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Isoniazid with Multiple Mode of Action on Various Mycobacterial Enzymes Resulting in Drug Resistance

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Abstract

Isoniazid (INH), is one of the drugs shown to be effective and has been extensively used in TB control. Interestingly tuberculosis showed predominant drug resistance to isoniazid and thus lead to multi drug therapy in TB treatment. However, isoniazid is still advocated in latent TB and use as prophylactic in HIV infection and in children for prevention of TB. It is of interest that different studies revealing interaction of isoniazid with around 117 enzymes of mycobacteria influencing metabolic pathways by number of ways in addition to inhibiting mycolic acid synthesis and thus affecting growth of mycobacteria. The purpose of this review is to present the various mechanisms of action of isoniazid at different enzymes of MTB causing drug resistance.

Keywords: Tuberculosis; Isoniazid; Drug resistance; *Mycobacterium*; Metabolic pathway

Introduction

Tuberculosis (TB), being an oldest infectious disease, has been a major health problem worldwide. It is caused by *Mycobacterium tuberculosis* (MTB) which infects around one third of the world's population. According to WHO Global Tuberculosis Report 2015, there were around 9.6 million people with active TB infection and amongst them 12% were HIV-positive. Further, in 2014 there were only 1,23,000 reported cases of multidrug-resistant TB (MDR-TB) amongst 4,80,000 cases [1]. The occurrences of extensively drug-resistant (XDR) tuberculosis have also been a rising risk in different regions around the globe [2]. The isoniazid (INH), also known as isonicotinyl hydrazine, one of the effective anti-TB drugs used for tuberculosis treatment is found to be resistant in different clinical strains of MTB [3]. Further, according to various studies, 82 different enzymes of mycobacteria associated with the interaction of INH, resulting in mutation and isoniazid drug resistance (Table 1) [4,5].

As INH has been used as a first-line drug in the prevention and treatment of TB [6], its mechanism of action has been studied for more than five decades. It is reported to produce various highly reactive compounds [7] which then target multiple enzymes of MTB [8]. Thus, the complex mode of action of isoniazid with number of enzymes needs study. Further, it is useful to understand how mutation in different MTB enzymes affects drug-enzyme interaction. This communication reviews the various mechanism of action of a single drug isoniazid at different MTB enzymes leading to drug resistance.

Mechanisms of Action of Isoniazid

Activation of INH by KatG and formation of INH-NAD(P) adduct

KatG of MTB encoded by Rv1908c has 740 amino acids in its protein sequence, is a multifunctional enzyme, showing both a catalase and a peroxynitritase activities [9,10]. Besides playing an important role in the intracellular survival of the pathogen within macrophages, it protects against reactive nitrogen and oxygen species produced by phagocytic cells [10]. Being a pro-drug, INH is activated by the catalase–peroxidase KatG and $MnCl_2$ and forms isonicotinoyl radical or anion which then reacts with NAD+ and NADP+ [11], and subsequently generates INH-NAD(P) adducts [12]. Amongst these adducts, the INH-NAD reported to inhibit the enoyl-ACP reductase enzyme (InhA) whereas INH-NADP inhibit dfrA - encoded dihydrofolate reductase [13] and MabA (3-oxoacyl-ACP reductase) [14].

Inhibition of InhA by INH-NAD adduct

INH-NAD adduct was reported to inhibit InhA of MTB encoded by Rv1484 which is reported to block the synthesis of mycolic acid, a major lipid of the mycobacterial cell wall.

Our *in silico* docking study between InhA and truncated INH-NAD adduct, demonstrated that the adduct binds with InhA by forming a hydrogen bond with its substrate binding residue Tyr158 [15] which correlates the *in vitro* study by Nguyen et al. reporting that the INH-NAD adduct as a potential inhibitor of InhA [16].

Truncated INH-NAD adduct

After profiling the MTB proteome using both the INH-NAD and INH-NADP adducts coupled to Sepharose solid supports, Argyrou et al. identified seventeen proteins (Table 2) that bind to these adducts with high affinity [13]. Further, the truncated form of INH–NAD adduct (4-isonicotinoylnicotinamide, 4-INN,) reported to have potential antimycobacterial activity [17]. The *in silico* docking study of truncated INH–NAD adducts with six MTB enzymes with known three-dimensional (3D) structure out of 17 proteins (Table 2), showed considerable binding affinity and thus revealing the truncated INH–NAD adducts as effective inhibitors for these proteins [15].

