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Supplementary materials

Molecular cloning, expression and characterization of acyltransferase from *Pseudomonas protegens*

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1. Screening of Pseudomonas wildtype strains

32 strains from the in-house culture collection (Table S1) were tested for their ability to catalyze a reversible acetylation and deacetylation of MAPG (Figure S1).

 Table S1. Pseudomonas strains investigated.

Entry	Strain	Designation
1	Pseudomonas acidovorans	ATCC 17438
2	Pseudomonas aureofaciens	ATCC 43051
3	Pseudomonas brassicacearum	DSM 13227
4	Pseudomonas chlororaphis	ATCC 9447
5	Pseudomonas cichorii	DSM 50259
6	Pseudomonas dehalogenans R	FCC 162
7	Pseudomonas elodea	ATCC 31461
8	Pseudomonas fluorescens	DSM 50106
9	Pseudomonas fluorescens	ATCC 17571
10	Pseudomonas fluorescens	ATCC 49838
11	Pseudomonas fluorescens	NRRL B 00010
12	Pseudomonas fluorescens Pf-5	ATCC BAA-477
13	Pseudomonas fragi	DSM 3456
14	Pseudomonas marginalis	FCC 177
15	Pseudomonas mephitica	FCC 178
16	Pseudomonas oleovorans	ATCC 29347
17	Pseudomonas ovalis	ATCC 00950
18	Pseudomonas pavonacea	NRRL B 00969
19	Pseudomonas protegens	DSM 19095
20	Pseudomonas pseudoalcaligenes	DSM 10086
21	Pseudomonas putida	FCC 145
22	Pseudomonas putida	ATCC 17453
23	Pseudomonas putida	ATCC 47054
24	Pseudomonas putida	DSM 12264
25	Pseudomonas rhodesiae	FCC 179
26	Pseudomonas sp.	DSM 6978
27	Pseudomonas sp.	DSM 12877
28	Pseudomonas sp.	NCIMB 11753
29	Pseudomonas stutzeri	DSM 17083
30	Pseudomonas syringae	DSM 50272
31	Pseudomonas syringae	DSM 1241
32	Pseudomonas thermotolerans	DSM 14292

Conditions: Lyophilized cells of the respective *Pseudomonas* strain (20 mg), KPi-buffer (50 mM, pH 7.5), MAPG (50 mM, forward reaction) or alternatively PG and DAPG (50 mM, reverse reaction), 3 h, 30 °C and 500 rpm.



EXAMPLE 7 Figure S1. TLC of the extracted products of the forward reaction after staining with cinnamaldehyde*HCl. References: DAPG (5, lane 1), MAPG (6, lane 2), PG (7, lane 7). Reactions (50 mM, 6): *P. protegens* DSM19095 (lane 3-4); *P. brassicacearum* DSM13227 (lane 5-6).

2. Plasmid construction of recombinant ATases

Primer sequences and plasmids used in this study are listed in Table S2. To construct the expression constructs PpATaseWT and PbATaseWT, the genomic DNA of the respective *Pseudomonas* wild-type served as template to amplify the ATase-encoding operon *phlACB* by PCR. The PCR products were digested (*KpnI/BamHI*), purified and ligated into target vector pASK-Iba3plus. The obtained expression vectors carry the ATase encoding genes *phlACB* under the control of the P_{Tet} promoter. To construct the recombinant *PpATaseCH* with the optimized sequence, the ATase encoding open-reading frames *phlA, phlC* and *phlB* of *P. protegens* were codon-optimized by manually matching the codon-frequency of the *Pseudomonas* wild-type with *E. coli*. To achieve this goal, codon-usage tables for *Escherichia coli* B and *Pseudomonas fluorescens* were obtained from the Kazusa-database (http://www.kazusa.or.jp/codon/). Ribosomal binding sites suitable for *E. coli* were introduced upstream of each start codon of each *phl* gene. The optimized *phl* genes were purchased as gene fragments (gBlocks©) and assembled with the double-digested pASKIBA3plus backbone (*EcoRI/Hind*III) by overlap extension-PCR (OE-PCR) and subsequent Gibson cloning (Gibson Assembly® master mix). The final expression vector carried the *E. coli* codon-optimized ATase encoding genes *phlACB* under the control of the *P_{Tet}* promoter.

Plasmids	Origin	Description/Comments
	(GenBankID)	
pASKIBA3plus	IBA-Lifescience	P _{Tet} , Amp ^r , ColE1 _{ori} , C-terminal StrepTag
pEG331	this study	Wild-type-derived phlACB genes of P. protegens DSM19095, isolated from
	(CP003190.1)	genomic DNA by PCR; PCR primers: <i>Pp</i> WT-Fow/Rev.
pEG330	this study	Wild-type-derived <i>phlACB</i> genes of <i>P. brassicacearum</i> DSM13227, isolated
	(KY173354)	from genomic DNA by PCR; PCR primers: <i>Pb</i> WT-Fow/Rev
pEG332	this study	Codon-optimized gene fragments <i>phlA</i> , <i>phlC</i> and <i>phlB</i> based on <i>phlACB</i> from
	(KY173355)	P. protegens DSM19095, assembled by Gibson cloning and overlap-extension
		PCR. PCR primers: OE1-4ATaseCH-Fow/Rev.
Primers	Origin	Sequence (5'→3')
<i>Pp</i> WT-Fow	Eurofins	ATATA <u>GGTACC</u> ATGAACGTGAAAAAGATAGGTATTG
<i>Pp</i> WT-Rev	Eurofins	ATATA <u>GGATCC</u> TTATATATCGAGTACGAACTTATAAG
<i>Pb</i> WT-Fow	Eurofins	ATATA <u>GGTACC</u> ATGAATAAAGTAGGAATTGTG
PbWT-Rev	Eurofins	ATATA <u>GGATCC</u> TTATTTCACCAGTACAAACTTATAG
OE1ATaseCH-Fow	IDT	ATATAA <u>GAATTC</u> aaggagatatacataTGATGAATGTGAAGAAAATAGGT
		ATCGTTAGC
OE2ATaseCH-Fow	IDT	CGCTGACCGCGTACCTCTAAGGTACCaaggagatatacataTGATGTGCGC
		ACGTCGCG
OE3ATaseCH -Rev	IDT	TGCGCACATCAtatgtatatctccttGGTACCTTAGAGGTACGCGGTCAGCG
		САТААТС
OE4ATaseCH -Rev	IDT	ATATAT <u>GAATTC</u> GCCGAGACGGCCATG
Bacterial Strains	Origin	GenBankID (phlACB gene locus)/comments
	(Strain ID)	
Pseudomonas	DSMZ	LT629713.1 (bp: 1051432-1054193) / phlACB from P. brassicacearum
brassicacearum	(DSM13227)	BS3663 are 100% to <i>phIACB</i> from <i>P. brassicacearum</i> DSM13227
Pseudomonas	DSMZ	CP003190.1 (bp: 6560049-6562816) / other designation: CHA0
protegens	(DSM19095)	
Pseudomonas	ATCC (ATCC	CP000076.1 (bp: 6766435-6769202)
fluorescens Pf5	BAA-477)	

