

Original Article

**Polymorphisms of twenty regulatory proteins between *Mycobacterium tuberculosis*
and *Mycobacterium bovis***

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Summary

Mycobacterium tuberculosis and *Mycobacterium bovis* are responsible for tuberculosis in humans or animals, respectively. Both species are closely related and belong to the *Mycobacterium tuberculosis* complex (MTC). *M. tuberculosis* is the most ancient species from which *M. bovis* and the other members of the MTC evolved. The genome of *M. bovis* is over 99.95% identical to that of *M. tuberculosis* but with seven deletions ranging in size from 1 to 12.7 kb. In addition, 1,200 single nucleotide mutations in coding regions distinguish *M. bovis* from *M. tuberculosis*. In this study we assessed 75 *M. tuberculosis* genomes and 23 *M. bovis* genomes to identify non-synonymous mutation in 202 coding sequences of regulatory genes between both species. We identified species-specific variants in 20 regulatory proteins and confirmed differential expression of hypoxia-related genes between *M. bovis* and *M. tuberculosis*.

Key words: *Mycobacterium bovis*, *Mycobacterium tuberculosis*, regulators, polymorphisms

Introduction

The *Mycobacterium* genus includes pathogens responsible for serious diseases such as tuberculosis and leprosy in mammals. Within this genus, the *Mycobacterium tuberculosis* complex (MTC) refers to a group of genetically related pathogenic species that can cause tuberculosis in several mammals, including humans. Indeed, *Mycobacterium bovis* is a MTC member of significant importance in livestock.

The complete genome sequence of *M. bovis* was published (1) five years after the publication of the genome sequence of *M. tuberculosis* (2). The genome of *M. bovis* is over 99.95% identical to that of *M. tuberculosis* but has seven deletions. The region containing the deletions is called Region of Difference (RD) and ranges from 1 to 12.7 kb.

This finding, therefore, could suggest that overall, the main evolutionary force shaping the genome of *M. bovis* gene has been the deletion. Interestingly, many of the missing or altered genes in *M. bovis* are missing in *M. leprae*, an obligate intracellular pathogen that suffered a considerable genome reduction (1).

Major advances have been achieved in the understanding of the evolutionary mechanisms that led to the emergence of species of MTC and particularly *M. bovis*. Nevertheless, it is still hard to explain how this bacterium, a microorganism whose genome has largely lost genomic regions and with no unique genes, inhabits a broader biological niche than its ancestor, *M. tuberculosis*. Probably, the presence of more than 1,200 single nucleotide polymorphisms (SNP) in coding regions or genes that distinguish *M. bovis* from *M. tuberculosis* may, in part, explain this characteristic. Garnier *et al.* have found that the most variable genes between both species are those related to cell wall and secreted proteins. In particular, they described polymorphisms in several genes involved in the synthesis or transport of lipid complexes (1) as well as in genes encoding the families of PE-PGRS and PPE proteins. PE-PGRS and PPE proteins are expressed on the surface of mycobacteria and may provide different antigenic variation causing immune responses in *M. tuberculosis*, their corresponding genes represent over 10% of the genome (3). In addition, *esxR* and *esxS* are genes encoding members of the Esx protein family and are absent from several *M. tuberculosis* strains (4). The Esx protein family consists of more than 20 proteins including antigens CFP7 and CFP10 (5) and many of them strongly stimulate T cell responses.

A polymorphism in *pncA* between *M. bovis* and *M. tuberculosis* was identified previous to the sequencing of mycobacterial genomes. *pncA* encodes the enzyme pyrazinamidase (PncA) (6) that activates the first-line antituberculous drug pyrazinamide into pyrazinoic. In *M. bovis* a point mutation produced the replacement of a histidine for an aspartate in a

specific position with the consequent loss of the enzyme activity. Although *M. tuberculosis* H37Rv carries an intact copy of *pncA*, some clinical isolates of *M. tuberculosis* show a variety of mutation in this gene (7).

