

Supplemental Material

Genomic and transcriptomic studies of an RDX-degrading actinobacterium

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Table S1. Predicted ORFs that were revised on pGKT2

deleted	KTR9_4813, KTR9_4830, KTR9_4845, KTR9_4870, KTR9_4929, KTR9_4930
inserted	KTR9_5096, KTR9_5103, KTR9_5108, KTR9_5109, KTR9_5110, KTR9_5117, KTR9_5124, KTR9_5134, KTR9_5140, KTR9_5146, KTR9_5154, KTR9_5158, KTR9_5164, KTR9_5170, KTR9_5171, KTR9_5172, KTR9_5173, KTR9_5189, KTR9_5202, KTR9_5213, KTR9_5218, KTR9_5236, KTR9_5254

Table S2. Classes of metabolic pathways predicted to occur in *Gordonia* sp. KTR9, *G. bronchialis* 3410^T, and *R. jostii* RHA1^a.

Type of pathway	KTR9 (5.9 Mb)	3410 ^T (5.3 Mb)	RHA1 (9.7 Mb)
Fatty acid and lipid catabolism	5	4	6
Fatty acid and lipid biosynthesis	20	12	21
Amines and polyamines catabolism	14	7	17
Aromatic compound catabolism	10	7	34
Cofactors, prosthetic groups, electron carriers biosynthesis	58	50	54
Amino acid biosynthesis	50	34	44
Total	277	206	338

^aData from www.biocyc.org.

Table S3. Genes differentially regulated in KTR9 growing on RDX vs. NH_4^+ . (Fold-change > 2 and $P < 0.5$).

Locus tag	Annotation	Fold change	P-value
Up-regulated			
KTR9_0011	bifunctional deaminase-reductase domain-containing protein	2.03	0.45
KTR9_0014	Ribosomal protein L31	2.42	0.49
KTR9_0055	Predicted metal-dependent hydrolase	2.23	0.11
KTR9_0111	hypothetical protein	2.31	0.25
KTR9_0131	hypothetical protein	3.28	5.76E-04
KTR9_0136	Predicted membrane protein	2.34	2.14E-09
KTR9_0164	hypothetical protein	2.14	0.37
KTR9_0181	glyoxalase/bleomycin resistance protein/dioxygenase	2.12	0.49
KTR9_0302	hypothetical protein	5.59	0.26
KTR9_0310	Transcriptional regulator	2.73	0.19
KTR9_0314	hypothetical protein	2.36	0.03
KTR9_0337	hypothetical protein	2.19	0.28
KTR9_0416	hypothetical protein	2.03	1.31E-06
KTR9_0425	hypothetical protein	2.51	0.13
KTR9_0426	Flp pilus assembly protein, ATPase CpaF	2.17	0.2
KTR9_0427	type II secretion system F domain protein	2.31	0.38
KTR9_0428	Flp pilus assembly protein	2.4	0.43
KTR9_0432	hypothetical protein	3.29	0.15
KTR9_0435	Cold shock proteins	2.16	1.73E-03
KTR9_0440	Predicted transcriptional regulators	3.05	0.33
KTR9_0464	Transcriptional regulators	2.59	6.77E-03
KTR9_0519	Multisubunit Na^+/H^+ antiporter, MnhG subunit	10.93	0.07
KTR9_0574	Transcriptional regulator	3.37	0.01
KTR9_0691	hypothetical protein	2.24	0.01
KTR9_0696	endoribonuclease L-PSP	2.18	0.44
KTR9_0706	Predicted aminoglycoside phosphotransferase	2	0.3
KTR9_0732	Protein-tyrosine phosphatase	5.42	0.34

KTR9_0769	DNA-directed RNA polymerase specialized sigma subunit, sigma24 -like protein	2.38	3.74E-03
KTR9_0771	Transcriptional regulator	2.18	0.44
KTR9_0827	Acyl-CoA dehydrogenase	2.03	0.47
KTR9_0988	hypothetical protein	2.05	0.16
KTR9_0989	hypothetical protein	10.05	1.68E-08
KTR9_0990	permease for cytosine/purines, uracil, thiamine, allantoin transporter	86.19	1.55E-14
KTR9_0991	N-methylhydantoinase B / acetone carboxylase, alpha subunit	95.35	0
KTR9_0992	N-methylhydantoinase A / acetone carboxylase, beta subunit	83.9	8.22E-15
KTR9_1021	hypothetical protein	11.19	0.36
KTR9_1030	DNA-directed DNA polymerase	2.4	0.01
KTR9_1105	hypothetical protein	2.54	3.11E-04
KTR9_1134	hypothetical protein	9.83	0.14
KTR9_1173	conserved hypothetical protein, DUF1469 superfamily	2.57	0.25
KTR9_1189	Choline-glycine betaine transporter	2.73	0.22
KTR9_1190	FAD dependent oxidoreductase	4.47	0.29
KTR9_1191	formyltetrahydrofolate deformylase	11.19	0.36
KTR9_1192	Sarcosine oxidase, gamma subunit	8.14	0.46
KTR9_1193	sarcosine oxidase, alpha subunit	2.71	0.49
KTR9_1196	Flavodoxin reductases (ferredoxin-NADPH reductases) family 1	4.58	0.5
KTR9_1218	Proline dehydrogenase	2.79	0
KTR9_1219	NAD-dependent aldehyde dehydrogenases	4.2	0
KTR9_1257	Methylase of polypeptide chain release factors	2.54	0.37
KTR9_1260	ABC-type sugar transport system, ATPase component	2.22	0.39
KTR9_1262	Ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenases	2.83	0.27
KTR9_1265	ring hydroxylating dioxygenase, alpha subunit	2.07	0.06
KTR9_1266	ring hydroxylating dioxygenase, beta subunit	2.72	0.05
KTR9_1343	hypothetical protein	2.37	0.48
KTR9_1359	hypothetical protein	2.88	0.46
KTR9_1360	hypothetical protein	4.07	0.46