Table S2. Plasmids and primers employed in this study. Mutagenized codons are shown in bold, restriction site are underlined. Ribosomal binding sites are shown in lowercase letters.

2.1. Protein sequence-alignment of PhIA, PhIC and PhIB

The *phlACB* operon from *P. protegens* DSM19095 and *P. brassicacearum* DSM13227 was amplified from the genomic DNA using primer sequences which were identified in a BLAST-search (Table S3).

Entry	Organism	Description	GenBank accession no.	Seq. identity [%]
1	Pseudomonas sp. YGJ3	phlACBDEFGHI complete cds	AB636682.1	100
2	P. protegens CHA0	complete genome	CP003190.1	99
3	P. brassicacearum NFM241	complete genome	CP002585.1	80
4	P. fluorescens J2	phlA, phlB, phlC, phlD complete cds	JN561597.1	80

Table S3. BLAST-search results.

Multiple sequence alignments of the *Phl*-subunits were performed using the T-COFFEE multiple sequence alignment program provided by EMBL-EBI.[1] *PhlA*, *PhlC* and *PhlB* originating from *P. protegens* (*Pp*), *P. brassicacearum* (*Pb*) and *P. fluorescens* Pf-5 (*Pf*5) were aligned to the *Phl*-subunits of *Pseudomonas* sp. YGJ3. The rendering of the sequence alignments was performed with ESPript 3.0 (http://espript.ibcp.fr).[2] The secondary structure elements of individual *Phl*-subunits from *P. protegens*, as present in the crystal structure, are depicted above the alignments (Figure S2-S4).

	β1	β2 α1	α2	β3
	ļ 10	20	3 0 4 0	5 ọ
YGJ3-PhlA	MNVKKIGIVSYGAGIP	VCRLKVQEVINV	WKNTDLKLVEENI	LGVTERAVLQ
PD-PhiA Pb-PhlA	MNVKKIGIVSIGAGIPV MNKVGIVSIGAGIPV	VCRLKVDDVIQVI	WKNTDLSLVKGQI	LGVLERAVLQ
Pf5-PhlA	MNVKKIGIVSYGAGIPV	VCRLKVQEVINV	WKNTDLKLVEENI	LGVTERAVLQ
	α3	f	34	α4 00000000
	6 Ó	7 Ģ 1	во о	100
YGJ3-PhlA Pp-PhlA	PDEDVITLGVLAAQRAI	LDKVPGHQIEAL	ILGTCTNPYDSR VLGTCTNPYDSR	ASA <mark>S</mark> IILEML
Pb-PhlA	PDEDVITLGVLAAQRAI	LDKAPPCSLEAL	YLGTCTNPYDSR#	ASAAIILEML
Pf5-PhlA	PDEDVITLGVLAAQRAI	LDKVPGHQIEAL	ILGTCTNPYDSR#	ASA <mark>S</mark> IILEML
	$\stackrel{\beta 5}{\longrightarrow} \begin{array}{c} \eta 1 \\ 2 2 0 0 \end{array}$	α5 2000000000000	εεβ6	
	110	120 1:	30 140	15 Q
YGJ3-PhlA Pp-PhlA	GSGYDAYCADVQFAGKS	SGTSALQI <mark>CQ</mark> AL	VASGMTGSALAIC	GADTINRNTA CADTINRNTA
Pb-PhlA	GCGYDAFCADVQFAGK	SGTSALQIAYAL	VASGMVGNALAVO	GADTINRNTA
Pf5-PhlA	GSGYDAYCADVQFAGK	SGTSALQI <mark>CQ</mark> ALV	VASGMTGSALAIC	GADTINRNTA
	η2 β7	→ <u></u>	\rightarrow $\xrightarrow{\beta9}$ $\xrightarrow{\beta1}$	ο β11
	160	170 11	Β Ϙ 19Ϙ	2 O O
YGJ3-PhlA Pp-PhlA	PGDLTESYAGAGAAALI PGDLTESYAGAGAAALI	LIGSODVIAEFD	ASFSCAADVADNJ ASFSCAADVADNI	I R P Q G D R Y I R
Pb-PhlA	PGDLTESYAGAGAAALI	LGTENVIAHFD	ASFSCAADVADNJ	IRPQGDRYIR
Pf5-PhlA	PGDLTESYAGAGAAALI	LIGSQDVIAEFD	ASFSCAADVADNI	IRPQGDRYIR
	 α6 ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο	α7 2000000000000	$\eta_{0}^{3} \xrightarrow{\beta_{12}} \eta_{12}^{3}$	0000
	210	220 23	3 0 24 0	250
YGJ3-PhlA Pp-PhlA	SGMGLGSDKNSIGLED	OTRRAAEGLMAK	LHTSPADYDYVVF	FQQNLVSTPY
Pb-PhlA	SGMGLGSDKNSIGLED	QTRRAASGLMAK	IHAQAGDFDYVVF	FQQNLVSTPY
Pf5-PhlA	SGMGLGSDKNSIGLEDÇ	QTRRAAEGLMAK]	LHTSPADYDYVVF	FQQNLVSTPY
	α8 000000		<u>α9</u> 000000000	β13
	2 6 Q	270 21	BQ 29Q	3 O O
YGJ3-PhlA	SLAKHLGFNPKQVEPGI	IYAGNVGDAGSAS	SPLLGLINVLDQA	ARPGQKILLV
Pb-PhlA	SLGKHLGFTTAQIEPGI	IYAQSVGDAGBA:	SPLLGLVNVLDQ#	ARPGERILVV
Pf5-PhlA	SLAKHLGFNPKQVEPGI	IYA <mark>GN</mark> VGDAG <mark>S</mark> A	SPLLGLINVLDQ#	ARPG <mark>QKILL</mark> V
	\rightarrow β_{14}	$\alpha 10$	$\alpha 11 \qquad \beta 15 \\ 0 0 0 0 \qquad \rightarrow \qquad ($	α12 2000000
	310	320 33	3 0 3 4 0	3 5 Q
YGJ3-PhlA	SYGFGAGSDAIALTVT	DAIEQYQKHNKPI		GTSIKYEFK
Pb-PhlA	SYGFGAGSDAIALTVTI	DAIEAYQKTNVPI	LRTLLEDKYYVD	YGTSIKYEFK
Pf5-PhlA	SYGFGAGSDAIALTVTI	DAIEQYQKHNKP1	LRELLESKIYVDY	Y GTSIKYEFK
	360			
YGJ3-PhlA Pp-Phla	YLRADYALTAYL VI.RADYAL TAYL			
Pb-PhlA	YLRPDYALTAYL			
Pf5-PhlA	Y L R A D Y A L T A Y L			