Because of their amplifying effect, the finding of mutations in genes encoding transcriptional regulators between *M. tuberculosis* and *M. bovis* is remarkable. For example, Peirs *et al.* (8) reported a gene coding for *PknD* (Rv0931c) that is truncated in *M. bovis* and Gonzalo-Asensio *et al.* (9) identified a mutation in the *phoR* gene of *M. bovis* isolates, which could explain the low transmission of this species to humans. PhoR together with PhoP form a two-component system that activates the expression of numerous proteins relevant for *M. tuberculosis* interaction with the host. The higher expression level of the humoral antigens MPB70 and MPB83 in *M. bovis* with respect to *M. tuberculosis* is also relevant. This difference is due to a mutation in *Rv0444c*, which encodes an anti-sigma factor K (10). The sigma factor K (SigK) positively regulates the transcription of *mpb70* and *mpb83* and a mutation in *RskA*, its repressor, therefore produces upregulation of *mpb70* and *mpb83* in *M. bovis*. Besides these few previously described polymorphisms in regulatory genes, no compiled genomic data are available on potential variants in regulatory proteins between *M. bovis* and *M. tuberculosis*. To fill this gap, in this study we search for conserved non-synonymous mutations in coding sequences for transcriptional regulators and two component systems in *M. bovis* compared to *M. tuberculosis*.

From 202 potential transcriptional regulators and two-component systems, we identified 20 genes with either non-synonymous SNPs or deletion/insertions (INDELs) between *M. bovis* and *M. tuberculosis*.

Materials and Methods

Genomic analysis: A total of 202 MTC regulatory genes were selected and downloaded from the TubercuList database server (<http://genolist.pasteur.fr/TubercuList>) and BoviList database server (genolist.pasteur.fr/BoviList/). These genes were selected because they encode for transcriptional regulators, two component systems or regulatory proteins. The sequence of each selected gene of *M. tuberculosis* H37Rv (Supporting Tables, List 1) was manually compared to that of *M. bovis* AF2122/97 by using the commercial software DNA Strider 1.4f13. The genes with non-synonymous polymorphisms between *M. tuberculosis* H37Rv and *M. bovis* AF2122/97 (Supporting Tables, List 2) were translated to protein, and the *M. bovis* variants of each protein were used as query sequences in a BlastX analysis (<https://blast.ncbi.nlm.nih.gov>). The query sequences (Supporting Tables, List 2) were aligned to 23 *M. bovis* sequenced proteomes and a list of proteins that conserved the non-synonymous polymorphisms (SNPs, IDELs) in all *M. bovis* protein sequences was generated (Supporting Tables, List 3). The *M. tuberculosis* H37Rv orthologous of these conserved proteins (Supporting Tables, List 3) were then used as query sequences for alignment to 75 *M. tuberculosis* sequenced proteomes (<http://blast.ncbi.nlm.nih.gov/Blast.cgi#>). The *M. tuberculosis* orthologous of the selected *M. bovis* proteins (Supporting Tables, List 3) that conserved the species-specific polymorphism in all *M. tuberculosis* protein sequences were considered as species-specific regulatory genes (Table 1).

Bacterial growth conditions: *M. bovis* isolates (*M. bovis* 04-303 and *M. bovis* 534) were grown under shaking conditions in 7H9 medium supplemented with 0.05% Tween 80, and 0.5% albumin, 0.4% dextrose and 0.4% pyruvate. *M. tuberculosis* H37Rv and *M. tuberculosis* CDC1551 strains were grown under shaking conditions in 7H9 medium supplemented with 0.05% Tween 80, albumin/dextrose and 0.4% glucose.

