

Supporting Information for:

Assessment of metabolic changes in *Mycobacterium smegmatis* wild type and *alr* mutant strains: evidence for a new pathway of D-alanine biosynthesis.

Darrell D. Marshall,^{1,†} Steven Halouska,^{1,†} Denise K. Zinniel,² Robert J. Fenton,² Katie Kenealy², Harpreet K. Chahal,² Govardhan Rathnaiah,² Raúl G. Barletta,^{2,3*} and Robert Powers,^{1,3*}

¹*Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304*

²*School of Veterinary Medicine and Biomedical Science, University of Nebraska–Lincoln, Lincoln, NE 68583-0905*

³*Center for Redox Biology, University of Nebraska-Lincoln, Lincoln, NE 68588-0662*

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Table S1: Proteins exhibiting a significant change in expression levels between TAM23 and mc²155 cells cultured in media without D-alanine.

#	Identified Proteins	Accession Number ^a	Molecular Wt.	Normalized Spectral Count	
				mc ² 155	TAM23
TAM23					
68	alanine dehydrogenase [Mycobacterium smegmatis str. MC2 155]	gi 118170099	39 kDa	0	28
99	adenosine deaminase [Mycobacterium smegmatis str. MC2 155]	gi 118169012	40 kDa	0	31
120	DeoR-family protein transcriptional regulator [Mycobacterium smegmatis str. MC2 155]	gi 118171152	35 kDa	0.92	12
42	alcohol dehydrogenase [Mycobacterium smegmatis str. MC2 155]	gi 118171987	38 kDa	0	86
55	regulatory protein, AsnC/Lrp [Mycobacterium smegmatis str. MC2 155]	gi 118173708	32 kDa	0	18
56	adenosylmethionine--8-amino-7-oxononoate transaminase [Mycobacterium smegmatis str. MC2 155]	gi 118175053	49 kDa	0	75
75	putative xylulose kinase [Mycobacterium smegmatis str. MC2 155]	gi 118173132	43 kDa	0	15
157	alcohol dehydrogenase [Mycobacterium smegmatis str. MC2 155]	gi 118171143	34 kDa	1	32
190	alcohol dehydrogenase, class IV [Mycobacterium smegmatis str. MC2 155]	gi 118172253	40 kDa	2	25
197	cytochrome P450 monooxygenase [Mycobacterium smegmatis str. MC2 155]	gi 118174179	50 kDa	2	15
208	phytoene dehydrogenase [Mycobacterium smegmatis str. MC2 155]	gi 118170654	55 kDa	2	10
245	eptc-inducible aldehyde dehydrogenase [Mycobacterium smegmatis str. MC2 155]	gi 118168881	56 kDa	5	17
299	alcohol dehydrogenase, iron-containing [Mycobacterium smegmatis str. MC2 155]	gi 118172943	46 kDa	0	17
506	putative acyl-CoA dehydrogenase [Mycobacterium smegmatis str. MC2 155]	gi 118171098	44 kDa	0	12
mc²155					
85	DNA-directed RNA polymerase, beta' subunit [Mycobacterium smegmatis str. MC2 155]	gi 118171055	147 kDa	18	1
100	ferredoxin-dependent glutamate synthase 1 [Mycobacterium smegmatis str. MC2 155]	gi 118169347	167 kDa	23	7
195	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase [Mycobacterium smegmatis str. MC2 155]	gi 118174880	38 kDa	16	1
199	ribosomal protein L20 [Mycobacterium smegmatis str. MC2 155]	gi 118172082	14 kDa	21	1
262	putative thiosulfate sulfurtransferase [Mycobacterium smegmatis str. MC2 155]	gi 118173744	33 kDa	16	0
275	CAIB/BAIF family protein [Mycobacterium smegmatis str. MC2 155]	gi 118173815	43 kDa	11	0
469	50S ribosomal protein L22 [Mycobacterium smegmatis str. MC2 155]	gi 118170402	16 kDa	11	0
mc²155 + TAM23					
73	methylmalonate-semialdehyde dehydrogenase [Mycobacterium smegmatis str. MC2 155]	gi 118171123	52 kDa	14	42

