The *rv1184c* locus encodes Chp2, an acyltransferase in *Mycobacterium tuberculosis* polyacyltrehalose lipid biosynthesis

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SUPPLEMENTAL MATERIAL

Supplemental Table S1. Bacterial strains and plasmids used in this study

Supplemental Table S2. Primers used in this study

Supplemental Table S1. Bacterial strains and plasmids used in this study

Strain	Name	Genotype	Source
M. tuberculosis Erdman			
M. tuberculosis Erdman		Δ*chp2*	This work
M. tuberculosis Erdman		Δ*chp2::chp2*	This work
M. tuberculosis Erdman		Δ*papA3*	Ref [1]
M. tuberculosis Erdman		Δ*papA3::papA3*	Ref [1]
M. tuberculosis Erdman	jcm110	Δ*mmpL10*	J. Cox
M. smegmatis mc^2155			

Plasmids

Reference name	Name	Description	Source
	pMV261	Kn^r, pAL5000 origin, ColE1 origin, multiple cloning site, P_{hsp60}	Ref [2]
	pMV306	Kn^r, *int*, attA5, ColE1 origin, multiple cloning site	Ref [3]
	pJSC407	oriE, cosA; HygR flanked by loxP sites	Ref [4]
Δ*chp2* KO construct		pJSC407; HindIII-804bp (first 156bp of *chp1*)-Hyg^r- 813bp (last 157bp of *chp1*)-KpnI	This work
	pRibo-BsaHind	pMV261 derivative; P_{hsp60}-theophylline riboswitch; BsaI for cloning	Ref [5]
Chp2-3xFLAG	pRibo Chp2-3xFLAG	pRibo-BsaHind derivative; contains *chp2* fused to 3xFLAG	This work
AP cytosolic control	pMB111	pMV261 derivative; contains *phoA*	M. Braunstein
AP secreted control	pMB124	pMV261 derivative; contains *fbpB*(aa2-40)-*phoA*	M. Braunstein
βGal cytosolic control	pHsp-*lacZ*	pMWS114 derivative; P_{hsp60}-*lacZ*	Ref [5]
βGal secreted control	sec-lacZ	pMB124 derivative; P_{hsp60}-*fbpB*(aa2-40)-*lacZ*	Ref [5]
full-length Chp2-βGal		pRibo-BsaHind derivative; contains full-length *chp2* fused to *lacZ*	This work
N-term Chp2-βGal		pRibo-BsaHind derivative; contains *chp2* (aa1-39) fused to *lacZ*	This work
cat Chp2-βGal		pRibo-BsaHind derivative; contains *chp2* (aa26-359) fused to *lacZ*	This work

Reference name	Name	Description	Source
full-length Chp2-AP		pRibo-BsaHind derivative; contains full-length *chp2* fused to *phoA*	This work
N-term Chp2-AP		pRibo-BsaHind derivative; contains *chp2*(aa1-39) fused to *phoA*	This work
cat Chp2-AP		pRibo-BsaHind derivative; contains *chp2* (26-359) fused to *phoA*	This work
chp2		pMV306 derivative; contains 1kb upstream of *fadD21*; *chp2*	This work
mmpL10		pMV306 derivative; contains 1kb upstream of *pks3/4*; *mmpL10*	This work
$P_{native2kb}$-*mmpL10*		pMV306 derivative; contains 2kb upstream of *pks3/4*	This work
P_{hsp60}-*mmpL10*		pMV306 derivative; P_{hsp60}-*mmpL10*	This work
P_{gs}-*mmpL10*		pMV306 derivative; P_{gs}-*mmpL10*	This work
	2BT	LIC vector with N-terminal 6xHis, TEV protease site, SspI site	S. Gradia
Chp2-cat		2BT derivative; 6XHis-TEV-*chp2*(aa26-359)	This work

References

1. Hatzios, S. K., et al. (2009) PapA3 is an acyltransferase required for polyacyltrehalose biosynthesis in *Mycobacterium tuberculosis*, *J Biol Chem 284*, 12745-12751.
2. Stover, C. K., et al. (1991) New use of BCG for recombinant vaccines, *Nature 351*, 456-460.
3. Stover, C. K., et al. (1993) Protective immunity elicited by recombinant bacille Calmette-Guerin (BCG) expressing outer surface protein A (OspA) lipoprotein: a candidate Lyme disease vaccine, *J Exp Med 178*, 197-209.
4. Stanley, S. A., et al. (2003) Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system, *PNAS 100*, 13001-13006.
5. Seeliger, J. C., et al. (2012) A Riboswitch-Based Inducible Gene Expression System for Mycobacteria, *PLoS ONE 7*, e29266.

Supplemental Table S2. Primers used in this study

Name (purpose)	**Sequence** (cloning strategy)
pJSC407 Δ*chp2* (knockout)	(by assembly PCR across HygR cassette)
HindIII 5' flank	ggatccacgaagcttCGAGACACGGCACACCTTTG
5' flank-loxP	ACGCTTCAACCGCCAAAGTCggtggcccggtataacttcg
5' flank-loxP revcomp	cgaagttataccgggccaccGACTTTGGCGGTTGAAGCGT
loxP-3' flank	GAACTGTACCGGATTTCGGACGCTGCAACCACAGATCGACG
loxP-3' flank revcomp	CGTCGATCTGTGGTTGCAGCGTCCGAAATCCGGTACAGTTC
3' flank KpnI revcomp	gactagagggtaccGTTACCCGGTCATGCTGAGG
Δ*chp2* PCR (knockout confirmation)	
chp2 KO confirm REV	CGTCATGTTGTCTTGGTGGCTC
fadD21 FOR	GGCCACATAAAGAGTCGCTTCC
hygR confirm REV	CAGGCTCGCGTAGGAATCATC
Δ*mmpL10* PCR (knockout confirm.)	
papA3 FOR	GCTGAAGTCTGTGTTCCAACGAGTC
mmpL10 internal REV	GTCTTCGTGCGCGCTGACATC
chp2 REV*	GTCGATCGTACGCTAGTTAACCGTGGATCCGGTGCGAG
hygR confirm REV	*same as above*
pMV306 *chp2* (complement)	(in two cloning steps via XbaI-HindIII and HindII-ClaI)
5' fadD21 promoter XbaI	GCTCTAGAgggtgctgcggtgctg
fadD21 promoter 3' HindIII	CTTCATAAGCTTCATtggtgctacattaccgtttc
chp2 5' HindIII for pMV306	ccaATGAAGCTTAAGCGAGTGATTGCGGGAG
chp2 3' ClaI for pMV306	GTCGACATCGATTTAGCCGCCGAAGGCGG
2BT *chp2*-cat (*E. coli* expression)	(by InFusion into SspI-cleaved 2BT)
5’ chp2-cat FOR	GTACTTCCAATCCAATGCAAGCGAACCCGCGTACC
3’ chp2-cat REV	TTATCCACTTCCAATtcattaGCCGCCGAAGGCGG
chp2 S141A QC FOR	GTCGGCCTAgCCCAGGGTTCCCTCGTGCTC
chp2 S141A QC REV	GGAACCCTGGGcTAGGCCGACGGCCGCGG
chp2 SA (Ser141Ala mutagenesis)	(by site-directed mutagenesis)
S141A FOR	GTCGGCCTAgCCCAGGGTTCCCTCGTGCTC
S141A REV	GGAACCCTGGGcTAGGCCGACGGCCGCGG

* Originally designed as a cloning primer. Only underlined portion is complementary to *chp2*.