RNA extraction: 50 ml of bacterial cultures (in duplicates) of *M. tuberculosis* H37Rv, *M. tuberculosis* CDC1551, *M. bovis* 04-303 and *M. bovis* 534 were harvested at the exponential phase of growth (Optical density 600_{nm}: 0.3-0.4). The cell pellets were immediately resuspended in 1 ml of Trizol (Sigma-Aldrich) and transferred to a 2-ml screw-cap microcentrifuge tube containing 0.1 mm diameter zirconium beads. Cells were disrupted with a Fastprep FP120 bead-beater (MP Biomedicals) for 20 s at a speed of 6 m s⁻¹. The samples were treated twice with 200 µl of chloroform, centrifuged at 9.000 g for 5 min and the nucleic acids present in the upper phases (aqueous phases) were precipitated with isopropanol. The RNA/DNA pellets were washed up with ethanol 70% and resuspended in Rnase-free water. Finally, the samples were cleaned up with RNeasy MinElute Cleanup Kit (Qiagen) and treated with DnaseI Ambion (Thermo Fisher Scientific) following the manufacture's specifications.

RT-qPCR: RT-qRT-PCR reactions were performed as previously described (11) using specific primers (Table 1) and DNA-free RNA (1µg) extracted from mid-exponential growth-phase cultures of *M. tuberculosis* H37Rv, *M. tuberculosis* CDC1551, *M. bovis* 04-303 and *M. bovis* 534. Briefly, RNA (1 µg) was mixed with 50 ng of random primers (Invitrogen) in 20 µl of final volume and reverse-transcribed to total cDNA with SuperScript II reverse transcriptase (Invitrogen, Life Technologies) following the manufacturer's instructions. Control reactions without reverse transcriptase were included. The cDNA (0.5 µl) was used as template for each real time quantitative PCR (RT-qPCR) reaction. All primers were designed using Primer 3 Software (bioinfo.ut.ee/primer3-0.4.0/) (Table 2). The qPCR reactions were performed with Taq Platinum DNA polymerase (Invitrogen, Life Technologies) and SYBR reagent (Thermo Fisher Scientific) following the manufacturer's instructions.

All reactions were performed in duplicate and the qPCR data were analyzed using the LinRegPCR software (12). Default settings were used for the LinRegPCR software. All samples without plateau or amplification and with very low Cq value were excluded for mean efficiency calculation. Strictly continuous log-linear setting was used for baseline estimation and the excluded samples were analyzed individually and corrected with the manual correction baseline option. The fold change was calculated using *sigA* as the reference gene. The final results and permutation statistical analysis were assessed with fg statistic software (13), which is part of Infostat software package. For the statistical test, the parameters were set to defaults with 5,000 permutations at random.

Results

***In silico* analysis of 202 regulatory genes of *Mycobacterium* sp.**

We first searched for total mutations in regulatory genes between the reference strains *M. bovis* AF2122/97 and *M. tuberculosis* H37Rv. The criterion to define a regulatory gene was that on the Tuberculist database (<http://tuberculist.epfl.ch/>), by combining genes classified as transcriptional regulator, two component systems and regulatory proteins. As a result, we obtained 202 genes (Supporting Tables, List 1). We then downloaded the 202 gene sequences from the TubercuList and BoviList databases and performed the pairwise comparisons (*M. bovis* AF122/97 vs *M. tuberculosis* H37Rv) with DNA Strider program. A total of 80 genes had nucleotide mutations between *M. bovis* AF2122/97 and *M. tuberculosis* H37Rv. From these genes, 25 were synonymous mutation (Supporting Table, List 2) and 55 were non-synonymous (Supporting Tables, List 2). Some polymorphisms that emerged during the divergence of *M. bovis* and *M. tuberculosis* should have had an impact in the niche adaptation of these species and therefore they should be conserved in all strains of the same species. With all this in mind, we looked for the species-specific