Figure S2. Protein sequence alignment of PhIA. Sequences of PhIA from *P. protegens (Pp), P. brassicacearum (Pb)* and *P. fluorescens (Pf-5)* were aligned to *Pseudomonas* sp. YGJ3.

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Pb-Ph1C	MSARRV	VAIVS	AAYI	C P K P	GSS	RVR	QTF	KE	MIV	VES	ΑΥÇ	ζAL	NA	IKI	ΜН	PRI	LQ.	A
Pf5-PhlC	MCARRY	JAIVS	JAAYI	C P K P	GSS	RVR	QTE	KE	MIN	VES	AY	(AL	KD.	A K	мн	PRI	E I Q	A
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Pb-Ph1C	VAYGYI	IGEGI	SEY	GLG	PTI	SDA	LGI	SP.	AP?	ГFM	STF	A N <mark>C</mark>	тS	SS	vs	FÕI	1AH	õ
Pf5-PhlC	VAYGYI	IGEGI	SEYC	GLG	PTI	SDA	LGI	SP.	AP:	ΓFM	ST	1 N C	тs	SS	vs	FQI	(GH	Q
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VGJ3-Ph1C	MVASCI	 	/T.C.G.	TEEK	мтр	HFN	YAT	т т т	- - -	ናጥም	CEN	- 7 D V	FL	Gт	នម	יחיד	FA	т.
Pp-Ph1C	MVASGI	EYDIV	LCG	GFEK	MTD	HFN	YAE	EYI	GS	STE	CEY	ZDY	FL	GI	Sн	TDA	FA	L
Pb-Ph1C	MVASGI	EYDIV	LCG	3 F E K	МTD	HIN	YAB	EYI)	GS	SТE	CEZ	ZDY	FL	GI	S H	TDA	AFA:	L
Pf5-PhlC	MVASGI	SYDIV	LCGO	3 F E K	MTD	H F N	YAE	EYI.	GSS	STE	CEZ	ZDY	FL	GI	SH	TD	AFA:	L
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Pp-PhlC	ATAEYI	FOKFO	YAGI	READ	VLA	TFG	RON	IR I	YA	N T NC	PTA	ATR	YG	ÕP	IP	SLI	VL	ĸ
Pb-Ph1C	ATAEYI	FÊKFG	YAG	READ	VLA	TFG	RÕN	(RI	YAJ	HNT	PTF	A T R	YG	ν́Р	ΙP	SLI	AL	к
Pf5-PhlC	ATAEYI	? <mark>Q</mark> K F G	YAGI	READ	VLA	ΤFG	RQI	(RI	Y A 🤇	рит	PTF	A T R	ΥG	QP	ΙP	SLI	C VL I	к
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YGJ3-PhlC Pp-PhlC	η7 2005 NSEACO NSEACO	2 210 35MLA 35MLA) L <mark>W</mark> GE	β6 ASGC	220 AIL AIL	β7 ➡_Ω VAE VAE	η8 22 HL2 HL2	2 AHK AHK	3 Q YTI YTI	DKP	VFV	β 2 7 R G 7 R G	3 4 0 CA CA		► GV GV	SHI	ης 25 25 (FG) Q T T
YGJ3-PhlC Pp-PhlC Pb-PhlC	η7 200 NSEACO NSEACO SSEACO	2 210 3SMLA 3SMLA 3SMLA 3SMLA) L <mark>W</mark> GE LWGE LWGE	β6 ASGC ASGC ASGC	220 AIL AIL AIL	β7 →♪ VAE VAE VAE	η8 ΔΔ HLZ HLZ	2 AHK AHK AHR	3 Q YTI YTI YTI	DKP DKP TQP	VF VF VF	β 2 7 RG 7 RG 1 RG	4 0 CA CA CA	YT(YT(YT)	► GV GV	SHI SHI SHI	η ^ς 25 25 (FG (FG (FG) Q T T T
YGJ3-PhlC Pp-PhlC Pb-PhlC Pf5-PhlC	η7 ΔΟΔ NSEACO SSEACO NSEACO	2 21 SMLA SSMLA SSMLA SSMLA) WGE WGE WGE WGE	β6 ASGC ASGC ASGC ASGC	220 AIL AIL AIL AIL	β7 ▼♪Ω VAE VAE VAE VAE	η8 22 HL2 HL2 HL2 HL2	2 AHK AHK AHK AHK	3 Q YTI YTI YTI YTI	D K P D K P I Q P D K P	VFV VFV VFJ	β 2 7 RG 7 RG 7 RG 7 RG	4 0 CA CA CA CA	YT YT YT YT	► GV GV GV	SHI SHI SHI SHI	η ^ς 25 (FG (FG (FG (FG)) 0 1 1 1
YGJ3-PhlC Pp-PhlC Pb-PhlC Pf5-PhlC Pf5-PhlC	η7 LOS NSEACC NSEACC SSEACC NSEACC	2 210 3SMLA 3SMLA 3SMLA 3SMLA 3SMLA) \WGE \WGE \WGE \WGE \WGE \	β6 ASGC ASGC ASGC ASGC	220 AIL AIL AIL AIL	β7 ▼♪Ω VAE VAE VAE VAE	η8 22 HL2 HL2 HL2	2 AHK AHK AHR AHR	3 Q YTI YTI YTI	D K P D K P I Q P D K P	VFV VFV VFJ VFV	β 2 7 RG 7 RG 7 RG 7 RG	4 0 CA CA CA CA	YT YT YT YT	GV GV GV GV	SHI SHI SHI SHI	ης 25 (FG (FG (FG) (FG) Q T T T T