Acetylation of INH by NAT

The arylamine N-acetyltransferase (NAT) of MTB also reported to have direct interaction with INH like KatG. As a drug-metabolizing enzyme, NAT acetylates INH and forms INH to a therapeutically inactive form i.e. N-acetylate INH [18]. Payton et al. observed that the over expression of NAT leads to increased INH resistance in *Mycobacterium smegmatis* [19]. Further, when the gene was knocked-out, the bacteria showed increased sensitivity to INH [19].

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S/No	Locus tag	Name	Protein Length	Gene name	PDB ID	Mutation	Pathway
1	Rv1772	hypothetical protein Rv1772	103	-	-	Thr4Ala	
2	Rv1909c	ferric uptake regulation protein furA (furA)	150	*furA*	-	Ser5Pro	
3	Rv0340	hypothetical protein Rv0340	179	-	-	Val163Ile	
4	Rv2428	alkyl hydroperoxide reductase subunit C	195	*ahpC*	2BMX	Inter-genic region G(-46)A	
5	Rv1483	3-oxoacyl-[acyl-carrier-protein] reductase	247	*fabG1*	1UZL	Ala5Pro, Val14Leu, Thr21Ala	Fatty acid biosynthesis
6	Rv1484	enoyl-(acyl carrier protein) reductase	269	*inhA*	1P44	Lys8Asn, Ile16Thr, Ile21Val/Thr, Ile47Thr, Val78Ala, Ser94Ala/ Leu, Ile95Pro, Ile95Thr, Ile194Thr, Arg202Gly, Glu217Asp, promoter region	Fatty acid biosynthesis
7	Rv3566c	arylamine n-acetyltransferase nat	283	*nat*	4BGF	Gly67Arg, Gly207Glu	• Nitrotoluene degradation • Metabolic pathways • Biosynthesis of secondary metabolites
8	Rv2243	acyl-carrier-protein S-malonyltransferase	302	*fabD*	2QC3	Ser275Asn	• Fatty acid biosynthesis • Metabolic pathways • Fatty acid metabolism
9	Rv0129c	secreted antigen 85-C FBPC (85C)	340	*fbpC*	4MQM	Gly158Ser -63(C/T), -23(A/C)	Glycerolipid metabolism
10	Rv2242	hypothetical protein Rv2242	414	-	-	Asp3Gly, Met323Thr	
11	Rv2245	3-oxoacyl-(acyl carrier protein) synthase II	416	*kasA*	4C6U	Asp66Asn, Met77Ile, Arg121Lys, Gly269Ser, Gly312Ser, Gly387Asp, Phe413Leu	Fatty acid biosynthesis
12	Rv1592c	hypothetical protein Rv1592c	446	-	-	Pro42Leu, Val430Ala	
13	Rv1854c	NADH dehydrogenase	463	*ndh*	-	Arg13Cys, Val18Ala, Thr110Ala, Leu239Pro, Arg268His	Oxidative phosphorylation
14	Rv3139	acyl-CoA dehydrogenase FADE24	468	*fadE24*	-	Insertion of 2 base pair (bp) at nucleotide position -64	
15	Rv2247	acetyl/propionyl-CoA carboxylase beta subunit AccD6	473	*accD6*	4FB8	Asp229Gly	• Fatty acid biosynthesis • Valine, leucine and isoleucine degradation • Pyruvate Metabolism • Glyoxylate and dicarboxylate metabolism • Propanoate Metabolism • Carbon Metabolism • Fatty acid metabolism
16	Rv0341	isoniazid inductible gene protein INIB	479	*iniB*	-	Deletion of 12 bp at nucleotide position 665	
17	Rv0343	isoniazid inductible gene protein INIC	493	*iniC*	-	Trp83Gly	
18	Rv2846c	integral membrane efflux protein EfpA	530	*efpA*	-	Ile73Thr	
19	Rv0342	isoniazid inductible gene protein INIA	640	*iniA*	-	Pro3Ala, Arg537His	
20	Rv1908c	catalase-peroxidase-peroxynitritase T KatG	740	*katG*	2CCA	Ser315Thr, Ser315Asn, Arg463Leu, Ser17Asn, Gly19Asp, Ser140Asn/ Arg, Gly279Asp, Gly285Asp, Gly316Asp, Ser457Ile, Gly593Asp	• Reactive oxygen species degradation • superoxide radicals degradation • Phenylalanine metabolism • Tryptophan metabolism • Metabolic Pathways • Biosynthesis of secondary metabolites
21	Rv3795	integral membrane indolylacetylinositol arabinosyltransferase EMBB	1098	*embB*	-	Tyr333His	• Cell wall biosynthesis • Mycolyl-arabinogalactan-peptidoglycan complex biosynthesis
22	Rv2427a	Transcriptional regulator OxyR', pseudogene		*oxyR'*	-	-	
23	Rv0236c	Alpha-(1>3)-arabino-furanosyltransferase	1,400	*aftD*	-	Thr797Ala	Cell wall polysaccharide biosynthesis
24	Rv0932c	Phosphate-binding protein	370	*pstS2*		Arg70Leu	• ABC transporters, • Two-component system • Tuberculosis
25	Rv0985c	Large-conductance mechano sensitive channel	151	*mscL*	2OAR	Gly55Ala	