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variants of regulatory genes among the 55 genes with non-synonymous mutations. For these purpose, we assessed 75 *M. tuberculosis* genomes (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch; Taxid 1773) and 27 *M. bovis* genomes. The *M. bovis* genomes correspond to nine strains isolated from Brazil, seven from Argentina (<http://www.ncbi.nlm.nih.gov/bioproject/214551>) and eleven from USA (unpublished results). Only 20 regulatory genes conserved the species-specific polymorphisms (SNP or INDEL) in the 23 *M. bovis* and 75 *M. tuberculosis* genomes (Table 2). Eight polymorphisms map in conserved predicted domains (Figure 1A and B). Two polymorphisms are located in the protein kinase domains of PknL and Mb3246c (Figure 1A) and one in the helix turn helix DNA binding domains (HTH) Mb3319c (Figure 1B). Several amino acid changes and a deletion of eight amino acids map in the region encompassing amino acid position 124 to 136 of Mb2801c, compared to its orthologous Rv2779c of *M. tuberculosis*. This highly polymorphic region is localized in a domain conserved in bacterial transcriptional regulatory proteins, AsnC. Finally, TcrA shows a polymorphism in a glycoside hydrolase family RRRD domain.

Experimental study of polymorphism consequences

To get insight into the potential impact of the observed polymorphisms on the function of regulatory proteins, we compared the transcription of some selected genes between *M. bovis* and *M. tuberculosis* strains. We chose one or two genes among those transcriptionally regulated by each of the five selected polymorphic regulatory proteins and assessed the gene expression by quantitative PCR using cDNAs obtained from total RNA of *M. tuberculosis* and *M. bovis*, as template. The expression level of *Rv3074* and *Rv1456c* whose expressions are regulated by LexA and BlaI (14)(15)(16), respectively, were similar in both mycobacterial species (Table 3). Remarkably, the expression of *hspX*

and *narK2*, which is regulated by DosT/R and the expression of *pks2* regulated by PhoP (17)(18), was higher in *M. tuberculosis* strains than in *M. bovis* isolates (Table 3), while the expression of *icl1*, which is regulated by Rv0465, was downregulated in *M. tuberculosis*.

Discussion

Reversible protein phosphorylation is one of the main signal transduction pathways by which both eukaryotic and prokaryotic cells regulate the metabolism in response to external stimuli. In bacteria, signal transduction events are performed by two-component regulatory systems and by specific protein kinases and protein phosphatases.

The *M. tuberculosis* genome encodes 11 eukaryotic-like serine/threonine protein kinases (PknA to PknL, except for PknC). All of these *pkn* genes encode functional serine/threonine kinases and some of them participate in the modulation of different cellular events such as environmental adaptation, differentiation and cell division (19).

Interestingly, in this study we found conserved polymorphisms in three protein kinases, PknD, PknL, PknF. We also identified polymorphisms in three two-component systems (DosT, PhoR and Mb3246c) and in phosphatases Mb0018 and Mb0158c|*ptbB*; particularly, Mb0158c|*ptbB* participates in host infection of *M. tuberculosis* (20). These findings suggest a relevant role of the signal transduction pathways mediated by protein phosphorylation in the adaptation of *M. bovis* to its animal hosts.

Particularly, *pknD* of *M. bovis* carries a nucleotide deletion that splits the gene in two parts, *pknDA* and *pknDB*. Greenstein *et al* (21) proposed a model by which PknD alters the transcriptional program of *M. tuberculosis* by stimulating phosphorylation of a sigma factor regulator. Furthermore, Vanzembergh *et al* (22) suggested that *pknD* is necessary for growing of *M. tuberculosis* in phosphate poor conditions. Indeed, *M. tuberculosis*

requires *pknD* to invade brain endothelia but not macrophages, lung epithelia, or other endothelia, thus highlighting a role of *PknD* in *M. tuberculosis*'s host cell specificity (23).

Interestingly, we found polymorphisms in PknL and its substrate, Mb2197c (Table 1). PknL is a protein of unknown function that possesses an original winged helix-turn-helix motif, which is indicative of a transcriptional regulator (Figure 1B) (24), while Mb2197c is a conserved regulatory protein of unknown function. This finding suggests that both polymorphisms have cosegregated in the adaptation of *M. bovis* to the animal hosts.

Structural or biochemical data are available for Mb0618c/TcrA (25) Mb2801c/Rv2779c (26), Mb2197c/Rv2175c (24), Mb2198|pknL (27), Mb3319c/Rv3291c (28) and PknD (29).