KTR9_1361	hypothetical protein	3.81	0.25
KTR9_1366	hypothetical protein	2.64	0.39
KTR9_1377	hypothetical protein	10.17	0.39
KTR9_1465	hypothetical protein	2.09	0.41
KTR9_1470	Predicted membrane protein	2.23	7.81E-05
KTR9_1473	phosphinothricin N-acetyltransferase	3.05	0.36
KTR9_1497	Flavodoxin reductases (ferredoxin-NADPH reductases) family 1	2.04	6.20E-04
KTR9_1532	hypothetical protein	7.29	0.09
KTR9_1544	Acyl dehydratase	3.39	0.08
KTR9_1566	Transposase and inactivated derivatives	3.05	0.36
KTR9_1567	Transposase and inactivated derivatives	2.75	0.37
KTR9_1612	Transcriptional regulators	3.52	0.18
KTR9_1622	Panθοthenate kinase	2.15	6.85E-12
KTR9_1661	transcription factor WhiB	2.43	2.44E-15
KTR9_1698	bacterioferritin-associated ferredoxin	2.89	8.37E-03
KTR9_1726	hypothetical protein	2.14	0.38
KTR9_1730	Formamidopyrimidine-DNA glycosylase	2.72	0.02
KTR9_1747	hypothetical protein	2.26	1.72E-03
KTR9_1756	Acyl-CoA dehydrogenases	4.83	7.08E-05
KTR9_1757	hypothetical protein	7.82	2.15E-04
KTR9_1758	large subunit of ring-hydroxylating dioxygenase	5.15	3.16E-05
KTR9_1759	hypothetical protein	4.95	5.21E-04
KTR9_1760	short-chain dehydorgenase/reductase	3.94	9.15E-03
KTR9_1761	Flavodoxin reductases (ferredoxin-NADPH reductases) family 1	4.92	0.03
KTR9_1765	Permease, MFS superfamily	3.98	3.84E-03
KTR9_1766	FMN-binding protein	4.91	3.08E-03
KTR9_1767	hypothetical protein	4.46	0.03
KTR9_2066	fatty acid desaturase type 2	3.23	3.65E-11
KTR9_2153	hypothetical protein	2.46	1.55E-09
KTR9_2169	hypothetical protein	2.25	1.15E-08
KTR9_2273	putative diaminohydroxyphosphoribosylaminopyrimidine deaminase, riboflavin biosynthesis	2.34	0.27
KTR9_2371	hypothetical protein	5.38	0.15

KTR9_2429	hypothetical protein	2.23	0.02
KTR9_2454	DNA G:T-mismatch repair endonuclease	3.05	0.43
KTR9_2586	hypothetical protein	3.39	0.38
KTR9_2600	Fe ²⁺ /Zn ²⁺ uptake regulation proteins	3.85	0.01
KTR9_2614	helix-turn-helix, AraC domain containing protein	2.68	0.11
KTR9_2615	bifunctional deaminase-reductase domain-containing protein	2.08	0.21
KTR9_2655	hypothetical protein	2.21	8.04E-04
KTR9_2686	Histone acetyltransferase HPA2 and related acetyltransferases	2.45	0.31
KTR9_2712	Arginine repressor	2.43	0.18
KTR9_2713	Ornithine carbamoyltransferase	2.57	2.65E-03
KTR9_2714	acetyloronithine aminotransferase	3.23	6.26E-03
KTR9_2715	Acetylglutamate kinase	3.35	1.29E-03
KTR9_2716	bifunctional ornithine acetyltransferase / N-acetylglutamate synthase	3.35	4.04E-04
KTR9_2717	N-acetyl-gamma-glutamyl-phosphate reductase	3.57	3.58E-03
KTR9_2724	Ribosomal protein L35	3.81	0.11
KTR9_2738	hypothetical membrane protein	2.31	0.29
KTR9_2744	hypothetical protein	4.41	0.26
KTR9_2752	Glycosyltransferase	2.25	0.18
KTR9_2753	hypothetical protein	2.26	0.21
KTR9_2819	Ferredoxin	3.89	0.09
KTR9_2825	ABC-type branched-chain amino acid transport systems, periplasmic component	2.6	0.43
KTR9_2826	hypothetical protein	10.68	0.21
KTR9_2828	Permeases, MFS superfamily	7.12	0.34
KTR9_2855	Enoyl-CoA hydratase \\ carnithine racemase	2.81	0.18
KTR9_2878	hypothetical protein	2.03	0.32
KTR9_2882	Predicted transcriptional regulators	2.15	0.29
KTR9_2926	hypothetical protein	2.14	0.04
KTR9_3086	Protein-tyrosine phosphatase	2.19	0.05
KTR9_3132	Predicted transcriptional regulators	2.11	0.03
KTR9_3133	hypothetical proteins	4.94	0.18
KTR9_3134	hypothetical protein	2.43	0.2

KTR9_3147	hypothetical protein	2.43	0.14
KTR9_3172	Transcriptional regulators	2.87	0.03
KTR9_3178	Transcriptional regulators	2.06	2.89E-10
KTR9_3179	H ⁺ /gluconate symporter and related permeases	2.57	4.54E-11
KTR9_3182	Alpha/beta hydrolase fold-3 domain protein	3.47	9.19E-04
KTR9_3183	Flavin reductase domain protein / FMN-binding protein	3.99	1.72E-04
KTR9_3184	Esterase / lipase	3.78	4.59E-05
KTR9_3185	Acyl-CoA dehydrogenases	4.3	3.06E-05
KTR9_3186	Acyl-CoA dehydrogenases	3.68	0.01
KTR9_3187	Coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase and related flavin-dependent oxidoreductases	4.03	0.03
KTR9_3188	Predicted flavoprotein involved in K ⁺ transport	4.62	0.01
KTR9_3189	Acyl-CoA synthetases (AMP-forming)/AMP-acid ligases II	5.57	0.01
KTR9_3190	Predicted aminoglycoside phosphotransferase	5.19	0.09
KTR9_3191	Acyl-CoA synthetases (AMP-forming)/AMP-acid ligases II	6.89	0.05
KTR9_3192	hypothetical protein	6.39	0.1
KTR9_3193	Enoyl-CoA hydratase / carnithine racemase	4.7	0.17
KTR9_3194	short-chain dehydrogenase/reductase	6.27	0.13
KTR9_3195	Acetyl-CoA acetyltransferase	4.75	0.14
KTR9_3196	hypothetical protein	7.63	0.15
KTR9_3197	Acyl dehydratase	6.87	0.19
KTR9_3240	Ribosomal protein L28	23.73	0.01
KTR9_3290	hypothetical protein	3.07	0.02
KTR9_3406	Coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase and related flavin-dependent oxidoreductases	2.03	0.26
KTR9_3428	Cytosine deaminase / adenosine deaminase	2.19	0.1
KTR9_3457	conserved hypothetical protein, DUF1707 superfamily	2.61	2.44E-03
KTR9_3467	hypothetical protein	2.48	6.99E-03
KTR9_3468	MoxR-like ATPases	2.8	0.03
KTR9_3469	hypothetical protein	2.93	0.05
KTR9_3503	hypothetical protein	2.21	0.25
KTR9_3552	Biotin carboxyl carrier protein	5.2	0.11
KTR9_3573	FAD/FMN-containing dehydrogenases	4.53	0.12

