Supplemental Tables and Figures

2 Table S1. List of primers

1

3 Chromosomal sequences are in upper cases and restriction sites are underlined.

Primers	Sequence 5'- 3'	Purpose
Ebm732	GCGGATATATGCCGTGTTGTTAAAAAAGTCTTGAGTCCAGGGTGtccatggaaaagagaag	IacP_TAP
Ebm734	ATAACAATTAATCTTATTCAATTGTTGTCAAGCGAGAGAAAAATAcatatgaatatcetcettag	tag
Ebm735	gaagaatteATGGCTTGCACATGCAGCGTT	iacP_TAP
Ebm736	ATCATCATATATTGCAGCCATAC	cloning
Ebm798	GATCTTTACGCTGACACATTGGATTTAATTG	IacP _{S38T}
Ebm799	CAATTAAATCCAATGTGTCAGCGTAAAGATC	mutagenesis
Ebm674	tctagaattcATGAATATGGATATTGAAGCAAGAGTC	iacP cloning
Ebm675	ttg <u>ctcgag</u> CTACACCCTGGACTCAAGAC	(2H, pKO3)
Ebm773	gaagaatteATGGCGATTCTCGGCCTGGGA	acpS cloning
Ebm774	ctc <u>ctcgag</u> CTAACTTTCCAGAATGACCGT	$(2H, pP_{TET})$
Ebm775	gaagaatteATGCTGACATCTCATTTTCCC	entD cloning
Ebm776	ctc <u>ctcgag</u> TTATCGGGGTATTGCGCTAAG	$(2H, pP_{TET})$
Ebm777	gaagaatteATGTACCAGGTCGTTCTGGGA	acpT cloning
Ebm778	ctc <u>ctcgag</u> TCATAGCGCTTTCGTTATCGT	$(2H, pP_{TET})$
Ebm779	gaagaatteATGGCGACTCACTTTGCCAGA	<i>yieE</i> cloning
Ebm780	ctc <u>ctcgag</u> TTATGAACGATTAAGCGTATA	$(2H, pP_{TET})$
Ebm904	GGCGGTGGCTGAAATGAAACGTTTGATGCTGGAAGCGCGCGGCTAAtcattggctggcaccaagcag	P _{BAD} acps
Ebm905	GCGGGCAATCTCTACAATATCCGTTCCCAGGCCGAGAATCGCCATcgtttcactccatccaaaaaaacggg	
Ebm133	gaccggatecAAATCACTGGCGCGCAAG	pKO3iacP
Ebm959	CGAGTTTTGATAGGAAATTTAAGAGTATGAATATGGATATTGAAGCAAGAG	
Ebm960	CTCTTGCTTCAATATCCATATTCATACTCTTAAATTTCCTATCAAAACTCG	

4

5 Table S2. List of plasmids

Name	Lab code	Description	Reference
pKD46	pEB267	Amp ^R , repA101ts & oriR101	(17)
pJL72	pEB793	Amp ^R , Kan ^R	(18)
pKT25link	pEB354	Kan ^R , p15A ori, Plac, T25	(20)
pT25_ACP	pEB375		(24)
pT25_ACP _{S36T}	pEB885		(24)
pT25_IacP	pJV4		This work
pT25_AcpS	pJV9		This work
pT25_EntD	pJV10		This work
pT25_AcpT	pJV11		This work
pT25_YieE	pJV12		This work
pT25_IacP _{S38T}	pJV17		This work
pUT18Clink	pEB355	Amp ^R , colE1 ori, Plac, T18	(20)
pT18_ACP	pEB379		(20)
pT18_ACP _{S36T}	pEB612		(20)
pT18_IacP	pJV1		This work
pT18_AcpS	pJV13		This work
pT18_EntD	pJV14		This work
pT18_AcpT	pJV15		This work
pT18_YieE	pJV16		This work
pT18_IacP _{S38T}	pJV18		This work
pUC18_IacP_TAP	pJV21	Amp ^R , pMB1 ori, MCS <i>lacZa</i>	This work
pUC18_IacP _{S38T} _TAP	pJV22		This work
pP _{TET}	pEB1242	Amp ^R , colE1 ori, P _{TET} , 6His	pASK-IBA37plus, IBA
pP _{TET} acpS	pJV37		This work
pP _{TET} entD	pJV38		This work
pP _{TET} acpT	pJV39		This work
pP _{TET} yieE	pJV40		This work
рКО3	pEB232	Cm ^R , repA(ts) ori, M13 ori, sacB	(22)
pKO3acpP	pEB1334		(30)
pKO3iacP	pEB1453		This work