^aGenBank genInfo identifier

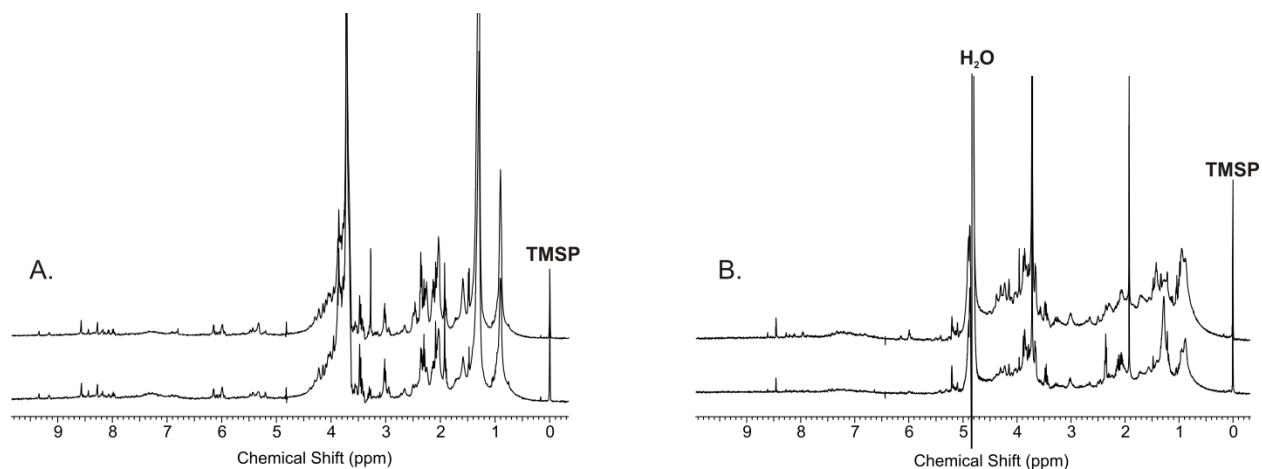


Figure S1. Representative examples of 1D ^1H NMR spectra of *M. smegmatis* metabolite extracts. 1D ^1H NMR spectra of metabolites extracted from (*top*) *alr* insertion mutant TAM23 compared to (*bottom*) wild type strain mc²155 (A) with and (B) without culture media supplemented with D-alanine.

