Supporting Information

The mycobacterial gene *cuvA/Rv1422* is required for optimal carbon-source utilization and virulence

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Supporting Methods

Time-lapse microscopy

600 μL sterile 1% agarose made in Middlebrook 7H9 medium containing 0.2% acetamide was poured into a rectangular chamber made of 4 Frame-Seals (Bio-Rad) on a glass slide and a coverslip was immediately placed over the agarose to smoothen the surface. After solidification of the agarose, the coverslip was removed and $5/6^{th}$ of the agarose pad was discarded. *M. smegmatis* containing plasmid pMV306Ac-*Rv1422-gfp* (RH558), grown in 7H9 broth to an $O.D_{600}$ of 0.5 and washed in minimal medium was then spotted on the remaining agarose pad. The chamber was closed with a new coverslip and the slide was incubated at $37^{o}C$ for 15 hrs before being observed under a TK spinning Disk Confocal microscope using 63x oil objective. The images were captured manually every 20 min using filters for GFP and phase contrast. The images were acquired using Slidebook 4 and a movie was created in imageJ.

Cellular Localization of PBP1

For localization of *M. smegmatis* PBP1 (encoded by *MSMEG_6900)*, a C-terminal MSMEG_6900-RFP fusion protein was expressed. The *MSMEG6900* gene was amplified by PCR from *M. smegmatis* genomic DNA using primers *MSMEG6900*-F and *MSMEG6900*-R. Similarly *rfp* was PCR-amplified using primers Rfp_F and Rfp_R. Overlap PCR was then carried out using the primers *MSMEG6900*-F and Rfp_R. The resulting fusion PCR product was cloned into the *NdeI* and *XbaI* sites in pMV261Ac 3' of the acetamidase promoter to obtain pMV261Ac-*MSMEG6900-rfp*. This construct was transformed into wild type and $cuvA_{Ms}$ deletion strains of M. smegmatis, cells were grown in liquid MM containing 0.2% glucose or 0.01% cholesterol for 6-8 hours and examined by DIC and fluorescence microscopy as described for localization of CuvA.

Table S1. Primers, plasmids and strains used in this study

Primer	Sequence
Rv1422AE	Ggatccattaatgaattcgatgaccgatggcatcgtcgcg
Rv1422rPac1	Cttctcctttgctagccatgttaattaagcgccacgcgtcgtcacccctcg
GFP3Pac1	Gacgacgcgtggcgcttaattaacatggctagcaaaggagaagaacttt
GFP4XK	Ggttctagaggtacctcagccatgtgtaatcccagcagcag
MSMEG3080-cond-1	Ctagtctagaacaggtggaccgggtatg
MSMEG3080-cond-2	Gttggcacagtccgttccttaattaagacgatgcgtcgtggtgtcat
MSMEG3080-cond-3	Ggaacggactgtgccaac
MSMEG3080-cond-4	Ctagtctagagactcggtgtcggggatg
MSMEG3080-us	Gcgcgtgctgcaccgggacctgggtcgcg
MSMEG3080-ds	Gctcagctcgtccttcacctcggctgtc
MSMEG3080-int-1	Gcgacgcatatgtgcttaattaacatgacaccacgcatcgtcgccctcgga
MSMEG3080-int-2	Ggacagatcgagaggggatgccccgtggcgaccatggtgatatcgacg
yrbE4A-F	Gtacaaggccgccgccttgatccaacaacttgcggttc
yrbE4A-R	Gcggatatcttactgcgccgagattcgcac
mCherry-F	Gcatgcttaattaagaaggagatatacatatggtgagcaagggcgaggag
mCherry-yrbE4A_R	Gatcaaggcggcggccttgtacagctcgtccatgc
mCherry_R	Gtcgcggatatctacttgtacagctcgtccatg
*MSMEG6900*_F	Ggaatccatatgaataacgaagggcgccac
*MSMEG6900*_R	Ggaggaggccatgttaattaacggaggcggcggggctccggg
Rfp_F	Tccgttaattaacatggcctcctccgaggacgtc
Rfp_R	Gcaagttctagatcaagcgccggtggagtggc
PbpA_F	Gaggatccatatgaacgcctctctgcgccg
PbpA_R	Ggccatgttaattaatggttccccctgcagtgc