7 Figure Legends

- Fig. S1: Post-translational modifications occurring on the acyl carrier protein ACP. Components
 are indicated on top and designations of ACP forms are indicated at the bottom.
- 10 Fig. S2: Purified IacP_CBP and IacP_{S38T}_CBP recovered using the TAP method, ran on SDS-
- 11 PAGE 15% and stained with Coomassie Blue.
- 12 Fig. S3: Phosphopantetheinyl transferase protein sequence alignment. The multiple sequence
- 13 alignment has been produced using ClustalW and displayed using Jalview. The consensus sequences
- 14 of the PPTase enzyme family defined by Lambalot and collaborators are framed (11) and the
- alignment has been manually corrected in the N-terminal part to optimize their alignment.

16 Fig. S4: Growth phenotypes related to AcpS depletion and complementation

- 17 A. Growth phenotypes of the IacP_TAP (JV48) and P_{BAD}acpS IacP_TAP (JV68) strains onto agar LB
- 18 plates containing 0.2% arabinose (inducing condition of PBAD), 0.2% glucose (repressing condition
- 19 of P_{BAD}) or agar LB without any sugar addition (non-inducing condition of P_{BAD}). **B.** Growth
- 20 phenotypes of P_{BAD}*acpS* IacP_TAP harboring the different PPTases genes cloned into pP_{TET}, onto agar
- LB plates containing 0.2% arabinose (inducing condition of PBAD) or agar LB (non-inducing
- 22 condition of P_{BAD}).
- 23 Fig. S5 Complementation of *acpP* mutant strains

24 A. The E. coli ACP temperature-sensitive strain (ACP^{ts}) was transformed with the indicated 25 plasmids used for bacterial 2-hybrid and carrying *acpP* or *iacP* coding sequences fused to the 26 T18 fragment of adenylate cyclase. Transformants were spread on LB plates containing 27 ampicillin and incubated at 30°C or 42°C for 3 days. Only strains able to complement the ACP temperature-sensitive phenotype could grow at 42°C. B. Using phage P1, the 28 $\Delta acpP$::Kan^R allele was transduced into *E. coli* MG1655 bearing the empty plasmid pKO3 or 29 pKO3 carrying *acpP* or *iacP* under the control of the *acpP* promoter. Transductants were 30 spread on LB plates containing kanamycin and grown at 30°C. Only strains able to 31

- 32 complement the absence of ACP could integrate the $\Delta acpP$::Kan^R deletion and grow on
- 33 kanamycin selective medium.