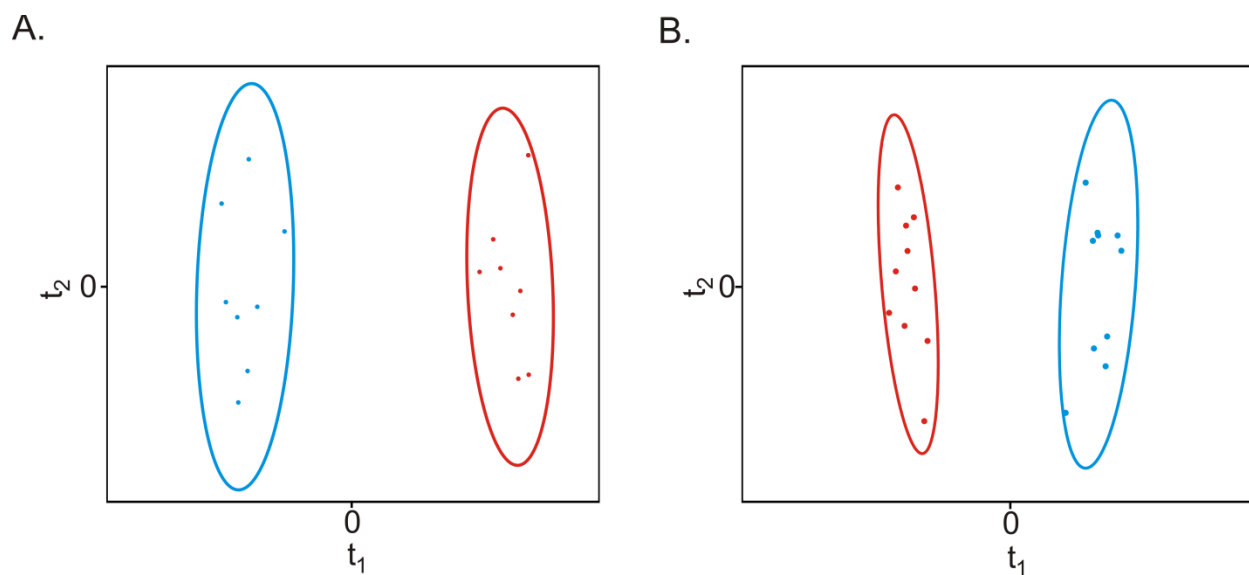


Figure S2. OPLS-DA model generated from the 1D ^1H NMR spectra obtained from *M. smegmatis* metabolite extracts. OPLS-DA scores plot comparing the mc²155 (blue) and TAM23 (red) metabolomes (A) without or (B) with media supplemented with D-alanine.

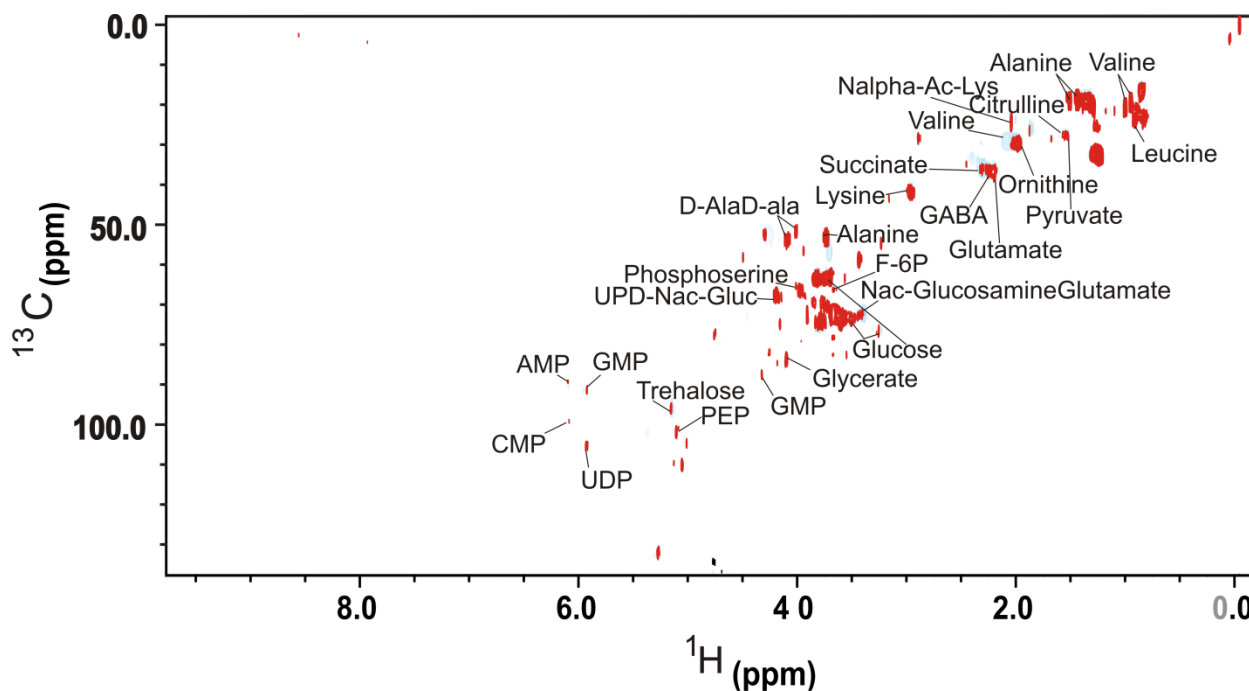


Figure S3. Overlay of 2D ^1H - ^{13}C HSQC₀ spectra from *M. smegmatis* metabolite extracts.

Metabolites were extracted from wild type strain *mc*²155 (blue) and *alr* insertion mutant TAM23 (red), which were grown in MADC supplemented with 100 μM of [$^{13}\text{C}_2$]-D-alanine. All of the labeled metabolites are derived from [$^{13}\text{C}_2$]-D-alanine and the absolute concentrations derived from the HSQC₀ experiment are summarized in Fig. 3.