YGJ3-PhlC Pp-PhlC Pb-PhlC Pf5-PhlC Pf5-PhlC	η7 ΔΟΔΔ NSEACO SSEACO NSEACO	2 210 3SMLA 3SMLA 3SMLA 3SMLA) LWGE / LWGE / LWGE / LWGE / LWGE /	β6 ASGC ASGC ASGC ASGC	220 AIL AIL AIL AIL	β7 ▼⊋ VAE VAE VAE VAE	η8 22 HL2 HL2 HL2	2 AHK AHK AHK	3 Q YTI YTI YTI YTI	D K P D K P I Q P D K P	VF VF VF VF VF	β 2 7 RG 7 RG 7 RG 7 RG	4 0 CA CA CA CA	YT YT YT YT	GV GV GV GV	SHI SHI SHI SHI	η ^ς 25 (FG (FG (FG (FG) Q T T T T
YGJ3-PhlC Pp-PhlC Pb-PhlC Pf5-PhlC	η7 ΔΟΔΔ NSEACO SSEACO NSEACO	2 21 3 5 MLA 3 5 MLA 3 5 MLA) LWGE / LWGE / LWGE / LWGE /	β6 ASGC ASGC ASGC ASGC	220 AIL AIL AIL AIL	β7 ▼♪Ω VAE VAE VAE	η8 20 HL2 HL2 HL2	2 AHK AHR AHR AHR	3 Q YTI YTI YTI	D K P D K P D K P D K P	VF VF VF VF	β 2 7 RG 7 RG 7 RG 7 RG	4 0 CA CA CA CA	YT YT YT YT	GV GV GV	shy shy shy shy β9	η ⁹ 25 (FG (FG (FG)) o t t t
YGJ3-PhlC Pp-PhlC Pb-PhlC Pf5-PhlC Pf5-PhlC	η7 ΔΟΔ NSEACC NSEACC SSEACC NSEACC	2 210 35 MLA 35 MLA 35 MLA 35 MLA 35 MLA) WGE2 WGE2 WGE2 WGE3	β6 ASGC ASGC ASGC ASGC	220 AIL AIL AIL AIL	$\beta 7$ $\downarrow 2$ VAE VAE VAE VAE	η8 20 HL2 HL2 HL2	2 AHK AHK AHR AHR a AHK	30 YTI YTI YTI YTI	D K P D K P I Q P D K P	VF VF VF VF VF	β 2 /RG /RG /RG /RG	4 0 CA CA CA CA	YT YT YT YT	GV GV GV GV	shi shi shi shi	η ⁹ 25 (FG (FG (FG) (FG)) 0 1 1 1 1 0
YGJ3-PhlC Pp-PhlC Pb-PhlC Pf5-PhlC YGJ3-PhlC	η7 ΔΟΔ NSEACC NSEACC SSEACC NSEACC	2 210 35MLA 35MLA 35MLA 35MLA 260	- WGE2 WGE2 WGE2 ★	β6 ASGC ASGC ASGC	220 AIL AIL AIL AIL 270 MAV	$\beta 7$ $\forall A E$ $V A E$ $V A E$ $Q A E$ $Q A E$ $Q A E$ $Q A E$			3 0 YTI YTI YTI 8 0 E 1		VF VF VF VF VF	β 2 7 RG 7 RG 7 RG 7 RG 7 RG	40 CA CA CA CA 90	YT YT YT YT		SHI SHI SHI β9	ης 25 (FG (FG (FG) (FG) (FG)	
YGJ3-PhlC Pp-PhlC Pb-PhlC Pf5-PhlC YGJ3-PhlC YGJ3-PhlC	η7 ΔΟΔΔ NSEACC SSEACC NSEACC NSEACC RFHNP	2 35 MLA 35 MLA 35 MLA 35 MLA 26 Q CL <u>HH</u> F CL <u>HH</u> F	GLPF	β6 ASGC ASGC ASGC ASGC	220 AIL AIL AIL 270 MAV MAV	$\beta7$ VAE VAE VAE VAE SAN SAN			3 0 Y T 1 Y T 1 Y T 1 S 0 E 1 1 E 1 1	DKP DKP DKP DKP	VF VF VF VF VF KA(β 2 7 RG 7 RG 7 RG 7 RG 7 RG 7 RG	4 0 CA CA CA CA CA A K	YT YT YT YT DI:		SHI SHI SHI SHI SHI SHI SHI	η ⁹ 25 (FG (FG (FG (FG) (FG) (FG) (FG) (FG) (F	
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Figure S3. Protein sequence alignment of *PhlC*. Sequences of *PhlC* from *P. protegens* (*Pp*), *P. brassicacearum* (*Pb*) and *P. fluorescens* (*Pf*-5) were aligned to *Pseudomonas* sp. YGJ3.