KTR9_3574	DNA-binding HTH domain-containing proteins	2.02	0.02
KTR9_3596	hypothetical protein	2.34	6.47E-03
KTR9_3683	ABC-type multidrug transport system, permease component	2.03	0.41
KTR9_3689	response regulator receiver	2.03	0.25
KTR9_3728	regulatory protein MerR	2.19	0.02
KTR9_3738	hypothetical protein	2.89	2.27E-04
KTR9_3739	Transcriptional regulator	2.97	0.28
KTR9_3764	NAD-dependent aldehyde dehydrogenases	2.54	0.46
KTR9_3765	Zn-dependent alcohol dehydrogenases, class III	2.22	0.48
KTR9_3801	hypothetical protein	2.42	0.02
KTR9_3871	conserved hypothetical protein, nitrobinding superfamily	2.06	4.92E-06
KTR9_3873	Cation transport ATPase	2.12	8.08E-09
KTR9_3885	Ferredoxin	2.62	0.48
KTR9_3889	hypothetical protein	8.14	0.29
KTR9_3930	TetR family transcriptional regulator	2.29	0.24
KTR9_4044	hypothetical protein	2.62	2.72E-03
KTR9_4069	ADP-ribosylglycohydrolase	2.07	0.01
KTR9_4073	hypothetical protein	2.54	0.27
KTR9_4096	Transcriptional regulator	2.88	0.46
KTR9_4122	Glycosyltransferase	2.08	0.36
KTR9_4177	hypothetical protein	3.46	0.41
KTR9_4270	Esterase/lipase	2.05	5.70E-05
KTR9_4276	Transcriptional regulator	2.36	9.96E-03
KTR9_4286	hypothetical protein	2.67	0.18
KTR9_4302	Universal stress protein UspA and related nucleotide-binding proteins	2.36	0.17
KTR9_4341	putative cyclohexanol dehydrogenase	2.29	1.60E-07
KTR9_4342	hypothetical protein	2.03	0.5
KTR9_4358	short-chain dehydrogenase/reductase	3.81	0.42
KTR9_4361	Gluconolactonase	2.22	0.48
KTR9_4370	conserved hypothetical protein, Cupin_2 superfamily	2.27	0.05
KTR9_4422	ABC-type Fe ³⁺ transport system, periplasmic component	2.1	0.44
KTR9_4450	DNA-directed RNA polymerase specialized sigma subunit, sigma24-like protein	2.32	4.66E-15
KTR9_4499	hypothetical protein	2.4	0.43

KTR9_4530	conserver hypothetical protein, FMN_red superfamily	2.49	0.45
KTR9_4548	Trans-aconitate methyltransferase	2.48	9.93E-06
KTR9_4570	Transcriptional regulator	2.17	0.09
KTR9_4574	Transcriptional regulator	2.02	0.09
KTR9_4611	putative HTH-type transcriptional regulator	2.01	0.27
KTR9_4697	hypothetical protein	3.25	0.44
KTR9_4748	hypothetical protein	2.2	0.46
KTR9_4808	hypothetical protein	3.05	0.11
KTR9_4811	hypothetical protein	2.44	0.34
KTR9_4820	hypothetical protein	2.24	0.49
KTR9_4822	hypothetical protein	2.69	0.25
KTR9_4833	hypothetical protein	5.09	0.46
KTR9_4869	hypothetical protein	3.05	0.47
KTR9_4880	integrase family protein	2.24	0.49
KTR9_4891	hypothetical protein	2.27	0.42
KTR9_4898	hypothetical protein	5.09	0.46
KTR9_4900	ATPases with chaperone activity, ATP-binding subunit'	3.05	0.31
KTR9_4908	hypothetical protein	2.94	0.18
KTR9_4911	hypothetical protein	2.06	0.26
KTR9_4913	hypothetical protein	2.62	9.42E-03
KTR9_4940	hypothetical protein	2.22	0.31
KTR9_4975	hypothetical protein	2.08	4.39E-07
KTR9_4982	hypothetical protein	2.03	0.44
KTR9_4984	hypothetical protein	2.13	0.26
KTR9_5014	non-hemolytic enterotoxin B	2.58	0.31
KTR9_5023	transposase IS3/IS911 family protein	2.59	3.95E-03
KTR9_5027	hypothetical protein	2.03	0.1
KTR9_5028	hypothetical protein	2.32	0.2
KTR9_5031	hypothetical protein	2.12	0.24
KTR9_5033	hypothetical protein	2.65	0.07
KTR9_5034	hypothetical protein	2.39	0.08
KTR9_5035	hypothetical membrane protein	3.17	0.02
KTR9_5036	hypothetical protein	2.21	0.02
KTR9_5037	hypothetical protein	3.56	0.13

