A periplasmic lanthanide mediator, lanmodulin, in Methylobacterium aquaticum strain 22A

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Fig. S1. Assessment of P_{fae1} and P_{mxaF} activity using a GFP-reporter. P_{fae1} and P_{mxaF} were each cloned into a GFP-reporter vector pHC42m, and the plasmids were introduced into strain 22A wild type. Their activity was assessed by measuring GFP fluorescence in strain 22A grown on methanol in the absence and presence of 30 μ M La³⁺. Strain 22A carrying pHC42m was regarded as control. The data are taken at 19 h. Data are presented as mean value ± standard deviation (SD), and analyzed with two-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test, and shown with compact letter display (p < 0.05, n = 3).

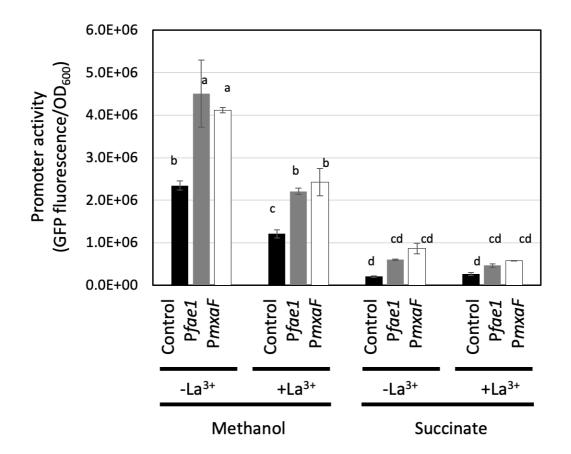
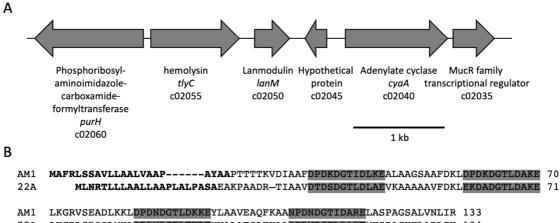


Fig. S2. A. Gene organization of a chromosomal region containing *lanM* in the *M. aquaticum* strain 22A genome. Arrows indicate gene orientation and size. The suffix of the accession numbers (Maq22A_) is not shown. B. Alignment of LanM amino acid sequences from strains AM1 and 22A. The putative signal peptide sequences are shown in bold. The EF-hand motif (D(or E, N)xDxDxxxxxE) is shaded.



22A LKGRLSRQQLMKADPDNDGTLTKDEYVALAESLFKAADPDNDGTLDAKEMKSPAGKTLARLIK 134

Fig. S3. Heterologous expression of His-LanM and GST-LanM in *E. coli*. A. Expression of His-LanM in *E. coli* DH5α (pCold-*lanM*) and purification. Lane 1, Protein marker; 2, *E. coli* DH5α (pCold-*lanM*) cell-free insoluble fraction; 3, *E. coli* DH5α (pCold-*lanM*) cell-free extract; 4, Flow-through fraction of Ni-NTA column; 5-9, elution fractions (1 ml each). Samples were analyzed by SDS-PAGE (18.2% gel). Theoretical mass of His-LanM is 14.0 kDa. B. Expression of GST-LanM in *E. coli* DH5α (pGEX-*lanM*). 1, Protein marker; 2, *E. coli* DH5α (pGEX-*lanM*) cell-free extract. The sample was analyzed by SDS-PAGE (10% gel). Theoretical mass of GST-LanM is 36.7 kDa. C. Purification of LanM cleaved from GST-LanM. 1, Protein marker; 2, LanM protein eluted from GST-ACCEPT by PreScission protease. The sample was analyzed by SDS-PAGE (18.2% gel). Theoretical mass of LanM is 12.3 kDa.

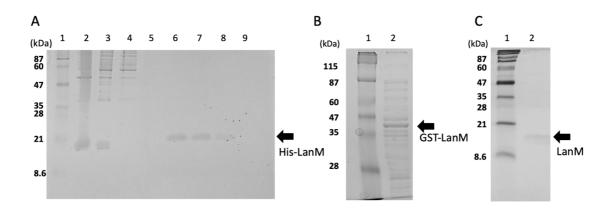


Fig. S4. Specific growth rate of various mutants on methanol and succinate in the absence/presence of $LaCl_3$. Data are presented as the mean value ± standard deviation (SD) (n = 3).

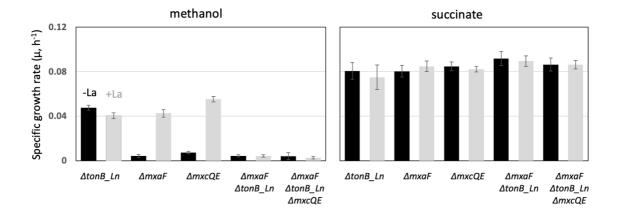


Fig. S5. SWATH proteome analysis of the wild-type strain 22A and $\Delta lanM$ grown on methanol in the absence/presence of LaCl₃. The peptide peak area of the identified and detected proteins are shown as a heatmap.