In addition, a function has been demonstrated or suggested for the *M. tuberculosis* homologous of Mb0474c (15), Mb1775|PknF (30), Mb1877c|BlaI (16), Mb0018c|PstP (31), Mb0158c|mPtbB (32), PhoPR (33) (34) (35), Mb2052c|DosT (36) (37) (38) (17) and Mb2739|LexA (39) (14). There is no available information on the probable two component sensor kinase Rv3220c|Mb3246c or on the putative transcriptional regulators Rv0078|Mb0080, Rv0823|Mb0846c, Rv1358|Mb1393, Rv1460|Mb1495 and Rv2621c|Mb2654c.

PtbB is a virulence factor that participates in the mechanism of *M. tuberculosis* immune evasion (40), whereas BlaI regulates the responses and resistance to beta-lactam antibiotics and ATP synthesis (24).

Remarkably, two of the polymorphic regulator genes described in this study, Mb0018c and PknF, participate in cell division process (41) (30). However, PknF may have other functions. For example, in *M. tuberculosis*, PknF phosphorylates GroEL1 (42), Rv1747 (43) and mtFabH (44). Rv1747 is an *in vivo* essential ABC transporter, whereas mtFabH is a key component of the mycolic acid pathway. On the other hand, GroEL1 is a conserved chaperone required for the folding of proteins in several microorganisms but with an

imprecise role in pathogenic Mycobacteria. GroEL1 seems to participate in the cytokine-dependent granulomatous response during *M. tuberculosis* infection and interacts with the β -ketoacyl-AcpM synthase KasA (42), which is another key component of the type II fatty acid synthase involved in mycolic acid biosynthesis. Therefore, PknF has multiple roles in the regulation of mycolic acid synthesis and also seems to control pathways of glucose utilization (30).

In contrast to *M. tuberculosis*, *M. bovis* does not grow in glucose or glycerol as unique carbon sources because of the inactive pyruvate kinase, PykA. Thus, the *M. bovis* metabolism seems to be highly dependent on fatty acids for energy production. The utilization of fatty acids requires a functional glyoxylate cycle with the key enzymes malate synthase and isocitrate lyase (Icl). Since Rv0465/Mb0474 represses the expression of *icl1* (45), mutations on Mb0474 and *pknF* may have allowed the adaptation of *M. bovis* to its biological niches. Consistently with this presumption, in this work we detected a non-synonymous polymorphism in Rv0465/Mb0474 and higher expression of *icl1* in *M. bovis* compared to *M. tuberculosis*.

Mb2801 encodes a possible transcriptional regulatory protein belonging to the leucine-responsive regulatory protein/asparagine synthase C products (Lrp/AsnC-family). This protein has a deletion of eight amino acids that maps in a conserved and predicted AsnC family domain. This class of regulators, which is widespread among prokaryotes, is involved in the regulation of amino-acid metabolism and related cellular processes. A preliminary X-ray analysis of Rv2779c, the *M. tuberculosis* homologous (26) of Mb2801, has been recently performed but no further information is available about the role of this protein in the bacilli. Another polymorphic Lrp/AsnC family member is Mb3319c or LprA with a SNP in the helix turn helix protein domain. This regulator is among the most connected regulatory hubs in the *M. tuberculosis* transcriptional regulatory network (46).

Gonzalo-Asensio et al (2014) have previously reported the low expression of *pks2* in *M. bovis*, compared to *M. tuberculosis* (9). Both DosT and Phop mediate the hypoxia response in *M. tuberculosis*; furthermore, Gonzalo-Asensio et al (2008) have suggested that PhoP regulates the dormancy/hypoxia regulon through crosstalking with DosR/T (47). Previous evidences have indicated differential behavior between *M. tuberculosis* and *M. bovis* under hypoxia conditions and one illustrative example of these differences is the unique capacity of *M. bovis* to disseminate extrapulmonary (48). In this study, the reduced *hspX* expression in *M. bovis* compared to *M. tuberculosis* may support these previous findings. However, to confirm this presumption it is required to compare the expression of *hspX* in both species under hypoxia conditions of growth.

