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Cardiolipin is dispensable for oxidative phosphorylation and non-fermentative growth of alkaliphilic *Bacillus pseudofirmus* OF4

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Abstract

Cardiolipin (CL), a membrane phospholipid in bacteria and mitochondria, has been hypothesized to facilitate movement of protons on the outer surface of membranes in support of respiration-dependent ATP synthesis, oxidative phosphorylation (OXPHOS). If so, the high levels of membrane CL found in alkaliphilic bacteria, such as *Bacillus pseudofirmus* OF4, might facilitate its robust OXPHOS at pH 10.5, where the bulk protonmotive (PMF) force is low. To address the role of CL in *Bacillus pseudofirmus* OF4, we studied strains in which genes (*cls*) potentially encoding a CL synthase (CLs) were deleted: three single (*AclsA*, *AclsB*, and *AclsC*), one double (*AclsA/B*), and one triple (*AclsA/B/C*) mutant. Two-dimensional thin layer chromatography analyses of lipid extracts from ³²P-labeled strains showed that the wild-type CL content was 15% of total phospholipids at pH 10.5 versus 3% at pH 7.5 during log phase. The % CL was higher (28-33%) at both pH values during stationary phase. The *clsA* gene plays a major role in CL biosynthesis as no detectable CL was found in *AclsA*-containing mutants, whereas the CL precursor phosphatidylglycerol was elevated. The *AclsB* mutant exhibited no significant reduction in CL, but *clsB* expression was up-regulated and appeared to support growth at pH 7.5. In the absence of detectable CL, the alkaliphile showed no significant deficits in non-fermentative growth, respiration-dependent ATP synthesis, or salt tolerance. Minor deficits in respiration and ATP synthase assembly were noted in individual mutants. In long term survival experiments, significant growth defects were found in *AclsA* strains and the *AclsC* strain at pH 10.5. © 2014 by The American Society for Biochemistry and Molecular Biology, Inc.

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