26	Rv0987	ABC transporter substrate-binding protein	855		-	Ala819Pro	
27	Rv1877	MFS-type transporter	687			Val660Phe	
28	Rv2576c	membrane protein	154		-	Hia128Arg	
29	Rv2999	Peptidase M23B	321	*lppY*		Met313Thr	
30	Rv3382c	4-hydroxy-3-methylbut-2-enyl diphosphate reductase 2	329	*ispH2*		Gln178Arg	• Terpenoid backbone biosynthesis • Metabolic Pathways • Biosynthesis of secondary metabolites • Biosynthesis of antibiotics
31	Rv3448	ESX-4 secretion system protein	467	*eccD4*	-	Ala193Pro	
32	Rv0194	Multidrug efflux ATP-binding/ permease protein	1194			Leu350Phe, Asp536His	
33	Rv0338c	FeS-binding protein	882			Lys490Asn	
34	Rv0517	Possible membrane acyltransferase	436			Ser408Gly	
35	Rv0793	Putative monooxygenase	101		1YOH	Gly81Asp	Antibiotic biosynthesis
36	Rv0886	Probable ferredoxin / ferredoxin-NADP reductase	575	*fprB*		Ile413Phe	• Metabolic Pathway • Photosynthesis
37	Rv1023	Enolase	429	*eno*		Ala348Ser	• Glycolysis / Gluconeogenesis • Methane Metabolism • Metabolic Pathways • Biosynthesis of secondary metabolites • Microbial Metabolism in diverse environments • Biosynthesis of Antibiotics • Carbon metabolism, • Biosynthesis of amino acids • RNA degradation
38	Rv1355c	molybdopterin biosynthesis protein	715	*moeY*		Ile710Val	Molybdopterin biosynthesis
39	Rv1555	Fumarate reductase subunit D	125	*frdD*		Ile103Thr	• Citrate cycle (TCA cycle) • Oxidative phosphorylation • Pyruvate Metabolism, • Butanoate Metabolism, • Metabolic Pathways • Biosynthesis of secondary metabolites • Microbial metabolism in diverse environments • Biosynthesis of Antibiotics • Carbon Metabolism
40	Rv1850	Urease subunit alpha	577	*ureC*		Asp336Gly	• Arginine biosynthesis • Purine Metabolism • Metabolic Pathways • Microbial metabolism in diverse environments
41	Rv2967c	Pyruvate carboxylase	1127	*pca*		Thr482Met	• Citrate cycle (TCA cycle) • Pyruvate metabolism • Metabolic pathway • Carbon metabolism • Biosynthesis of amino acid
42	Rv3401	glycosyl hydrolase	786			Leu114Pro	Metabolic pathway
43	Rv3537	3-oxosteroid 1- dehydrogenase	563	*kstD*		Ala148pro	• Steroid Degradation • Metabolic pathway • Microbial metabolic in environments
44	Rv0574c	Probable polyglutamine synthesis accessory protein	380		-	Val16Ile	Capsule biosynthesis
45	Rv1118c	Conserved hypothetical protein	286			Gly30Cys	
46	Rv1504c	Conserved hypothetical protein	199			Glu73Gly	
47	Rv1896c	S-adenosyl-L-methionine-dependent methyltransferase	303			Lys132Glu	Methylation