Figure S4. Protein sequence alignment of *PhlB*. Sequences of *PhlB* from *P. protegens* (*Pp*), *P. brassicacearum* (*Pb*) and *P. fluorescens* (*Pf*-5) were aligned to *Pseudomonas* sp. YGJ3.

2.2. Cloning of *Pb*ATaseWT (pEG330) and *Pp*ATaseWT (pEG331)

PCR-amplification of the wild-type *phlACB* operon. The ATase encoding *phlACB* operon (approx. 2770 bp) was amplified from the genomic DNA of *P. protegens* or *P. brassicacearum*. The genomic DNA was isolated according to the manufacture's protocol (PureLink® Genomic DNA Minikit, Thermo Fischer). The following primers were used (restriction site underlined):

<i>Pb</i> ATase-FW:	5'-ATATA <u>GGTACC</u> ATGAATAAAGTAGGAATTGTG-3'
PbATase-REV:	5'-ATATA <u>GGATCC</u> TTATTTCACCAGTACAAACTTATAG-3'
PpATase-FW	5'-ATATA <u>GGTACC</u> ATGAACGTGAAAAAGATAGGTATTG-3'
PpATase-REV	5'-ATATAGGATCCTTATATATCGAGTACGAACTTATAAG-3'

The PCR reaction mixture consisted of the following components:

- $26 \,\mu L$ H₂O sterile
- 10 μ L Phusion GC buffer (5×)
- $1 \ \mu L$ template (genomic DNA)
- 5 μ L primer forward (5 nmol μ L⁻¹)
- 5 μ L primer reverse (5 nmol μ L⁻¹)
- 1.5 µL DMSO (3 vol%)
- $1 \ \mu L$ dNTPs (0.2 nmol μL^{-1})
- 0.5 μ L Phusion DNA polymerase (2 U μ L⁻¹)

The following PCR program was used:

1× 98 °C 2:00 min

	^{98 °C} (0:20 min
25×	{ 58 °C	0:15 min
	^C 72 °C	2:00 min
$1 \times$	72 °C	3:00 min
	4 °C	∞

Column purification of the PCR-products was performed (Qiagen®-PCR purification kit). The approximate concentration of the PCR products was determined by agarose gel electrophoresis.

Restriction and ligation. The PCR products $(0.2 \ \mu g)$ and the pASK-IBA3plus vector backbone $(1 \ \mu g)$ were digested with *KpnI* and *Bam*HI (FastDigest, Thermo Fischer). The DNA was gel-purified prior to ligation.

The ligation consisted of the following components:

 $12 \ \mu L$ H2O sterile $4 \ \mu L$ insert $1 \ \mu L$ vector $2 \ \mu L$ ligation buffer (10×) $1 \ \mu L$ T4 ligase

The reaction was incubated overnight at 4 °C. 5 μ L of the ligation mix was transformed into *E. coli* DH5a and streaked onto LB plates containing 100 μ g mL⁻¹ ampicilin for selection. Isolated plasmids (QIAprep Spin Miniprep Kit, Qiagen®) of randomly picked clones were controlled by restriction digest and sequencing.

2.3. Cloning of *Pp*ATaseCH (pEG332)

The genes *phlA*, *phlC* and *phlB* originating from *P. protegens* were codon-harmonized by manually matching the codon-frequency of *Pseudomonas* to *E. coli*. Ribosomal binding sites (RBS) were introduced at the 5'-end of each *phl* gene. The optimized *phl** genes were ordered as gene strings (gBlocks®, IDT). Cloning of the gene strings into the pASK-IBA3plus expression vector was accomplished by Gibson assembly and overlap extension PCR (OExPCR).

Gibson cloning. A four-fragment Gibson assembly [5] between the pASK-IBA3plus vector (*Eco*RI/*Bam*HI digested, gel-purified) and the gene strings, *phlA**, *phlC** and *phlB** was carried out (Figure S5 and Table S4) using the Gibson assembly master mix® (New England Biolabs).



Figure S5. Cloning strategy. Four-fragment Gibson assembly of the optimized *phl** genes and the pASK-IBA3 backbone.

The following amounts were applied (Table S4):

Table S4. Calculations	for the Gibson	assembly sample	e preparation.
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Fragment	Size (bp)	pmol	Mass (ng)	
pASK-IBA3plus vector	3226	0.048	100	
phlA*	1238	0.048	38.4	
$phlC^*$	1346	0.048	41.7	
phlB*	590	0.048	18.3	

The assembly was performed according to the manufacture's protocol followed by direct transformation into *E*. *coli* DH5α.

Colony-PCR. Gibson assembled clones were verified by colony-PCR using primers which flank the desired insert:

IBA3-FW:	5'-GAGTTATTTTACCACTCCCT-3'
IBA3-REV:	5'-CGCAGTAGCGGTAAACG-3'

The PCR reaction mixture consisted of the following components:

7.5 μL H ₂ C) sterile	
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- 12.5 μL DreamTaq PCR-mastermix (2×)
- 2.5 μ L primer forward (5 nmol μ L⁻¹)
- 2.5 μ L primer reverse (5 nmol μ L⁻¹)

The following PCR program was used:

$1 \times$	95 °C	3:00 min
(- 94 °C	0:30 min
25× {	58 °C	0:30 min
ι	∽ 72 °C	2:50 min
$1 \times$	72 °C	5:00 min
	4 °C	œ

Selected clones were restreaked onto LB plates containing 100 μ g mL⁻¹ ampicillin for selection and the isolated plasmids were sequenced. Misassembled DNA stretches within *phlA** and *phlC** of the Gibson assembled plasmid pEG332_C20 (Figure S6, a) were corrected by OEx PCR to establish the correct *phlACB** construct (pEG332).