DosR/T also regulates the expression of *narK2*, which encodes a nitrate transporter, and the transcription of *narK2* is induced by hypoxia in *M. tuberculosis* but not in *M. bovis* (49),(50). Chauhan and collaborators have demonstrated that the deficient induction of *narK2X* operon in *M. bovis* under hypoxia condition is due to a t6c SNP in the -10 promoter element of *narK2X* operon (51). However, we can not conclude that the low expression of *narK2* here detected in *M. bovis* is a consequence of the reported promoter 's mutation because the growth conditions assayed were different between the Chauhan's study and this report. The study of Chauhan and collaborators has also demonstrated that complementation of *M. bovis* with both *narGHJI* and *narK2X* genes from *M. tuberculosis* failed to restore nitrate reductase activity in *M. bovis* in both aerobic and hypoxia conditions (51), suggesting that additional regulatory mechanisms for nitrate reduction are altered in *M. bovis*. From these previous findings and the results of this study, we hypothesize that the mutation in DosT might contribute to the failure of the nitrate reductase activity in *M. bovis*.

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Rehren et al. (2007) defined a set of genes that are differentially expressed between *M. bovis* and *M. tuberculosis* under standard conditions of growth (52). Interestingly, in their study, the transcription of *Rv0465c* and *Rv2779c*, which are both encoding polymorphic transcriptional regulators, was upregulated in *M. bovis*. Based on the premise that most of the transcriptional regulators regulate their own synthesis, we speculate that the regulatory capacities of *Rv0465c* and *Rv2779c* are impaired in *M. bovis* because of the mutations in their genes. In addition, in Rehren et al.'s study, the expression of *Rv1588c*, a member of the LexA regulon, was upregulated in *M. bovis*.

The finding of non-synonymous mutations in 20 regulatory genes of *M. bovis* strains compared to *M. tuberculosis* strains suggests that punctual polymorphisms may explain or contribute to the phenotypic differences between *M. bovis* and *M. tuberculosis*. In addition, here we found evidence that can explain the differential behaviour between *M. bovis* and *M. tuberculosis* under hypoxia environments. However, further studies need to be done to determine the impact of these polymorphisms in the niche specialization of both pathogens.

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Disclosure

None declared.

Competing interests

There are not competing interests.

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Figure Legends

Figure 1: Schematic representation of regulatory proteins. A) Two component systems, protein kinases and protein phosphatases. B) Other regulatory proteins. Amino acid changes (*M. tuberculosis*/*M. bovis*) are indicated.

Title of Supporting Tables

List 1: Genes of transcriptional regulators and regulatory proteins of *M. tuberculosis*

H37Rv

List 2: Genes with non-synonymous and synonymous SNP/Indel between *M. tuberculosis*

H37Rv and *M. bovis* AF2121/97

List 3: Polymorphic proteins conserved in *M. bovis* strains

List of abbreviations

MTC: *Mycobacterium tuberculosis* complex

RD: region of difference

SNP: single nucleotide polymorphisms

Table 1: primers sequences of selected genes used in RT-qPCR experiments

<i>Primers</i>	<i>Sequences</i>
<i>hptX</i>	F: GACATTATGGTCCGCGATG R: GCCTTAATGTCGTCCTCGTC
<i>pks2</i>	F: ATCGGTGACCCCATGAATA R: GACTGGGTGTGTCCGAAGTT
<i>Rv3074</i>	F: TGGTTTACGAGATGCCACAC R: ACATCCAGACATGCGCTTT
<i>iclI</i>	F: CCAAGTTCCAGAAGGAGCTG R: TTCCTGCAGTTCGACATACG
<i>Rv1456c</i>	F: TGTTGGTTGCCTACCTTGC R: CCGTGAAGTATTGCGTGGT
<i>narK2</i>	F: GTGACCTGGGAGATGTCGTT R: AGAACCCGTAGATCGTGGTG

sigA was the calibrator gene

Table 2: Genes with conserved non-synonymous mutation between *M. tuberculosis* H37Rv and *M. bovis* AF2122/97