KTR9_5103	hypothetical protein	2.48	0.04
KTR9_5108	hypothetical protein	2.09	0.42
KTR9_5134	hypothetical protein	3.62	2.28E-05
KTR9_5154	hypothetical protein	2.29	0.27
KTR9_5164	hypothetical protein	2.28	0.07
KTR9_5170	hypothetical protein	7.63	0.31
KTR9_5293	hypothetical protein	3.08	0.05
KTR9_5294	hypothetical protein	3.45	0.05
KTR9_5295	hypothetical protein	2.09	0.41
KTR9_5296	hypothetical membrane protein	2.03	0.5
KTR9_5297	hypothetical protein	2.93	0.07
KTR9_5298	hypothetical protein	2.89	0.09
KTR9_5299	hypothetical protein	2.08	0.38
KTR9_5301	hypothetical protein	3.36	0.26
KTR9_5319	hypothetical protein	3.17	8.36E-03
KTR9_5352	helix-turn-helix domain-containing protein	4.58	0.34
KTR9_5356	hypothetical protein	2.08	0.08
KTR9_5370	hypothetical protein	2.71	0.49
KTR9_5374	Transposase and inactivated derivatives	2.54	0.37
KTR9_5378	AraC-type DNA-binding domain-containing proteins	2.78	0.02
KTR9_5381	Uncharacterized NAD(FAD)-dependent dehydrogenases	2.08	0.38
KTR9_5382	Transglutaminase-like protein, putative cysteine protease	2.29	0.44
KTR9_5411	ABC-type transport system involved in resistance to organic solvents, periplasmic component	2.12	0.49
KTR9_5417	Transposase and inactivated derivatives	2.2	0.25
KTR9_5419	helicase-associated protein	2.5	0.4
KTR9_5427	hypothetical protein	2.11	0.46
KTR9_5429	hypothetical protein	15.26	0.26
KTR9_5436	membrane protein	15.26	0.26
KTR9_5438	hypothetical protein	2.19	0.45
KTR9_5445	hypothetical protein	2.32	0.33
KTR9_5450	hypothetical protein	2.11	4.56E-04
KTR9_5452	hypothetical protein	2.37	0.48
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Down-regulated			
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KTR9_0023	Permease, MFS superfamily	-2.23	0.15

KTR9_0068	putative molybdopterin oxidoreductase	-2.77	7.98E-03
KTR9_0076	Zn-dependent alcohol dehydrogenases, class III	-2.34	9.45E-04
KTR9_0077	beta-lactamase domain protein	-2.06	9.46E-03
KTR9_0084	ATP-dependent DNA ligase	-2.46	0.09
KTR9_0095	NAD-dependent aldehyde dehydrogenases	-2.52	0.01
KTR9_0100	Hypothetical protein	-2.25	1.71E-05
KTR9_0130	Hypothetical protein	-2.95	0.3
KTR9_0142	Hypothetical protein	-2.27	0.12
KTR9_0169	Hypothetical protein	-2.13	0.48
KTR9_0194	Putative <i>N</i> -acetylmuramoyl-L-alanine amidase	-2.32	7.20E-09
KTR9_0207	Predicted esterase	-2.02	1.96E-11
KTR9_0208	Hypothetical protein	-2.79	9.03E-06
KTR9_0209	Hypothetical protein	-3.75	3.52E-04
KTR9_0225	Cell wall arabinan synthesis protein	-2.51	0.16
KTR9_0226	Cell wall arabinan synthesis protein	-2.48	0.23
KTR9_0279	Transcriptional regulators	-2.5	0.2
KTR9_0317	Predicted redox protein, regulator of disulfide bond formation	-2.04	0.48
KTR9_0371	Permeases of the drug/metabolite transporter, DMT superfamily	-2.25	1.31E-03
KTR9_0382	Nitroreductase	-2.4	0.31
KTR9_0383	Permease, MFS superfamily	-4.47	4.62E-06
KTR9_0384	Transcriptional regulator	-2.59	0.25
KTR9_0390	Gluconate kinase	-2.3	0.16
KTR9_0391	H ⁺ /gluconate symporter and related permeases	-3.02	0.04
KTR9_0394	Ferritin Dps family protein	-2.47	3.33E-15
KTR9_0468	UDP-glucose 4-epimerase	-2.11	0.05
KTR9_0493	Acyl carrier protein	-2.95	0.49
KTR9_0604	Chaperonin GroEL (HSP60 family)	-4.15	1.91E-14
KTR9_0605	Lactoylglutathione lyase and related lyases	-3.93	0.27
KTR9_0608	Predicted acetyltransferase	-2.5	0.23
KTR9_0668	Hypothetical protein	-2.02	0.02
KTR9_0734	Conserved hypothetical protein, glyoxalase superfamily	-2.14	0.06
KTR9_0852	Cytochrome bd-type quinol oxidase, subunit 1	-2.69	1.08E-05
KTR9_0853	Cytochrome bd-type quinol oxidase, subunit 2	-3.09	4.35E-05
KTR9_0854	ABC-type transport system involved in cytochrome bd biosynthesis, ATPase and permease components	-2.78	8.55E-03

KTR9_0855	ABC-type multidrug transport system, ATPase and permease components	-2.44	0.02
KTR9_0997	Ribosomal protein L10	-2.17	3.70E-04
KTR9_0998	Ribosomal protein L7/L12	-3.32	6.83E-04
KTR9_1009	Hypothetical protein	-2.7	0.1
KTR9_1047	Hypothetical protein	-2.34	0.26
KTR9_1093	4-hydroxyacetophenone monooxygenase	-2.02	0.41
KTR9_1130	Transglycosylase domain-containing protein	-2.15	0
KTR9_1153	ABC-type phosphate/phosphonate transport system, ATPase component	-3.11	0.42
KTR9_1205	Cytosine/adenosine deaminases	-3.2	0.34
KTR9_1329	Acetyltransferase	-2.08	0.2
KTR9_1427	Transglycosylase domain-containing protein	-2.22	0.12
KTR9_1428	Dimethyladenosine transferase (rRNA methylation)	-2.53	0.33
KTR9_1502	Stress protein	-2.64	0.07
KTR9_1503	Hypothetical protein	-2.12	2.60E-04
KTR9_1504	Predicted multitransmembrane protein	-2.15	0.06
KTR9_1505	ABC-type metal ion transport system, periplasmic component / surface antigen	-3.93	0.47
KTR9_1508	Coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase and related flavin-dependent oxidoreductases	-2.89	0.22
KTR9_1515	Predicted esterase	-2.55	3.33E-08
KTR9_1632	Fumarase	-2.17	2.29E-04
KTR9_1633	Tartrate dehydratase alpha subunit/Fumarate hydratase class I, N-terminal domain	-2.57	0.04
KTR9_1659	Co-chaperonin GroES (HSP10)	-2.81	2.57E-08
KTR9_1660	Chaperonin GroEL (HSP60 family)	-2.04	2.38E-04
KTR9_1794	DNA-directed RNA polymerase specialized sigma subunit, sigma24 -like protein	-3.78	2.22E-15
KTR9_1839	Membrane protein	-5.64	6.23E-03
KTR9_1840	Hypothetical protein	-2.01	0.14
KTR9_1845	Hypothetical protein	-2.78	6.80E-03
KTR9_1855	Protein chain release factor A	-2.01	0.08
KTR9_1861	F0F1-type ATP synthase, subunit a	-2.99	1.72E-10
KTR9_1863	F0F1-type ATP synthase, subunit b	-2.16	2.02E-12
KTR9_1864	F0F1-type ATP synthase, delta subunit (mitochondrial oligomycin sensitivity protein)	-2.11	2.85E-11