,	wт	∆lanM		
La ³⁺				
Las	. т		Maq22A_c05815	AsnC family transcriptional regulator
L.			Maq22A_c05985	2-dehydro-3-deoxy-6-phosphogalactonate aldolase
d e			Maq22A_c25240	phytoene/squalene synthetase
-44			Maq22A_c20240 Maq22A_c10370	chemotaxis protein cheZ
			Maq22A_c12225	transmembrane glycoprotein N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase
		- -	Maq22A_c12285	inositol monophosphatase
			Maq22A_c12695	fumarate hydratase
卢飞			Maq22A_c14425	blue light sensor protein
			Maq22A_c12405	UDP-diphospho-muramoylpentapeptidebeta-N-acetylglucosaminyltransferase
			Mag22A 1p30010	urocanate hydratase
	-		Maq22A_1050010 Maq22A_c16070	NAD(P)H-quinone oxidoreductase
			Mag22A_c24025	peptide ABC transporter ATP-binding protein
			Maq22A_c09905	hypothetical protein
			Maq22A_c14865	biotin carboxyl carrier protein
			Maq22A 1p36015	cell wall metabolism sensor kinase, associated with heavy metal resistance efflux system
			Maq22A_c10925	methionine ABC transporter ATP-binding protein
			Mag22A_c05245	dienelactone hydrolase
[[-			Maq22A_c11880	FMN reductase (NADPH)
			Mag22A c04290	radical SAM protein, hypothetical protein, predicted secreted protein, NUDIX hydrolase
			Maq22A_c07415	leucine/isoleucine/valine transporter ATP-binding subunit
			Mag22A c01380	TRAP-type C4-dicarboxylate transport system, periplasmic component
4└			Mag22A_c17685	hypothetical protein
			Maq22A_c07095	Tol-pal system protein
			Maq22A_c10125	6-phosphogluconolactonase
			Maq22A_c07785	glutathione S-transferase
			Maq22A_c07255	4-hydroxybutyrate dehydrogenase
			Maq22A_2p41960	3-hydroxyacyl-CoA dehydrogenase
			Maq22A_4p60130	hypothetical protein
			Maq22A_c06945	50S ribosomal protein L36
			Maq22A_c17650	D-alanyl-D-alanine carboxypeptidase
	-		Maq22A_3p50230	hypothetical protein
			Maq22A_c02325	anthranilate synthase component I
	_		Maq22A_c05765	2-dehydro-3-deoxy-L-arabinonate dehydratase
		_	Maq22A_c20750	general stress protein CsbD
		_	Maq22A_c20090	amino acid ABC transporter ATP-binding protein
- F			Maq22A_c12525	2-deoxy-D-gluconate 3-dehydrogenase
			Maq22A_c01775	Protein of unknown function (DUF3616)
			Maq22A_c16420	ABC transporter ATP-binding protein
니너			Maq22A_1p32125	phosphate starvation protein PhoH
		1000	Maq22A_1p33360	imidazole glycerol phosphate synthase subunit HisH
		100	Maq22A_1p33200	MxaK protein
2.5		6		

Peak area (log10)

Fig. S6. Confocal microscopic images of MVs from $\Delta lanM$, wild-type, and lanM-OX strains grown on methanol in the presence of 30 μ M LaCl₃. MVs collected by a commercial kit (upper panel) and by ultracentrifugation (lower panel) are shown. Bar, 2 μ m.

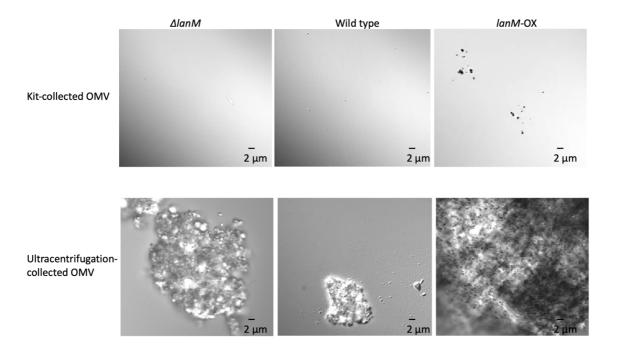


Fig. S7. La content in the MV fractions collected by a commercial kit from the wild-type, $\Delta lanM$, and lanM-OX strains. The data were analyzed with one-way ANOVA followed by Tukey's multiple comparisons test. Data are presented as the mean value ± standard deviation (SD) (n = 3).