FIGURE S1



FIGURE S2



FIGURE S3

		10	20	30	40	50	60	70
АсрТ/	1-192		LGKVSTLSAGO	T.PDAT.TAOAP-			VI.I.SRAI.SPI.F	EMVYG
YieE/	1-251	MATHEARC	TLTECOLVSTR	LSSSCHTEARN		LASPCLLAFL	MEMLYCISEL	ETTTO
EntD/	1-234	MT.TSHEDT.DEACH	RLHTVDEDASS	FREHDLLWLPH	HDRLRSACRI	KRKAEHLACR	TAAVHALREVO	
AcpS/	1-126							
- ·								
		80	90	100	110	120	130	140
AcpT/	1-192	EOCKPAESACAPL		ALLISDECEVC	CDTEVT			EHAEM
VioF/	1_251	AKCKPAFRDKNLP	FSTSYACNMV	GVALTTEGECC			PHSLERHPES	RNENT
Fn+D/	1_231	CMCDKROPLWPDGI	LFCSISHCATT	ALAVISBORIC			TELAPSTIDSD	
AcnS/	1_126				TDIVETABLE	TAVISRSCH	ERLARRVI.SDN	
перь/	1-120							
		150	160	170	180	190	200	210
Ларт/	1_102							210 ا T.T.POT
AcpT/	1-192	150 EAER <mark>P</mark> EQQLAAFWI	160 RIWTRKEAIVK	170 QR <mark>GG</mark> SAWQIVS	180 VDSTLPSALS		200 SLAVCTPTPT	210
AcpT/ YieE/	1-192 1-251	150 EAERPEQQLAAFW WVANQNDPNEARA(OASLLEEPLLALT	160 RIWTRKEAIVK QLITLRQSVLK	170 QR <mark>GG</mark> SAWQIVS LTG-DVMNDDF AFS-DRVTLPG	180 VDSTLPSALS RELQLLPVA	190 SVSQCQLDTLS RLKCAHVTQJ	200 SLAVCTPTPFT LEAVCDAEDVI	210 LT <mark>P</mark> QT VWSVT
AcpT/ YieE/ EntD/	1-192 1-251 1-234	150 EAER <mark>P</mark> EQQLAAFW WVANQNDPNEARA QASLLPFP-LALT	160 RIWTRKEAIVK QLITLRQSVLK LAFSAKESVYK	170 QRGGSAWQIVS LTG-DVMNDDF AFS-DRVTLPG	180 VDSTLPSALS RELQLLPVAC FNSAKVTSL FNOFFVEND	190 SVSQCQLDTLS GRLKCAHVTQI FATHISLHLLI	200 SLAVCTPTPFT LEAVCDAEDVI PAFAATMAERT	210 LTPQT VWSVT VRTEW
AcpT/ YieE/ EntD/ AcpS/	1-192 1-251 1-234 1-126	150 EAER <mark>P</mark> EQQLAAFWI WVANQNDPNEARA QASLLPFP-LALT ETHQQPVRFLA	160 RIWTRKEAIVK QLITLRQSVLK LAFSAKESVYK KRFAVKEAAAK	170 QRGGSAWQIVS LTG-DVMNDDF AFS-DRVTLPG AFGTGIRNGLA	180 VDSTLPSALS RELQLLPVA FNSAKVTSL FNQFEVFNDF	190 SVSQCQLDTLS GRLKCAHVTQJ FATHISLHLLJ EL <mark>G</mark> KPRLRLW(200 SLAVCTPTPFT LEAVCDAEDVI PAFAATMAERT SEALTLAEKLC	210 LT <mark>P</mark> QT VWSVT VRTEW VAHMH
AcpT/ YieE/ EntD/ AcpS/	1-192 1-251 1-234 1-126	150 EAERPEQQLAAFWI WVANQNDPNEARAG QASLLPFP-LALT ETHQQPVRFLA 220	160 RIWTRKEAIVK QLITLRQSVLK LAFSAKESVYK KRFAVKEAAAK	170 QRGGSAWQIVS LTG-DVMNDDF AFS-DRVTLPG AFGTGIRNGLA 240	180 VDSTLPSALS RELQLLPVA FNSAKVTSL FNQFEVFNDF 250	190 SVSQCQLDTLS RLKCAHVTQI FATHISLHLLI ELGKPRLRLWC 260	200 SLAVCTPTPFT LEAVCDAEDVI PAFAATMAERT GEALTLAEKLG	210 LT <mark>P</mark> QT VWSVT VRTEW VAHMH