KTR9_1865	F0F1-type ATP synthase, alpha subunit	-2.14	3.30E-12
KTR9_1886	Inner membrane protein	-2.16	4.40E-04
KTR9_1932	Na ⁺ /H ⁺ -dicarboxylate symporters	-2.03	4.30E-05
KTR9_1957	Putative thioesterase	-2.02	0.41
KTR9_1969	DsbA oxidoreductase	-2.1	0.01
KTR9_1972	Formamidopyrimidine-DNA glycosylase	-2.31	0.27
KTR9_1974	Hypothetical protein	-5.02	1.20E-03
KTR9_1994	L-ectoine synthase	-2.46	1.72E-06
KTR9_1995	L-2,4-diaminobutyric aminotransferase	-2.86	6.21E-12
KTR9_2090	Hypothetical protein	-4.67	3.00E-15
KTR9_2221	Preprotein translocase subunit SecD	-2.04	7.73E-03
KTR9_2226	Peptidyl-prolyl cis-trans isomerase (rotamase) - cyclophilin family	-2.15	0.03
KTR9_2392	Hypothetical protein	-2.09	0.04
KTR9_2393	Predicted metal-dependent hydrolase with the TIM-barrel fold	-2.16	0.19
KTR9_2434	Hypothetical protein	-2.29	0.43
KTR9_2475	Peptidase_M23 superfamily protein	-6.88	0.1
KTR9_2531	Predicted GTPases	-2.08	0.13
KTR9_2535	Hypothetical protein	-2.11	0.24
KTR9_2545	Hypothetical protein	-2.33	0.21
KTR9_2549	DNA-directed RNA polymerase specialized sigma subunit, sigma24-like protein	-2.03	0.45
KTR9_2551	Short-chain dehydrogenase/reductase	-3.34	0.43
KTR9_2562	Ribulose 1,5-bisphosphate carboxylase, large subunit	-3.77	0.33
KTR9_2587	Zn-dependent hydrolases, including glyoxylases	-3.79	0.29
KTR9_2590	Hypothetical protein	-3.07	0.36
KTR9_2596	Pyridoxamine 5'-phosphate oxidase family protein	-2.95	0.49
KTR9_2682	Chorismate mutase	-2.24	0.35
KTR9_2721	Transcriptional accessory protein	-2.05	0.25
KTR9_2779	Hypothetical protein	-2.56	0.41
KTR9_2784	Acyl-CoA synthetases (AMP-forming)/AMP-acid ligases II	-2.09	0.11
KTR9_2956	Hypothetical protein	-2.37	0.17
KTR9_2957	Nicotinate-nucleotide pyrophosphorylase	-2.62	4.56E-04
KTR9_2958	Quinolate synthase	-2.8	1.02E-13
KTR9_2992	Hypothetical protein	-2.14	2.80E-04
KTR9_3039	Cell wall-associated hydrolases (invasion- associated	-2.57	0.04

	proteins)		
KTR9_3040	Cell wall-associated hydrolases (invasion- associated proteins)	-2.06	6.18E-04
KTR9_3071	Glutamine synthetase, type I	-2.19	0
KTR9_3119	Beta-lactamase class C and other penicillin binding proteins	-2.08	0.16
KTR9_3163	Sulfite reductase	-2.6	7.78E-07
KTR9_3221	Gamma-aminobutyrate permease and related permeases	-2.35	0.32
KTR9_3233	Predicted drug exporters, RND superfamily	-2.14	0.23
KTR9_3234	Hypothetical protein	-2	0.35
KTR9_3237	DUF1994 domain-containing protein	-2.53	0.5
KTR9_3254	3-isopropylmalate dehydratase large subunit	-2.04	0.02
KTR9_3262	D-3-phosphoglycerate dehydrogenase	-2.02	0.02
KTR9_3318	Coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase and related flavin-dependent oxidoreductases	-2.18	1.41E-03
KTR9_3424	Hypothetical protein	-2.75	6.85E-11
KTR9_3425	Formate/nitrite family of transporters	-2.35	0
KTR9_3434	Trypsin-like serine proteases	-15.23	1.77E-14
KTR9_3435	Lactoylglutathione lyase and related lyases	-2.94	3.63E-07
KTR9_3436	Peptide methionine sulfoxide reductase	-4.92	2.62E-05
KTR9_3437	Predicted GTPase, probable translation factor	-2.2	0.05
KTR9_3493	Cell division protein	-2.92	0.02
KTR9_3494	Predicted ATPase involved in cell division	-2.03	0.1
KTR9_3515	Predicted flavoprotein involved in K ⁺ transport	-2.65	9.51E-05
KTR9_3531	Predicted methylated DNA-protein cysteine methyltransferase	-3.15	9.01E-10
KTR9_3532	Hypothetical protein	-2.64	2.44E-15
KTR9_3533	Uroporphyrinogen-III synthase	-2.69	1.32E-14
KTR9_3538	Adenylyltransferase, thiamin (thiazole moiety) biosynthesis protein \\ molybdopterin synthase sulfurylase protein	-2.09	1.78E-06
KTR9_3553	Anti-sigma factor	-2.17	4.17E-05
KTR9_3594	Xanthine permeases / uracil permeases	-2.3	8.88E-16
KTR9_3753	Predicted esterase	-2.58	0.13
KTR9_3755	CDP-diacylglycerol pyrophosphatase	-2.02	0.18
KTR9_3803	Hypothetical protein	-2.25	0.14
KTR9_3806	Predicted flavoprotein involved in K ⁺ transport	-3.04	0.29
KTR9_3807	Regulatory protein TetR	-2.32	0.45