Plasmid	Characteristics	Source
pRH1351	An Integrating vector that contains the counter-selection marker *sacB*, a gentamicin resistance gene, a catechol 2,3-dioxygenase gene (*xylE*) and a temperature sensitive mycobacterial replication origin.	(1)
pMV306Ac	A mycobacterial integrating vector containing the acetamidase promoter, Kan^R	This study
pMV306A*c-gfp*	An integrating vector having *gfp* cloned downstream to the acetamidase promoter,	This study

pMV306Ac-*Rv1422-gfp*	An Integrating vector having *Rv1422-gfp* cloned downstream to the acetamidase	This study
pJEB402	Integrating vector containing the *hsp70* (MOP) promoter, Kan^R	(2)
pJEB-*Rv1422*	*Rv1422* cloned downstream to MOP promoter in pJEB402, Kan^R	This study
pMind	Replicating shuttle vector with tet promoter, coli EI ori, pAL1500 ori, Kan^R, Hyg^R	(3)
pMind-MSMEG3080	MSMEG3080 cloned downstream to tet promoter in pMind, Kan^R	This study
pMV261-P_{tet}-*mCherry-yrbE4A*	*mCherry-yrbE4A* cloned downstream to tet promoter in replicating vector, Hyg^R, Kan^R	This study
pMV261-P_{tet}-*mCherry*	*mCherry* cloned downstream to tet promoter in replicating vector, Hyg^R	This study
pMV261Ac-*MSMEG6900-rfp*	*MSMEG6900-rfp* cloned downstream to the acetamidase promoter in replicating vector,	This study
pMV261Ac-*pbpA-rfp*	*pbpA-rfp* cloned downstream to the acetamidase promoter in replicating vector,	This study
pMV261Ac-*rfp*	*rfp* cloned downstream to acetamidase promoter in replicating vector, Hyg^R	This study

Strain	**Characteristics**	**Source**
E. coli Top10	Cloning strain	Invitrogen
M. smegmatis mc^2155	Wild type	(4)
M. tuberculosis H37Rv	Wild type	Lab collection
M. tuberculosis mc^26206	Δ*leuD*Δ*panCD* double auxoptroph of *M. tuberculosis* H37Rv	(5)
RH477	*MSMEG3080* deletion mutant in *M. smegmatis* mc^2155	This study
RH478	*Rv1422* deletion strain in *M. tuberculosis* H37Rv	This study
RH479	RH478 containing pJEB402 integrated at *attB* site, Kan^R , Hyg^R	This study
RH480	RH478 containing plasmid pJEB-Rv1422 integrated at *attB* site, Kan^R, Hyg^R	This study
RH481	Wild type strain harboring plasmid pJEB402 integrated at *attB* site, Kan^R	This study
RH558	Wild type *M. smegmatis* mc^2155 containing plasmid pMV306Ac-*Rv1422-gfp* integrated at *attB* site, Kan^R	This study
RH555	Wild type *M. smegmatis* mc^2155 containing pMV261Ac-*rfp*, Hyg^R	This study
RH661	Wild type *M. smegmatis* mc^2155 containing plasmid pMV261-P_{tet}-*mCherry-YrbE4A*, Hyg^R,	This study

RH741	Wild type *M. smegmatis* mc^2155 containing plasmid pMV261Ac-*pbpA-rfp*, Kan^R	This study
RH773	*MSMEG3080* deletion strain of *M. smegmatis* containing pMV261Ac-*MSMEG6900-rfp*, Kan^R	This study
RH861	Wild type *M. smegmatis* mc^2155 containing plasmid pMV261-P_{tet}-*mCherry*, Hyg^R	This study
RH862	*MSMEG3080* deletion strain of *M. smegmatis* containing pMV306-P_{tet}-*mCherry-YrbE4A*, Hyg^R, Kan^R	This Study
RH893	*M. tuberculosis* mc2-6206 containing pMV306Ac-*Rv1422-gfp* inserted at *attB* site,	This study

Supporting Methods References

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