the lipid remodelling
216 process in natural marine systems (**Figure 6**). Dissolved manganese concentrations are in
217 the low nanomolar range (0.1-4 nM) in marine surface waters and it is already known that
218 manganese is important for maintaining optimum photosystem II (PSII) activity in marine
219 phytoplankton (31, 32). Thus, the wide occurrence of ABC-type manganese transporters in
220 SAR11 and marine Roseobacter clades might suggest potential competition with marine
221 phytoplankton for manganese (9). Nevertheless, our observation of PlcP dependence on
222 manganese reiterates more generally the importance of trace metals in regulating the activity
223 of enzymes playing key roles in nutrient transformations in marine systems e.g. zinc in
224 alkaline phosphatase (8) and iron in nitrogenase (23). Moreover, the fact that PlcP appears
225 able to degrade several native bacterial lipids which are common in marine bacteria, *e.g.* PG

226 and PE, suggests that PlcP undertakes a generic lipid remodelling role among marine

227 heterotrophic bacteria adapted to low P environments.

228 **Experimental procedures**

229

230 **Cloning, expression and purification of PlcP**

231 The wild-type *plcP* from *Pelagibacter* sp. HTCC7211 and the wild type *plcP* and site directed
232 *plcP* mutants (H12A, D40A, D43A, N81A, H82A, H82N, H82R, H117A, D119A, H201A,
233 H203A and D220A) from *Phaeobacter* sp. MED193 were codon-optimized and chemically
234 synthesized by Genscript. The genes were then inserted into the pET28a expression vector
235 using *Nde*I and *BamH*I restriction sites and transformed into *E. coli* BL21(DE3)-CodonPlus-
236 RIL. To induce the expression of PlcP, 2% (v/v) overnight *E. coli* culture grown on lysogeny
237 broth (LB) was inoculated into 600 ml fresh LB broth. Kanamycin was added to LB medium
238 to a final concentration of 50 mg l^{-1}. The cultures were then incubated at 37℃ with shaking
239 (200 r.p.m). When the OD_{600} reached ~ 0.5, IPTG was added to a final concentration of 0.2
240 mM and cells harvested after 4 h at 37℃ for $PlcP_{193}$ and its mutants or 8 h at 30℃ for
241 $PlcP_{7211}$. Cells were then harvested by centrifugation and re-suspended in buffer containing
242 50 mM Tris–HCl, 200 mM NaCl, pH 8.0. Cells were disrupted by sonication. Cell debris was
243 removed by centrifugation at 20,000 ×g for 20 min and the supernatant loaded onto a nickel
244 column (GE healthcare). After washing with buffer containing 20 mM Tris-HCl pH 8.0, 200
245 mM NaCl, 100 mM imidazole, proteins were eluted with elution buffer (20 mM Tris-HCl, pH
246 8.0, 200 mM NaCl, 300 mM imidazole). Purified protein was analysed by SDS-PAGE and
247 protein concentrations were determined using the Bradford assay.

248

249 **Bioinformatics and homology modelling**

250 The homologous sequence and conserved domain were identified using the BLASTp software
251 provided by the National Center for Biotechnology Information
252 (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignment analyses of the deduced amino acid
253 sequences were performed by ClustalW2 (http://www.ebi.ac.uk/ Tools/clustalw2/index.html).
254 Phylogenetic analysis used the neighbour-joining analysis method using Molecular
255 Evolutionary Genetic Analysis 7.1 software (MEGA, version 7.1) (13). The three-dimensional

256 model structures of $PlcP_{193}$ and the H82A, H82N and H82R mutants were generated by
257 submitting the respective amino acid sequences to the Phyre 2 protein modelling and structure
258 prediction server (10) and selecting the overall best scoring model in terms of coverage and
259 confidence. All protein structure models were visualised in Chimera (22).

260

261 **PlcP activity assay using artificial substrates**

262 PlcP activity was measured in 96 well microplates using 1 mM *p*-nitrophenylphosphorylcholine
263 (NPPC) or *p*-nitrophenylphosphate (PNPP) as a substrate, containing 1 µM purified enzyme in
264 50 mM Tris-HCl (pH 9.5), 60% (w/v) sorbitol, 1 mM $MnCl_2$. Enzyme activity was measured at
265 65 ℃ for 30 min and absorbance was monitored at 405 nm for the formation of *p*-nitrophenol.
266 One unit of phospholipase activity was defined as the amount of enzyme releasing 1 µmol *p*-
267 nitrophenol per min under the standard conditions. K_m and V_{max} values were calculated using
268 Hanes-Wolff plots at varying concentrations of substrates (0.02-1.0 mM) in three replicates.