KTR9_3810	ABC-type nitrate/sulfonate/bicarbonate transport systems, periplasmic components	-2.75	0.27
KTR9_3811	ABC-type nitrate/sulfonate/bicarbonate transport system, permease component	-3.11	0.25
KTR9_3821	Hypothetical protein	-3.38	0.02
KTR9_3952	Hypothetical protein	-2.95	0.3
KTR9_3953	Selenocysteine lyase	-2.32	0.45
KTR9_3975	Hypothetical protein	-2.15	0.24
KTR9_3982	Permeases, MFS superfamily	-2.27	0.42
KTR9_4005	4-aminobutyrate aminotransferase and related aminotransferases	-25.25	0
KTR9_4006	FAD/FMN-dependent dehydrogenases	-46.94	0
KTR9_4007	Succinate-semialdehyde dehydrogenase	-53.88	0
KTR9_4008	Xaa-Pro aminopeptidase	-17.03	0
KTR9_4009	Transcriptional regulators	-10.49	0
KTR9_4010	Putative acetylornithine deacetylase	-2.36	2.01E-06
KTR9_4011	Permeases, MFS superfamily	-6.26	3.77E-15
KTR9_4012	Transcriptional regulators	-4.16	3.44E-15
KTR9_4013	Hypothetical protein	-35.75	1.81E-09
KTR9_4014	Asp-tRNA ^{Asn} /Glu-tRNA ^{Gln} amidotransferase A subunit and related amidases	-28.73	0
KTR9_4015	Maleate cis-trans isomerase	-16.01	1.64E-05
KTR9_4023	PPOX class putative F420-dependent enzyme	-2.22	0.18
KTR9_4024	Maleate cis-trans isomerase	-8.32	2.02E-04
KTR9_4092	Acyl CoA:acetate / 3-ketoacid CoA transferase, beta subunit	-2.32	0.39
KTR9_4093	Acyl CoA:acetate / 3-ketoacid CoA transferase, alpha subunit	-2.79	0.22
KTR9_4095	Acetyl-CoA acetyltransferase	-2.95	0.3
KTR9_4116	ABC-type Fe ³⁺ -hydroxamate transport system, periplasmic component	-8.36	0.28
KTR9_4138	Hypothetical protein	-3.39	0.02
KTR9_4140	Peptide methionine sulfoxide reductase A	-2.88	2.32E-09
KTR9_4141	Peptide methionine sulfoxide reductase B	-3.34	0
KTR9_4172	DnaJ-class molecular chaperone	-2.37	1.28E-06
KTR9_4173	Molecular chaperone GrpE	-2.87	1.60E-10
KTR9_4174	Molecular chaperone	-2.9	9.99E-16
KTR9_4181	ATP-dependent Lon protease, bacterial type	-2.91	3.53E-06
KTR9_4220	Predicted dinucleotide-binding protein	-2.11	8.14E-03

KTR9_4265	Cytosine permease	-2.15	0.03
KTR9_4348	Hypothetical protein	-3.2	0.5
KTR9_4388	ABC-type dipeptide/oligopeptide/nickel transport systems, permease components	-2.35	2.17E-05
KTR9_4390	ABC-type dipeptide transport system, periplasmic component	-2.3	8.03E-05
KTR9_4401	Short-chain dehydrogenase/reductase	-5.41	0.44
KTR9_4467	Arsenite efflux pump, permeases	-2.82	0.25
KTR9_4469	Protein-tyrosine phosphatase	-2.36	0.46
KTR9_4483	Short-chain dehydrogenase/reductase	-3.4	0.08
KTR9_4484	Permease, MFS superfamily	-2.11	0.37
KTR9_4507	Hypothetical protein	-2.83	1.47E-05
KTR9_4525	Aspartate oxidase	-2.99	7.02E-04
KTR9_4584	2,5-didehydrogluconate reductase	-2.09	2.37E-03
KTR9_4594	Hypothetical protein	-9.24	2.98E-09
KTR9_4712	Hypothetical protein	-2.36	0.01
KTR9_4713	Putative stress protein	-2.58	0.08
KTR9_4714	Hypothetical protein	-2.49	0.05
KTR9_4715	Hypothetical protein	-2.65	0.1
KTR9_4716	Hypothetical protein	-3.31	0.1
KTR9_4717	Membrane protein	-4.52	0.07
KTR9_4726	Predicted Na ⁺ -dependent transporter	-2.28	0.19
KTR9_5003	Glutaredoxin-like proteins	-2.27	9.01E-07
KTR9_5329	Hypothetical protein	-3.25	0.08

Table S4. Potential sRNAs in *Gordonia* sp. KTR9.^a

Name	Position	Start	End	Length (bp)	Highest read number	Comment ^b
1A	pGKT1	50302	50561	259	1637	
1B	pGKT1	86952	86673	279	5560	predicted promoter: ccgaca...ggggaatt
2A	pGKT2	4437	4700	263	4177	predicted promoter: gtgcga...cctcaact
3A	pGKT3	82763	82356	407	7043	predicted promoter: ctgcat...tgttattca
CA	KTR9	135953	136287	334	7877	
CB	KTR9	491121	491437	316	4264	
CC	KTR9	500382	500795	413	31597	overlaps KTR9_0435 (hypothetical protein)
CD	KTR9	943964	944269	305	5141	overlaps KTR9_0848 (cold shock protein)
CE	KTR9	1371813	1372100	287	8191	overlaps KTR9_1211 (hypothetical protein)
CF	KTR9	1503764	1504241	477	41873	
CG	KTR9	1710855	1711354	499	31424	overlaps KTR9_1510 (hypothetical protein)
CH	KTR9	3561225	3560902	323	13714	
CI	KTR9	3876611	3877016	405	8369	predicted promoter:ttcaca...accctaaact
CJ	KTR9	3941913	3942409	496	9389	overlaps KTR9_3451 (hypothetical protein); predicted promoter: gtgtct...ctttctaat
CK	KTR9	3980817	3980474	343	408170	74% nucleotide sequence identity with tmRNA of <i>Corynebacterium diphtheriae</i> NCTC 13129 (NC_002935)

^asRNAs were identified as transcripts in RNA-seq studies (>1500 reads) that had no protein coding sequence in any of the 6 reading frames. The orientation of predicted sRNAs was based on a BLAST search of the sRNA database (<http://bioinfo.mikro.bio.med.uni-giessen.de/sRNAdb/Blast>), the minimum free energy prediction for the sRNA secondary structure (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) and the prediction of sRNA promoter location (<http://linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb>).

^bOverlaps with annotated ORFs are noted. The nucleotide sequences of the predicted promoters represent the -35 and -10 regions.

Table S5. Annotation of cholesterol catabolic genes of KTR9^a.