AcpT/ YieE/ EntD/ AcpS/	1-192 1-251 1-234 1-126	150 EAERPEQQLAAFWI WVANQNDPNEARAA QASLLPFP-LALTI ETHQQPVRFLA	160 RIWTRKEAIVK QLITLRQSVLK LAFSAKESVYK KRFAVKEAAAK	170 QRGGSAWQIVS ITG-DVMNDDF AFS-DRVTLPG AFGTGIRNGLA 240	180 VDSTLPSALS RELQLLPVA FNSAKVTSL FNQFEVFNDF 250	190 SVSQCQLDTLS RLKCAHVTQI FATHISLHLLI ELGKPRLRLWC 260	200 SLAVCTPTPFT LEAVCDAEDVI PAFAATMAERT GEALTLAEKLG	210 ITTPQT IVWSVT VRTEW VAHMH
AcpT/ YieE/ EntD/ AcpS/ AcpT/	1-192 1-251 1-234 1-126	150 EAERPEQQLAAFWI WVANQNDPNEARAQ QASLLPFP-LALTI ETHQQPVRFLA 220 ITKAL	160 RIWTRKEAIVK QLITLRQSVLK LAFSAKESVYK KRFAVKEAAAK 230	170 QRGGSAWQIVS ITG-DVMNDDE AFS-DRVTLPG AFGTGIRNGLA 240	180 VDSTLPSALS RELQLLPVAC FNSAKVTSL FNQFEVFNDF 250 	190 SVSQCQLDTLS RLKCAHVTQI FATHISLHLLI ELGKPRLRLWC 260	200 SLAVCTPTPFT LEAVCDAEDVI PAFAATMAERT SEALTLAEKLC	210 LT <mark>P</mark> QT VWSVT VRTEW VAHMH
AcpT/ YieE/ EntD/ AcpS/ AcpT/ YieE/	1-192 1-251 1-234 1-126 1-192 1-251	150 EAERPEQQLAAFWI WVANQNDPNEARAG QASLLPFP-LALTI ETHQQPVRFLAI 220 ITKAL	160 RIWTRKEAIVK QLITLRQSVLK LAFSAKESVYK KRFAVKEAAAK 230 	170 QRGGSAWQIVS LTG-DVMNDDE AFS-DRVTLPG AFGTGIRNGLA 240 LQTRANEPTGR	180 VDSTLPSALS RELQLLPVAC FNSAKVTSL FNQFEVFNDF 250 LMRFAQLPAA	190 SVSQCQLDTLS RLKCAHVTQI TATHISLHLLJ ELGKPRLRLWC 260 	200 SLAVCTPTPFT LEAVCDAEDVI PAFAATMAERT SEALTLAEKLC	210 LT <mark>P</mark> QT VWSVT VRTEW VAHMH
AcpT/ YieE/ EntD/ AcpS/ AcpT/ YieE/ EntD/	1-192 1-251 1-234 1-126 1-192 1-251 1-251	150 EAERPEQQLAAFWI WVANQNDPNEARAG QASLLPFP-LALT ETHQQPVRFLA 220 ITKAL	160 RIWTRKEAIVK QLITLRQSVLK LAFSAKESVYK KRFAVKEAAAK 230 GKLGWKSLPD TRVPHDRSAP	170 QRGGSAWQIVS LTG-DVMNDDF AFS-DRVTLPC AFGTGIRNGLA 240 LQTRANEPTGR ASILSAIPR	180 VDSTLPSALS RELQLLPVAC FNSAKVTSL AFNQFEVFNDF 250 LMRFAQL <mark>P</mark> AA	190 SVSQCQLDTLS RLKCAHVTQI TATHISLHLLI ELGKPRLRLWC 260 	200 SLAVCTPTPFT LEAVCDAEDVI PAFAATMAERT SEALTLAEKLC	210 LT <mark>P</mark> QT VWSVT VRTEW VAHMH

FIGURE S4



FIGURE S5