269

270 **PlcP activity assay using phospholipids**

271 A range of phospholipids were used to test PlcP specificity, including 1,2-dipalmitoyl-sn-
272 glycero-3-phospho-rac-(1-glycerol) sodium salt (PG), 1, 2-dipalmitoyl-sn-glycero-3-
273 phosphocholine (PC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (PE), 1, 2-
274 dipalmitoyl-sn-glycero-3-phosphate sodium salt (PA), 1,2-dipalmitoyl-sn-glycero-3-phospho-L-
275 serine sodium salt (PS) and 1, 2-dipalmitoyl-sn-glycero-3-phospho-1'-myo-inositol ammonium
276 salt (PI) (7). The standard reaction mixture (300 µl) contained 2.5 µM purified enzyme in 50
277 mM Tris-HCl (pH 9.5), 1 mM $MnCl_2$ and phospholipids (0.1-0.8 mM), which was incubated for
278 30 min at 65 ℃. Phospholipids and the common degradation product, diacylglycerol (DAG)
279 were extracted according to the Folch method using methanol:chloroform:water at a ratio of
280 1:2:0.6 (v/v/v). The lipid extract was dried under nitrogen gas at room temperature. The dried
281 lipids were re-suspended in acetonitrile and ammonium acetate (10 mM, pH 9.2) at a ratio of
282 95:5 (v/v), and analysed by liquid chromatography-mass spectrometry (LC-MS).

283

284 **Phospholipid characterization and quantification by LC-MS**

285 Phospholipids and PlcP-hydrolysed lipid products were analysed by LC-MS using a Dionex
286 UltiMate 3000 LC system (Thermo Scientific, Walham, MA) coupled to a Bruker amazon SL
287 electrospray- ion trap mass spectrometer (Billerica, MA). A BEH Amide XP column (2.5 μm,
288 3 mm ×150 mm) was obtained from Waters (Milford, MA) and used for the chromatographic
289 separation using a mobile phase consisting of acetonitrile (solvent A) and 10 mM ammonium
290 acetate, pH 9.2 (solvent B). The column was equilibrated for 10 min with 95:5 (v/v) A:B prior
291 to sample injection. The separation of phospholipids was conducted using a stepwise
292 gradient starting from 95% (v/v) A: 5% (v/v) B to 70% (v/v) A: 30% (v/v) B after 15 min with a
293 constant flow rate of 150 μl min^{-1}. Instrument settings for the positive ion ESI/MS and MS/MS
294 analysis of phospholipids were as follows: capillary voltage of 4500 V; end plate offset of 500
295 V; 8 L min^{-1} drying gas at 250 ℃; nebulizing gas pressure of 15 psi. Data analysis was
296 performed using the Compass DataAnalysis and QuantAnalysis 4.2 software (Bruker,
297 Billerica, MA). PlcP activity towards phospholipids was measured by quantifying 1,2-
298 dipalmitoyl-sn-glycerol (DAG) formation. A standard calibration curve for DAG was
299 generated by correlating peak area to DAG quantity. A concentrated stock solution of DAG
300 was prepared by dissolving in chloroform at a concentration of 0.5 mg ml^{-1}. The concentrated
301 stock was further diluted in chloroform to generate standards at 0.005, 0.01, 0.02, 0.04, 0.06,
302 0.08, and 0.1 mg ml^{-1}.

303

304 **Acknowledgements**

305 This project has received funding from the European Research Council (ERC) under the
306 European Union's Horizon 2020 research and innovation programme (grant agreement no.
307 726116). We also thank the Program of Study Abroad for Young Scholars sponsored by
308 Zhengzhou University of Light Industry, China to W.T., the National Natural Science
309 Foundation of China (NSFC, no. 31728001, 31630012, U1706207) and a Royal Society
310 International Exchanges grant (IEC\NSFC\170213).

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431 al., (2017) Manganese in the west Atlantic Ocean in the context of the first global
432 ocean circulation model of manganese. *Biogeosci.* 14:1123-1152.

433 **Table 1** Kinetic parameters of $PlcP_{7211}$ wild type, $PlcP_{193}$ wild type, and $PlcP_{193}$ mutant

434 enzymes following the hydrolysis of *p*-nitrophenylphosphorylcholine (NPPC)[a].

435

Protein	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (s^{-1} mM^{-1})
$PlcP_{7211}$	41.8 ± 6.3	275.6 ± 5.7	151.7 ±5 .9
$PlcP_{193}$	38.6 ± 5.2	234.2 ± 6.3	164.8 ± 5.6
H82R	25.5 ± 3.8	182.5 ± 5.3	140.0 ± 3.9
H82N	18.6 ± 4.2	154.8 ± 4.8	120.1 ± 4.5
H82A	0.12 ± 0.1	17.2 ± 2.3	7.0 ± 2.5

436 [a] Values are mean ± standard deviation of three independent experiments.

Figure legends

Figure 1 Multiple sequence alignment and functional domain analyses of PlcP proteins. PLC_{193}, PlcP of *Phaeobacter* sp. MED193; PLC_{7211}, PlcP of *Pelagibacter* sp. HTCC7211; $PlcP_{Sm}$, PlcP of *Sinorhizobium meliloti* (30).