Gene name	Locus tag	Annotation	Identity ^b (%)
<i>kstR</i>	KTR9_0788	Transcriptional regulator, TetR family	68
<i>fadE34</i>	KTR9_0789	Acyl-CoA dehydrogenase	47
<i>fadE28</i>	KTR9_0790	Acyl-CoA dehydrogenase	48
<i>fadE29</i>	KTR9_0791	Acyl-CoA dehydrogenase	73
	KTR9_0792	Conserved hypothetical protein	61
	KTR9_0793	Conserved hypothetical protein	73
<i>ltp2</i>	KTR9_0794	Branched-chain β -ketoacyl-CoA thiolase	77
<i>hsd4B</i>	KTR9_0801	2-Enoyl acyl-CoA hydratase	59
<i>kstD</i>	KTR9_0802	3-Ketosteroid- Δ^1 -dehydrogenase	64
<i>hsaE</i>	KTR9_0810	2-Hydroxypenta-2,4-dienoate hydratase	65
<i>hsaG</i>	KTR9_0811	Acetaldehyde dehydrogenase	78
<i>hsaF</i>	KTR9_0812	4-Hydroxy-2-ketovalerate aldolase	78
	KTR9_0818	Conserved hypothetical protein	40
<i>kshA</i>	KTR9_0819	3-Ketosteroid-9 α -hydroxylase, oxygenase component	61
<i>hsaA</i>	KTR9_0820	3-Hydroxy-9,10-seconandrosta-1,3,5(10)-trien-9,17-dione (3-HSA) hydroxylase, oxygenase component	75
<i>hsaC</i>	KTR9_0821	3,4-Dihydroxy-9,10-seconandrosta-1,3,5(10)-trien-9,17-dione (3,4-DHSA) dioxygenase	78
<i>hsaB</i>	KTR9_0822	3-HSA hydroxylase, reductase component	58
<i>fadE33</i>	KTR9_0827	Acyl-CoA dehydrogenase	40
<i>fadE32</i>	KTR9_0828	Acyl-CoA dehydrogenase	49
<i>fadE31</i>	KTR9_0829	Acyl-CoA dehydrogenase	64
<i>choD</i>	KTR9_1668	Cholesterol oxidase	68
<i>kshB</i>	KTR9_3740	3-Ketosteroid-9 α -hydroxylase, reductase component	55
<i>mce4F</i>	KTR9_3876	MCE family protein	42
<i>mce4E</i>	KTR9_3877	MCE family protein	30
<i>mce4D</i>	KTR9_3878	MCE family protein	40
<i>mce4C</i>	KTR9_3879	MCE family protein	40
<i>mce4B</i>	KTR9_3880	MCE family protein	45
<i>mce4A</i>	KTR9_3881	MCE family protein	39
<i>supB</i>	KTR9_3882	ABC transporter permease subunit	70
<i>supA</i>	KTR9_3883	ABC transporter permease subunit	69

<i>hsd4A</i>	KTR9_3884	Hydroxysteroid dehydrogenase	64
<i>fdxD</i>	KTR9_3885	Ferredoxin	50
<i>fadE26</i>	KTR9_3886	Acyl-CoA dehydrogenase	74
<i>fadE27</i>	KTR9_3887	Acyl-CoA dehydrogenase	45
<i>fadD17</i>	KTR9_3888	Acyl-CoA synthetase	56
<i>fadD19</i>	KTR9_3892	Acyl-CoA synthetase	58
<i>echA19</i>	KTR9_3893	Enoyl-CoA hydratase	76
	KTR9_3895	Flavin Coenzyme F420 dependent oxidoreductase	69
	KTR9_3896	Conserved hypothetical protein	59
<i>ltp4</i>	KTR9_3897	β -Ketoacyl-CoA thiolase	58
<i>ltp3</i>	KTR9_3898	SCPx-related β -ketoacyl-CoA thiolase	79
<i>cyp125A1</i>	KTR9_3902	Cytochrome P450	61
<i>fadA5</i>	KTR9_3903	β -Ketoacyl-CoA thiolase	72
	KTR9_3904	Conserved hypothetical protein	51
	KTR9_3911	Short chain dehydrogenase/reductase SDR	63
	KTR9_3912	Short chain dehydrogenase/reductase SDR	59
<i>echA20</i>	KTR9_3913	Enoyl-CoA hydratase	75
<i>ipdA</i>	KTR9_3914	CoA transferase, alpha subunit	68
<i>ipdB</i>	KTR9_3915	CoA transferase, beta subunit	67
	KTR9_3916	Oxidoreductase	71
<i>kstR2</i>	KTR9_3920	Transcriptional regulator, TetR family	63
<i>fadA6</i>	KTR9_3922	β -Ketoacyl-CoA thiolase	71
	KTR9_3923	Oxidoreductase	72
<i>fadE30</i>	KTR9_3924	Acyl-CoA dehydrogenase	66
<i>fadD3</i>	KTR9_3925	Acyl-CoA synthetase	59
<i>hsaD</i>	KTR9_4392	4,5-9,10-Diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10),2-dien-4-oate (4,9-DSHA) hydrolase	35

^a Annotation is based on assignments described in (21, S1 and S2).