48	Rv1977	Conserved hypothetical protein	348			Ser2Pro	
49	Rv2184c	hypothetical protein	379			Pro294Leu	
50	Rv2432c	hypothetical protein	136		-	Tyr117His	
51	Rv2917	Alanine / arginine-rich protein	626			Thr95Ala	Cell wall synthesis
52	Rv3181c	Antitoxin protein	150	*vapB49*		Val39Gly	
53	Rv0131c	acyl-CoA dehydrogenase	447	*fadE1*		Ala35Val	• Fatty acid Degradation • Valine, leucine ,isoleusine degradation • Beta alanine metabolism • Metabolic Pathway • Biosynthesis of secondary metabolism • Biosynthesis of Antibiotic • Carbon metabolism • Fatty acid Metabolism • Propanoate Metabolism
54	Rv1527c	Polyketide synthase	2,108	*pks5*		Gly2040Asp	• Lipid Biosynthesis • Polyketide biosynthesis
55	Rv1729c	S-adenosylmethionine-dependent methyltransferase	312			His238Arg	Lipid metabolism
56	Rv2383c	phenyloxazoline synthase	1,414	*mbtB*		His1251Pro	• Mycobactin biosynthesis • Siderophore biosynthesis
57	Rv2384	bifunctional salicyl-AMP ligase/salicyl-S-ArCP synthetase	565	*mbtA*		Gly18Ser	• Polyketide biosynthesis • Mycobactin biosynthesis • Siderophore biosynthesis
58	Rv3392c	Cyclopropane mycolic acid synthase 1	287	*cmaA1*	1KP9	Gln99Glu	Mycolic acid biosynthesis
59	Rv3480c	diacyglycerol O-acyltransferase	497			Glu315Ala	Triacylglycerol biosynthesis
60	Rv3649	DEAD / DEAH box helicase domain containing protein	771			Asp459Gly	Information pathway
61	Rv0355c	PPE family protein	3.300	*PPE8*		Leu1213Pro	lipid metabolism.
62	Rv2659c	Prophage integrase	375			Val235Ala	
63	Rv1198	ESAT-6-like protein	94	*esxL*	4GZR	Gln20Leu	
64	Rv1362c	Mce-associated membrane Protein	220		-	Asp95Ala	
65	Rv2869c	Zinc metalloprotease	404	*Rip1*		Lys95Thr	
66	Rv2911	D-alanyl-D-alanine carboxy Peptidase	291	*dacB2*	4RYE	Leu220Gln	Peptidoglycan biosynthetic
67	Rv0086	Hydrogenase	488	*hycQ*		Ala322Val	Metabolism and respiration
68	Rv1844c	6-phosphogluconate dehydrogenase	485	*gnd1*		Ala400Thr	• Pentose phosphate pathway • Glutathione Metabolism • Metabolic Pathways • Biosynthesis of secondary metabolites • Microbial metabolism in diverse environments • Biosynthesis of antibiotics • Carbon metabolism
69	Rv2296	Haloalkane dehalogenase 1	300	*dhmA1*		Ala211Val	• Chlorocyclohexane and chlorobenzene degradation • Chloroalkane and chloroalkene degradation • Metabolic pathways • Microbial metabolism in diverse environments
70	Rv3299c	Probable arylsulfatase	970	*AtsB*		Arg439Trp	Sphingolipid metabolism
71	Rv0104	Hypothetical protein	504			Ile13Leu	Metabolic pathway
72	Rv1069c	Conserved hypothetical protein	587		-	Val465Met	
73	Rv2955c	Hypothetical protein	321		-	Phe315Ile	