A) Multiple sequence alignment of PlcP and closely related LpxH enzymes. The 6 conserved motifs are highlighted in grey. The conserved histidine residue in PlcP (H82) is highlighted in green.

B) Neighbour-joining phylogenetic analysis between members of the metallophosphoesterase family (PFam 00149) including proteins closely related to PlcP: PaLpxH, LpxH from *Pseudomonas aeruginosa* (19); HiLpx, LpxH from *Haemophilus influenza* (5). LpxH displays pyrophosphatase activity and acts on UDP-2, 3-diacylglucosamine to produce lipid X, a key precursor for the formation of lipid A in lipopolysaccharide biosynthesis. More distantly related members of the metallophosphoesterase family include: MJ0936, which represents a group of novel phosphodiesterases which do not degrade phosphomonoesters (4); Mre11/SbcD which are bacterial and archaeal DNA phosphodiesterases involved in DNA repair (21, 29); Dbr1, which is a group of phosphodiester nucleases that act on RNA (12); CpdA, CpdB and cAMP phosphodiesterases which are cyclic nucleotide phosphodiesterases; ApaH, which represents a group of enzymes with pyrophosphatase and protein phosphatase activities (1); YfcE, which represents a group of small metallophosphoesterases showing phosphodiesterase activity (17); Sphingomyelinase, which is a group of hydrolases responsible for breaking down sphingomyelin to phosphocholine and ceramide (6). Numbers indicate bootstrap values (only values >50 are shown).

463 **Figure 2** Homology modelling showing the predicted structure of $PlcP_{193}$ and the metal-
464 binding pocket. The signature arginine residue in LpxH (Arg81) is substituted by a histidine
465 residue in PlcP (His82).
466
467 **Figure 3** PlcP displays manganese-dependent phosphomonoesterase and
468 phosphodiesterase activities.
469 **A**) Over-expression and purification of PlcP from *Phaeobacter* sp. MED193 and *Pelagibacter*
470 sp. HTCC7211. M, protein molecular weight marker. Lane 1, cell-free supernatant induced
471 with Isopropyl β-D-1-thiogalactopyranoside (IPTG); lane 2, cell-free supernatant without
472 IPTG induction; lane 3, purified PlcP protein (molecular weight estimated to be ~ 27 kDa).
473 **B)** PlcP activity assays in the presence of various divalent metal ions (1 mM). Values are
474 mean ± standard deviation of three replicated measurements.
475 **C)** PlcP activity assays using *p*-nitrophenylphosphorylcholine (NPPC) or *p*-
476 nitrophenylphosphate (PNPP). Values are mean ± standard deviation of three replicated
477 measurements.
478
479 **Figure 4** Homology modelling prediction of the metal-coordination centre in the $PlcP_{193}$
480 enzyme (**A**) and the site-directed mutants of His82 to Ala82 (**B)**, Arg82 **(C)** and Asn82 **(D).**
481 Specific activities of site directed mutants of $PlcP_{193}$ are measured using *p*-
482 nitrophenylphosphorylcholine (NPPC) as the substrate (**E**). Values are mean ± standard
483 deviation of three replicated measurements.
484
485
486 **Figure 5** Relative activity of the degradation of phospholipids by $PlcP_{193}$ and $PlcP_{7211}$.
487 Activity was measured by quantifying the formation of the common product diacylglycerol
488 (DAG) in these reactions. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG,
489 phosphatidylglycerol; PA, phosphatidic acid; PI, phosphatidylinositol; PS,

490 phosphatidylserine. Values are mean ± standard deviation of three replicated
491 measurements.
492
493
494 **Figure 6** A schematic overview of the PlcP-mediated lipid remodelling pathway and its
495 regulation in representative marine bacteria. The major lipids in *Phaeobacter* sp. MED193
496 and *Pelagibacter* sp. HTCC7211 during P replete conditions are two phospholipids
497 (highlighted in grey), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). During
498 P stress, the two component signal transduction system PhoBR is activated and the
499 phosphorylated PhoB activates not only the expression of the high affinity ABC transporter
500 for phosphate (PstSABC) but also the transcription of the *plcP* gene. A conserved phoB
501 binding site in the *plcP* promoter has previously been identified in these bacteria (26). The
502 purified PlcP protein requires manganese for activity (Figure 3). Manganese is likely
503 transported into the cell through either the SitABCD or the MntX transporter systems that are
504 present in these marine heterotrophic bacteria (9). Active PlcP can convert PE, PG or its
505 biosynthesis precursor phosphatidic acid (PA) to diacylglycerol (DAG), which serves as the
506 building block for the biosynthesis of alternative P-free surrogate lipids (highlighted in blue),
507 including diacylglyceryl trimethylhomoserine (DGTS) and the glycolipids monoglycosyl
508 diacylglycerol (MGDG) and glucuronic acid diacylglycerol (GADG) (3, 26).