OExPCR. The OExPCR consisted of 3 steps: (i) extension PCR 1 & 2, (ii) overlap extension PCR, (iii) purification PCR [4] (Figure S6, b).



Figure S6. Cloning strategy to "repair" the defective pEG332_C20 plasmid. (**a**) Defective pEG332_C20 obtained *via* Gibson assembly containing random DNA stretches (\approx 100 bp) within *phlA**. (**b**) Overview of the OExPCR to establish the correct *phlACB** construct (pEG332).

(i) Extension PCR. Areas of homology (OE-sequences) required for the subsequent overlap PCR were introduced to the flanking regions of the *phlA** and *phlC** gene strings. The following primers were used (restriction sites underlined; RBS small letters):

OE1ATaseCH-FW: 5'-ATATAA<u>GAATTC</u>aaggagatatacataTGATGAATGTGAAGAAAATAGGTATCGTTAGC-3' OE2ATaseCH-REV: 5'-CGCTGACCGCGTACCTCTAAGGTACCaaggagatatacataTGATGTGCGCACGTCGCG-3' OE3ATaseCH-FW: 5'-TGCGCACATCAtatgtatatctccttGGTACCTTAGAGGTACGCGGTCAGCGCATAATC-3' OE4ATaseCH-REV: 5'-ATATAT<u>GAATTC</u>GCCGAGACGGCCATG-3' The PCR reaction mixture consisted of the following components:

- $25 \,\mu L$ H₂O sterile
- 10 μ L Phusion GC buffer (5×)
- 2.5 µL template (2.5 ng, *phlA** or *phlC** gene strings)
- 2.5 μ L primer forward (5 nmol μ L⁻¹)
- 2.5 μ L primer reverse (5 nmol μ L⁻¹)
- 1.5 µL DMSO (3 vol%)
- 5 μ L dNTPs (0.2 nmol μ L⁻¹)
- 0.5 μL $\,$ Phusion DNA polymerase (2 U $\mu L^{\text{-1}})$

The following PCR program was used:

$1 \times$	95 °C	0:45 min
ſ	- 94 °C	0:10 min
25× {	60 °C	0:20 min
l	72 °C	0:40 min
$1 \times$	72 °C	3:00 min
	4 °C	∞

The products of the extension PCR (*phlA**_*OE* & *phlC**_*OE*) were gel-purified, blunt-end ligated into pJET1.2 according to the manufacture's protocol (CloneJET-PCR Cloning Kit, Thermo Scientific) and transformed into *E. coli* DH5 α . Isolated plasmids of selected clones were sent for sequencing.

(ii) Overlap PCR. The products of the extension PCR, *phlA*_OE* and *phlC*_OE*, were spliced together in a primerless overlap PCR yielding *phlAC*_OE* (15 cycles). The amounts of template DNA were calculated based on 10.0 ng of the biggest fragment using the following equation:

$$m_{\text{Insert_small}} = \frac{m_{\text{insert_big}}[ng] * \text{size}_{\text{insert_small}}[bp]}{\text{size}_{\text{insert_big}}[bp]}$$

The PCR reaction mixture consisted of the following components:

32.3 µL H₂O sterile

10 µL	Phusion GC buffer $(5\times)$
1 μL	template 1 (10 ng, <i>phlA*_OE</i>)
0.75 µL template	e 2 (7.5 ng, <i>phlC</i> *_ <i>OE</i>)
1.5 μL	DMSO (3 vol%)
4 μL	dNTPs (0.2 nmol μ L ⁻¹)
0.5 μL	Phusion DNA polymerase (2 U $\mu L^{\text{-1}})$

The following PCR program was used:

$1 \times$	98 °C	0:30 min
ſ	98 °C	0:10 min
15× {	60 °C	0:20 min
Ĺ	72 °C	0:40 min
$1 \times$	72 °C	7:00 min
	4 °C	∞

(iii) **Purification PCR**. Flanking primers (OE1ATaseCH-FW & OE4ATaseCH, *vide supra*) were directly added to the overlap PCR mix in order to amplify the spliced gene product *phlAC_OE* (20 cycles).

The PCR reaction mixture consisted of the following components:

50 µL	overlap-PCR mix	
5.65 μ L H ₂ O ste	rile	
4 μL	Phusion GC buffer $(5\times)$	
4 μL	primer forward (5 nmol μ L ⁻¹)	
4 μL	primer reverse (5 nmol μL^{-1})	
0.6 μL	DMSO (3 vol%)	
1.25 μL dNTPs (0.2 nmol μL ⁻¹)		
0.5 μL	Phusion DNA polymerase (2 U $\mu L^{\text{-1}})$	

The following PCR program was used:

$1 \times$	98 °C	0:30 min
ſ	- 98 °C	0:10 min
20× {	60 °C	0:20 min
l	∼ 72 °C	0:40 min
$1 \times$	72 °C	7:00 min
	4 °C	∞

The entire reaction mixture was loaded onto an agarose gel and the desired gene product $phlAC^*OE$ (approx. 1986 bp) was gel-purified (Figure S7).

: OK	
	←1986 bp

Figure S7. Different products obtained by OExPCR. The strong band (\approx 1986 bp) belongs to the desired gene product *phlAC**_*OE*.

Restriction and ligation. The misassembled DNA stretch in pEG332_C20 was removed by restriction digest (*Eco*RI). The remaining backbone pEG332_C20 was recovered and gel-purified. Ligation with *phlAC*_OE* finally established the correct *phlACB** construct (pEG332).