74	Rv0564c	Glycerol-3-phosphate dehydrogenase 2 [NAD(P)+]	341	*gpdA1 / gpsA*		Pro131Ser	• Glycerophospholipid metabolism • Biosynthesis of secondary metabolites • CDP-diacylglycerol biosynthesis I • CDP- diacylglycerol biosynthesis II
75	Rv0726c	S-adenosyl-L-methionine-dependent methyltransferase	367			Leu258Pro	Lipid metabolism
76	Rv0667	DNA-directed RNA polymerase subunit beta	1,178	*rpoB*		Asp435Val	• Information Pathways • Purine Metabolism • Pyrimidine Metabolism • Metabolic pathway • RNA polymerase
77	Rv1189	ECF RNA polymerase sigma factor	290	*sigI*		Arg76Cys	Information pathway
78	Rv0578c	PE-PGRS family protein	1,306	*PE_ PGRS7*		Ala785Thr	lipid metabolism
79	Rv1753c	PPE family protein	1,053	*ppe24*		Thr669Ser	lipid metabolism
80	Rv0094c	Conserved hypothetical protein	317			Lys315Glu	
81	Rv1358	transcriptional regulatory protein	1159			TAG*1160Ser	Cyclic nucleotide biosynthesis
82	Rv0175	Mce associated membrane protein	213		-	Met138Thr	

Table 1: Mutations in MTB Genes / Proteins reported to be associated with Isoniazid resistance [4,5].

Isoniazid Drug Resistance

Mutation in different mycobacterial enzymes associated with INH resistance

The mechanism of INH resistance has been the focus of extensive study. It is broadly reported that INH resistance in MTB has been associated with mutations in different genes [7] such as katG, inhA, kasA, ahpC etc. Mutation in NADH dehydrogenase, encoded by ndh also reported to be linked with INH resistance [20]. Further, mutation in promoter region of inhA strongly linked with extensively drug-resistant tuberculosis [21].

Besides 22 genes of MTB reported in Tuberculosis Drug Resistance Mutation Database, which were associated with INH resistance [4], Shekar et al. identified 60 novel genes associated with INH resistance in INH-resistant clinical isolates of MTB by their whole genome sequencing [5]. Most of the genes are associated with important metabolic pathways of MTB such as biosynthesis of secondary metabolites, antibiotics, amino acid, fatty acid; carbon metabolism; cell wall biosynthesis; glycolysis/gluconeogenesis, glutathione metabolism, glyoxylate and dicarboxylate metabolism, lipid biosynthesis, lipid metabolism, metabolic pathway, microbial metabolism in diverse environments, mycolic acid, nitrotoluene degradation, oxidative phosphorylation etc [22,23].

As KatG is associated with activation of INH, mutation in its gene plays an important role in INH resistance. Among various mutations identified in KatG, mutation at S315T and S315N has been widely reported in INH resistance strains. Our computational studies also showed that KatG mutations (S315T/S315N) prevent free radical formation that leads to drug resistance [24].

Two mutations in NAT enzymes (G207E and G67R), reported in MTB clinical isolates associated with INH resistance. From the molecular dynamics (MD) simulation analysis of NAT wild type and mutants (G67R and G207E) models, it was observed that these mutations increases the stability of the binding interfaces of enzyme by providing extra electrostatic interaction with neighboring amino acids. This stability might facilitate in rapid acetylation of INH and detoxification and leads to isoniazid resistance [25].

The mycobacterial NADH pyrophosphatase (NudC) reported to have an important role in the degradation of INH-NAD adduct (the active forms of isoniazid) and ETH-NAD adduct (active form of ethionamide (ETH)) that leads to INH and ETH inactivation. The polymorphism P237Q leads to loss of enzymatic activity and thus leads to INH and ETH resistance [26,27]. Further, a silent mutation in mabA (Rv1483), at nucleotide position 609 (g609a), leads to INH resistance in MTB [28].

Mycothiol (MSH), a major low molecular mass thiol in mycobacteria has antioxidant activity as well as the ability to detoxify a variety of toxic compounds. Four genes such as mshA (Rv0486), mshB (Rv1170), mshC (Rv2130c) and mshD (Rv0819) involves in Mycothiol biosynthesis. Buchmeier et al. observed that the MTB mutant (613 bp deletion within the mshB gene) showing increase resistance to Isoniazid [29]. Further, the mshA (Rv0486) gene of MTB encoding glycosyltransferase involved in the first step of mycothiol biosynthesis [10]. Jagielski et al. observed that a defective mshA gene (frame shift mutation - insC1283) might contribute to the increase in isoniazid resistance [30].