^b Amino acid identity with the *Mtb* H37Rv orthologue

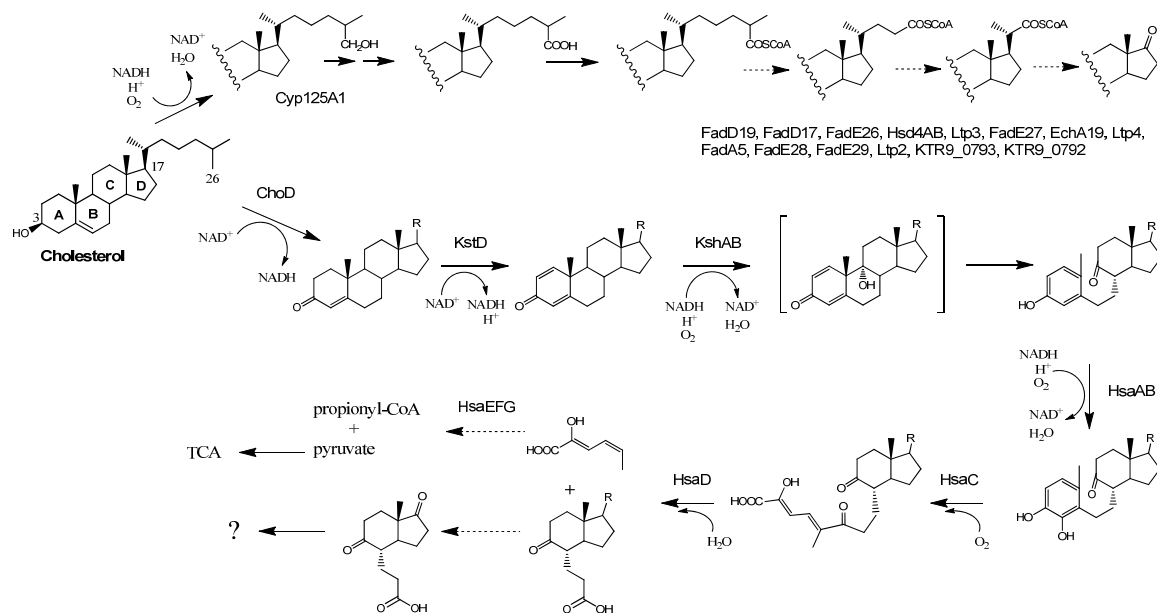


Figure S1. Cholesterol catabolic pathway in KTR9 based on metabolic reconstruction. Dashed arrows represent multiple steps. ChoD is tentatively assigned to the initial transformation of ring A. R can either be a partially degraded side chain containing 3, 5, or 8 carbons, and a CoA moiety, or a keto group. TCA, tricarboxylic acid cycle.

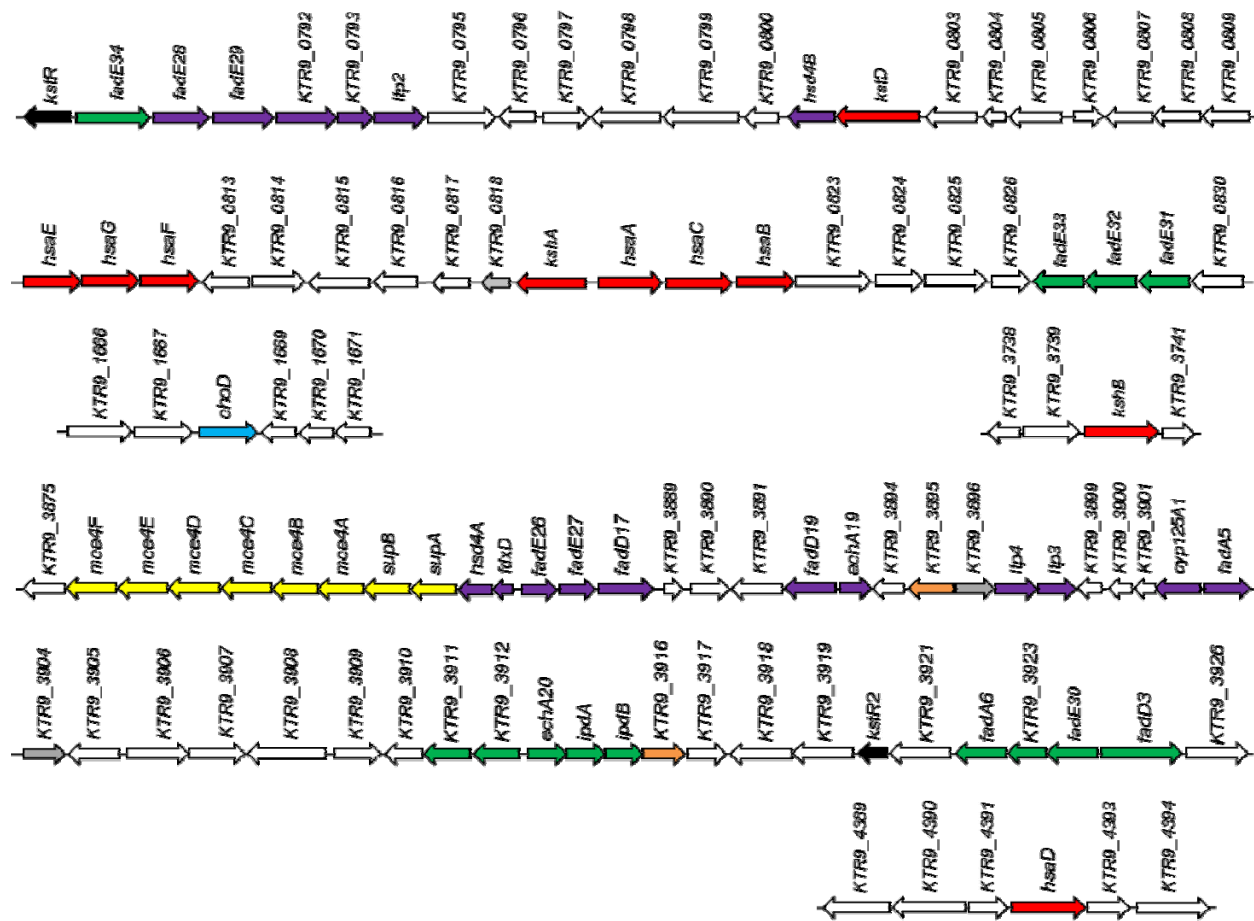


Figure S2. Predicted cholesterol catabolic genes in KTR9. Most of the genes are arranged in two clusters spanning KTR9_0788 to KTR9_0829 and KTR9_3876 to KTR9_3925, respectively. Three other genes occur elsewhere in the genome. Genes are color-coded as follows: red, genes involved in degradation of rings A and B; purple, genes involved in side chain degradation; green, genes involved in degradation of rings C and D; yellow, genes comprising the Mce4 cholesterol importer; black, transcriptional regulator; blue, *choD*, encoding a proposed cholesterol oxidase; orange, encoding oxidoreductases of unknown function conserved in the RHA1 and *Mtb* cholesterol catabolic cluster; grey, encoding conserved hypothetical protein; white, genes of no known function in cholesterol catabolism.

Supplemental References

S1. Griffin, J.E., J.D. Gawronski, M.A. Dejesus, T.R. Ioerger, B.J. Akerley, and C.M. Sassetti. 2011. High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. *PLoS Pathog.* **7**:e1002251.

S2. Ouellet, H., J.B. Johnston, and P.R. Ortiz de Montellano. 2011. Cholesterol catabolism as a therapeutic target in *Mycobacterium tuberculosis*. *Trends Microbiol.* **19**:530-539.