The ligation consisted of the following components:

8.7 μL	insert (186 ng, <i>phlAC*_OE</i>)
4 μL	vector (100 ng, pEG332_C20)
4.3 μL	H ₂ O sterile
2 μL	ligation buffer (10×)
1 μL	T4 ligase

The reaction was incubated overnight at 4 °C. 5 μ L of the ligation mix was transformed into *E. coli* DH5 α and streaked onto LB plates containing 100 μ g mL⁻¹ ampicillin for selection. Isolated plasmids of randomly picked clones were controlled by restriction digest and sequencing.

2.4. Cloning of *Pp*ATaseCH (pCAS1)

Restriction and ligation. Cloning of the codon-harmonized *phlACB** construct from pEG332 into the T7-regulated pCAS1 expression vector was accomplished by restriction digest with *Eco*RI and *Bam*HI (1 μ g DNA). The fragments were gel-purified prior to ligation.

The triple ligation (1:1:1 ratio) consisted of the following components:

6.1 μL	insert 1 (30.3 ng)
4.6 μL	insert 2 (60.6 ng)
2.3 μL	vector (100 ng)
4 μL	H ₂ O sterile
2 μL	ligation buffer (10×)
1 μL	T4 ligase

The reaction was incubated overnight at 4 °C. 5 μ L of the ligation mix was transformed into *E. coli* DH5 α and streaked onto LB plates containing 100 μ g mL⁻¹ ampicillin for selection. Isolated plasmids of randomly picked clones were controlled by restriction digest and sequencing.

3. SDS-PAGE analysis

Cell-free extract was analyzed by SDS-PAGE (Figure S8)



Figure S8. SDS-PAGE analysis of the cell-free *E. coli* extract containing the recombinant *Pp*ATaseWT, *Pb*ATaseWT or *Pp*ATaseCH. Empty *E. coli* BL21 (DE3) host cells served as positive control (C). The ATase encoding genes *phlA*, *phlC* and *phlB* of all ATases were overexpressed in soluble form.

4. Alternative ATase Preparations and Storage Types

Lyophilized cells/KPi or PBS. The harvested cells were washed and resuspended in KPi-buffer (50 mM, pH 7.5) or PBS-buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄), shock-frozen in liquid nitrogen and lyophilized. The cells were stored at 4 °C (optional: inert storage under Ar) until further use for biotransformations.

Lyophilized cell-free extract. The harvested cells were suspended in buffer (7 mL buffer to 1 g wet cells; KPibuffer, 50 mM, pH 7.5) and disrupted to obtain the cell-free extract. The cell-free extract was shock-frozen in liquid nitrogen, lyophilized and stored at 4 °C (optional: inert storage under Ar) until further use for biotransformations.

Cell-free extract. The harvested cells were suspended in buffer (7 mL buffer to 1 g wet cells; KPi-buffer, 50 mM, pH 7.5) and disrupted to obtain the cell-free extract. The cell-free extract was shock-frozen in liquid nitrogen and stored at 4 °C, -20 °C or -80 °C until further use for biotransformations.

Culture media: LB-, TB- and YENB-media.

LB-media (*1 L*): 10 g tryptone (OxoidTM), 7 g NaCl (Roth), 5 g yeast extract (OxoidTM), optional: 1 mM ZnCl₂, fill up to 1 L with H₂O, autoclave.

TB-media (*1 L*): 12 g tryptone (OxoidTM), 24 g yeast extract (OxoidTM), 4 mL glycerol, fill up to 900 mL with H₂O, autoclave. Add 100 mL of a sterile solution of 0.17 M KH₂PO₄, 0.72 M K₂HPO₄. *YENB-media* (*1 L*): 0.8% nutrient broth (DifcoTM), 0.75% Bacto yeast extract (DifcoTM), 12 N NaOH (adjust pH 7.5), fill up to 1 L with H₂O, autoclave.

5. Modified procedure to test the impact of bivalent metals

The chloride salt of Ca^{2+} , Mg^{2+} , Zn^{2+} , Cu^{2+} , Co^{2+} , Mn^{2+} , Sr^{2+} or Ni^{2+} (5.0 or 8.0 mM final concentration) was added to the reaction mixture containing ATase and substrate (Figure S9).



Figure S9. The influence of bivalent metals on the biocatalytic reaction catalyzed by PpATaseCH. Chloride salts were added to the reaction at different concentrations, *i.e.* 5.0 mM (black columns) or 8.0 mM (red columns). The control reaction was performed in the absence of metal salts (dashed grey line). Assay conditions: Lyophilized cell of *E. coli* extract containing the recombinant PpATaseCH (20 mg), HEPES-buffer (50 mM, pH 7.5), solution of M^{2+} (5.0 mM or 8.0 mM, prepared in HEPES-buffer using the corresponding metal chloride salt MCl₂), resorcinol (**1b**, 10 mM), DAPG (15 mM), 35 °C, 30 minutes, 750 rpm.

The bioacetylation of $\mathbf{8}$ with DAPG was performed as described in the manuscript. The influence of various temperatures on expression visibility are shown in Figure S10.



Figure S10. SDS-PAGE of the *Pp*ATaseCH and the *Pp*ATaseCH (pCAS1-construct) in comparison to the *Pp*ATaseWT after expression in the presence or absence of $ZnCl_2$ (1 mM) for 21 h at different temperatures: *Pp*ATaseCH 30 °C, Zn^{2+} , supernatant (lane 1), pellet (lane 2); *Pp*ATaseWT 30 °C, Zn^{2+} , supernatant (lane 3),

pellet (lane 4); PageRuler Prestained Protein Ladder (lane 5, 13, 26); *Pp*ATaseCH 37 °C, Zn²⁺, supernatant (lane 6), pellet (lane 7); *Pp*ATaseCH 25 °C, Zn²⁺, supernatant (lane 8), pellet (lane 9); *Pp*ATaseCH 20 °C, Zn²⁺, supernatant (lane 10), pellet (lane 11); *Pp*ATaseCH-pCAS1 37 °C, Zn²⁺, supernatant (lane 12), pellet (lane 14); *Pp*ATaseCH-pCAS1 25 °C, Zn²⁺, supernatant (lane 15), pellet (lane 16); *Pp*ATaseCH-pCAS1 20 °C, Zn²⁺, supernatant (lane 17), pellet (lane 18); negative control, empty pASK-IBA3 vector (lane 19); *Pp*ATaseCH 37 °C, w/o Zn²⁺, supernatant (lane 20), pellet (lane 21); *Pp*ATaseCH 25 °C, w/o Zn²⁺, supernatant (lane 22), pellet (lane 23); *Pp*ATaseCH 20 °C, Zn²⁺, supernatant (lane 24), pellet (lane 25).