Other Enzymes Associated with INH Resistance

The MTB glf (Rv3809c) gene encoding UDP-galactopyranose mutase, catalyzes the conversion of UDP-galactopyranose into UDP-galactofuranose through a 2-keto intermediate. It was reported that the over expression of Glf enzyme bound with the modified form of INH or by sequestering a factor such as NAD+ required for INH activity and thus might contribute to INH resistance [31]. Further, Pasca et al. observed that over expression of transmembrane transport protein MmpL7 encoded by mmpL7 (Rv2942) gene, in *Mycobacterium smegmatis* leads to high level INH resistance [32]. Yang et al. observed that InbR, a transcriptional regulatory protein which is encoded by Rv0275c, directly bind with INH and involved in isoniazid resistance [33]. Pandey et al. observed the over expression of Rv1475c (acn) gene, encoding Aconitate hydratase-A enzyme in clinical isolate of MTB resistant to rifampicin, isoniazid, ethambutol and kanamcyin [34] which suggested that Rv1475c might have some important role in INH resistance [35].

Though INH resistance is mostly due to the chromosomal

S/No	Locus_tag	Name	Protein Length	Locus	PDB ID	Pathway
1	Rv3248c	S-adenosyl-L-homocysteine hydrolase	495	sahH	3DHY	• Cysteine and methionine • Metabolism • Metabolic pathways
2	Rv0753c	Methylmalonate-semialdehyde dehydrogenase	510	mmsA		• Valine, leucine and isoleucine • Degradation • beta-Alanine metabolism. • Inositol phosphate metabolism • Propanoate metabolism • Metabolic pathways • Carbon metabolism
3	Rv1187	pyrroline-5-carboxylate dehydrogenase ROCA	543	rocA	4IHI	• Alanine, aspartate and glutamate Metabolism • Arginine and proline metabolism • Metabolic pathways
4	Rv0155	NAD(P) transhydrogenase subunit alpha	366	pntAa/ pntAA		• Nicotinate and nicotinamide metabolism • Metabolic pathways
5	Rv2623	Universal stress protein	297	TB31.7	3CIS	
6	Rv1996	hypothetical protein	317			
7	Rv0468	3-hydroxybutyryl-CoA dehydrogenase	286	fadB2		• Phenylalanine metabolism • Benzoate degradation • Butanoate metabolism • Metabolic pathways • Microbial metabolism in diverse environments
8	Rv1484	enoyl-ACP reductase	269	inhA	1P44	• Fatty acid biosynthesis • Metabolic pathways
9	Rv2691	TRK system potassium uptake protein CEOB	227	ceoB / trkA		
10	Rv0091	bifunctional 5-methylthioadenosine nucleosidase / S-adenosylhomocysteine nucleosidase	255	Mtn / pfs		• Cysteine and methionine metabolism • Metabolic pathways • Biosynthesis of amino acids,
11	Rv2858c	Aldehyde dehydrogenase	455	aldC		• Glycolysis / Gluconeogenesis • Pentose and glucuronate interconversions • Ascorbate and aldarate metabolism • Fatty acid degradation • Valine, leucine and isoleucine degradation • Lysine degradation • Arginine and proline metabolism • Histidine metabolism • Tryptophan metabolism • beta-Alanine metabolism • Glycerolipid metabolism • Pyruvate metabolism • Chloroalkane and chloroalkene degradation • Limonene and pinene degradation • Metabolic pathways • Biosynthesis of secondary metabolites • Microbial metabolism in diverse environments • Biosynthesis of antibiotics
12	Rv1059	Hypothetical protein	354			
13	Rv3777	Oxidoreductase	328			
14	Rv2971	Oxidoreductase			4OTK	
15	Rv2766c	3-ketoacyl-ACP reductase	260			
16	Rv2671	Possible bifunctional enzyme riboflavin biosynthesis protein RibD	258	ribD		• Riboflavin metabolism • Metabolic pathways • Biosynthesis of secondary metabolites
17	Rv2763c	Dihydrofolate reductase (DHFR)	159	dfrA/ folA	4KL9	• One carbon pool by folate • Folate biosynthesis • Metabolic pathways
18	Rv1483	3-oxoacyl-ACP reductase	247	fabG1 / mabA	1UZL	• Fatty acid biosynthesis • Biotin metabolism • Biosynthesis of unsaturated fatty acids • Metabolic pathways

Table 2: High Affinity INH-NAD (P) - binding proteins from Mycobacterium tuberculosis [13,14].

mutations in the target genes, around 20-30% of INH resistant MTB isolates do not have mutations in any of the genes linked with resistance to INH [36] which suggests that other mechanism(s), namely efflux pump systems of MTB may be involved in INH resistance. The over expression of efflux pump genes such as efpA, mmpL7, mmr, p55 and the Tap-like gene Rv1258c etc are shown to contribute for INH resistance [36]. Further, efpA, jefA (Rv2459), drrA, drrB, mmr, Rv1250, Rv1634 and Rv0849 were reported to be over expressed under isoniazid

S/No	Locus_tag	Name	Protein Length	Locus	PDB ID	Pathway
1	Rv2243	Malonyl CoA-acyl carrier protein transacylase	302	FabD	2QC3	• Fatty acid biosynthesis • Lipid metabolism
2	Rv2244	Meromycolate extension acyl carrier protein	115	acpM	1KLP	
3	Rv2245	3-oxoacyl-[acyl-carrier-protein] synthase 1	416	kasA	4C6U	• Fatty acid biosynthesis • Lipid metabolism
4	Rv2246	3-oxoacyl-[acyl-carrier-protein] synthase 2	417	kasB		fatty acid biosynthesis Lipid metabolism
5	Rv2247	Propionyl-CoA carboxylase beta chain 6	473	AccD6	4FB8	• Fatty acid biosynthesis • Valine, leucine and isoleucine degradation • Pyruvate metabolism • Glyoxylate and dicarboxylate metabolism • Propanoate metabolism • Metabolic pathways • Biosynthesis of secondary metabolites • Microbial metabolism in diverse environments • Biosynthesis of antibiotics • Carbon metabolism • Fatty acid metabolism
6	Rv0129c	Diacylglycerol acyltransferase/mycolyl-transferase Ag85C	340	fbpC	4MQM	• Glycerolipid metabolism • Metabolic pathways
7	Rv3140	Acyl-CoA dehydrogenase	401	FadE23		• Fatty acid degradation • Valine, leucine and isoleucine degradation • Beta-Alanine metabolism • Propanoate metabolism • Metabolic pathways • Biosynthesis of secondary metabolites • Biosynthesis of antibiotics • Carbon metabolism • Fatty acid metabolism
8	Rv3139	Butyryl-CoA dehydrogenase	468	FadE24		
9	Rv2428	Alkyl hydroperoxide reductase subunit C	195	ahpc	2BMX	• Glutathione metabolism • Metabolic pathways
10	Rv2846c	MFS-type transporter EfpA	530	efpA		
11	Rv1592c	Probable inactive lipase	446			
12	Rv1772	Hypothetical protein	103			
13	Rv0341	Isoniazid-induced protein	479	iniB		
14	Rv0342	Isoniazid-induced protein	640	iniA		
15	Rv0343	Isoniazid-induced protein	493	iniC		

Table 3: Genes induces by INH or ethionamide treatment of an INH-sensitive strain [38].

or rifampicin stress [37]. Besides some other genes of MTB (Table 3) of an INH-sensitive strain, are also observed to be induced by isoniazid or ethionamide treatment [38].

Though, the in depth molecular mechanism of INH resistance in number of mycobacterial proteins from drug resistance strains is yet to be thoroughly understood, many studies proposed the association of INH with number of MTB proteins in different ways such as direct activation by KatG, acetylation by NAT, inhibiting different enzymes through adduct formation, inducing different enzymes etc. Further, mutations in many enzymes of MTB are reported to be associated with INH resistances which are supposed to associate with different important metabolic pathways of MTB. The consequence of mutation of those enzymes on respective pathways needs further study so as to reveal other mechanisms of INH resistance.

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