Different water-immiscible (toluene, cyclohexane), moderately water-miscible (MTBE, DIPE, Et₂O, EtOAc), aprotic water-miscible (DMSO, DMF, THF, 1,4-dioxane, acetone, MeCN) and protic water-miscible (MeOH, EtOH, glycerol, ethylene glycole) solvents were tested for the bioacetylation of model substrate **8** either at 5 vol% (Figure S11, black columns) or 20 vol% (Figure S11, grey columns) and the compatibility with PpATaseCH was determined based on the formation of *C*-acetyl product **9**.



Figure S11. Co-solvent-study for the acetylation of 8 employing *Pp*ATaseCH at 5 vol% (black columns) or 20 vol% (grey columns) of solvent. Assay conditions: Cell-free *E. coli* extract containing the recombinant *Pp*ATaseCH (vol. \equiv to 20 mg lyophilisate), KPi-buffer (50 mM, pH 7.5), resorcinol (**8**, 10 mM), DAPG (15 mM), co-solvent (5 or 20 vol%), 35 °C, 30 minutes, 750 rpm.

6. Modified procedure to test the impact of inhibitors/additives

A small aliquot of cell-free *E. coli* extract containing the recombinant ATase (50 μ L) was pretreated with the respective inhibitor/additive for 40 minutes at 28 °C: dithiothreitol (DTT, 0.5 or 2 mM), 2-mercaptoethanol (β -Met, 1 or 2 mM), phenylmethanesulfonyl fluoride (PMSF, 1 mM), iodoacetic acid (IAA, 1 or 2 mM), p-chloromecuribenzoic acid (pCMB, 1 mM), diethylpyrocarbonate (DEPC, 2 or 3 mM), EDTA (5 mM), Triton-X100 (0.5 w/v%), Tween-40 (0.5 w/v%). The residual activity of the pretreated ATase was determined by performing the bioacetylation of 8 with DAPG for 30 minutes at 35 °C as described in manuscript.

Additionally, the effect of phloroglucinol (PG, 10 mM), resorcinol (8, 10 mM), monoacetylphloroglucinol (MAPG, 15 mM), 2,4-dihydroxyphloroglucinol (DAPG, 15 mM) was examined. A solution containing the recombinant ATase (50 μ L), KPi-buffer (50 mM, pH 7.5, total volume: 0.5 mL), phloroglucinol (PG, final conc. 10 mM) or resorcinol (8, final conc. 10 mM) was incubated for 40 minutes at 28 °C. After this time, the bioacylation was started by addition of DAPG (1.58 mg, 0.0075 mmol, 15 mM final concentration) dissolved in DMSO (50 μ L). The final reaction mixture (0.5 mL, 10 vol% DMSO) was shaken for 30 min at 35 °C and 750 rpm in an Eppendorf benchtop shaker. The reaction was aborted by addition of HPLC-grade MeCN (0.45 mL) and vigorous shaking. The precipitated protein was removed by centrifugation (20 min, 14,000 rpm, 18,407 x g) and the supernatant (800 μ L) was directly subjected to HPLC for determination of conversions.

In the case of MAPG (final conc. 15 mM) or DAPG (final. Conc. 15 mM) after incubation with enzyme, the bioacylation was started by addition of resorcinol (8, 10 mM final conc.) dissolved in DMSO (50 μ L).

7. Synthesis of 2,4-diacetylphloroglucinol (DAPG, 5)

 $\begin{array}{c} O \quad OH \quad O \\ 1 \\ 1b \quad 1a \quad 3a \quad 3b \\ HO \quad 6 \quad 4 \quad OH \end{array}$

 $HO_{5}^{6} HO_{5}^{4} OH$ According to a literature procedure,[4] phloroglucinol (500 mg, 4.0 mmol) was dissolved in BF₃·2CH₃COOH (2.5 mL, 18.0 mmol) and the resulting mixture was refluxed for 3 h. After cooling the mixture to room temperature, a solution of 0.5 M aqueous KOAc (50 mL) was added dropwise and stirring was continued for further 30 minutes. The crude precipitate was filtered and recrystallized from MeOH/H₂O (1:1) affording 2,4-diacetylphloroglucinol **5** as orange needle-shaped crystals (767 mg, 3.65 mmol, 91 %). R_F = 0.8 (CHCl₃/MeOH, 80:20), m.p 143-145 °C (173-174 °C). NMR data is in accordance with literature.[3] ¹H-NMR (300 MHz, DMSO-*d*6): δ [ppm] = 2.57 (s, 6 H, 1b-H, 3b-H), 5.85 (s, 1 H, 5-H), 13.19 (s, 2 H, Ar-OH), 16.27 (s, 1 H, Ar-OH). ¹³C-NMR (75 MHz, DMSO-*d*6): δ_{C} [ppm] = 32.97 (C-1b), 95.05, 104.0, 169.1, 171.6 (4 × arom. C), 204.0 (C-1a). GC-MS (EI⁺, 70 eV): m/z (%) = 210 [M+] (67), 195 [C₉H₈O5⁺] (100), 177 [C₁₀H₉O₃⁺] (64).

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