Rv number/ Mb number	Gene	Description	INDEL /SNP*	AA change
<i>Rv0018c/Mb0018c</i>	<i>pstP</i>	Phosphoserine/threonine phosphatase PstP	g1363t c1387t	S455A P463S
<i>Rv0078/Mb0080</i>		Probable transcriptional regulatory protein	a337g	I113V
<i>Rv0153c/Mb0158c</i>	<i>ptbB</i>	Phosphotyrosine protein phosphatase PTPB (protein-tyrosine-phosphatase) (PTPase)	a314g	D105G
<i>Rv0465c/Mb0474c</i>		Probable transcriptional regulatory protein	g977a	R326Q
<i>Rv0602c/Mb0618c</i>	<i>tcrA</i>	Two component DNA binding transcriptional regulatory protein TcrA	g82a	V28I
<i>Rv0758/Mb0781</i>	<i>phoR</i>	Possible two component system response sensor kinase membrane associated PhoR	g211a g212t	G71I
<i>Rv0823c/Mb0846c</i>		Possible transcriptional regulatory protein	g388t t988c	G130C F330L
<i>Rv0931c/Mb0955c</i>	<i>pknD</i>	Transmembrane serine/threonine-protein kinase D PknD (protein kinase D) (STPK D)	a828ins	<i>T277fsTer14</i>
<i>Rv1358/Mb1393</i>		Probable transcriptional regulatory protein	a1325c	D442A
<i>Rv1460/Mb1495</i>		Probable transcriptional regulatory protein	c797t	A266V
<i>Rv1746/Mb1775</i>	<i>pknF</i>	Anchored-membrane serine/threonine-protein kinase PknF (protein kinase F) (STPK F)	g973a	A325T
<i>Rv1846c/Mb1877c</i>	<i>blaI</i>	Transcriptional repressor BlaI	g274t	D92Y
<i>Rv2027c/Mb2052c</i>	<i>dosT</i>	Two component sensor histidine kinase DosT	t46g	L16V
<i>Rv2175c/Mb2197c</i>		Conserved regulatory protein	c50t	P17L
<i>Rv2176/Mb2198</i>	<i>pknL</i>	Probable transmembrane serine/threonine-protein kinase L PknL (protein kinase L) (STPK L)	t154g	S52A
<i>Rv2621c/Mb2654c</i>		Possible transcriptional regulatory protein	g581_g583del	G195del
<i>Rv2720/Mb2739</i>	<i>lexA</i>	Repressor LexA	t350c	V117A
<i>Rv2779c/Mb2801c</i>		Possible transcriptional regulatory protein (probably Lrp/AsnC-family)	t368_ g391del	V123_A130del
<i>Rv3220c/Mb3246c</i>		Probable two component sensor kinase	g139a	D47N
<i>Rv3291c/Mb3319c</i>	<i>lrpA</i>	Probable transcriptional regulatory protein LrpA	c65t	A22V

(Lrp/AsnC-family)

* Nucleotide positions in *M. tuberculosis* H37Rv

Table 3: Transcription of selected genes in *M. tuberculosis* and *M. bovis*

GENE	FOLD CHANGE*	SD	P-VALUE	Trancriptional Regulator
<i>pks2</i>	11.79	1.20	0.0319	PhoP/R
<i>Rv1456</i>	3.71	2.42	0.1156	BlaI
<i>hspX</i>	27.98	2.96	0.0050	DosR/T
<i>narK2</i>	54.34	0.0031	0.0072	DosR/T
<i>Rv3074c</i>	2.05	1.35	0.1067	LexA
<i>icl1</i>	0.13	0.06	0.0082	Rv0465

* Expression ratio of *M. tuberculosis*/*M. bovis*

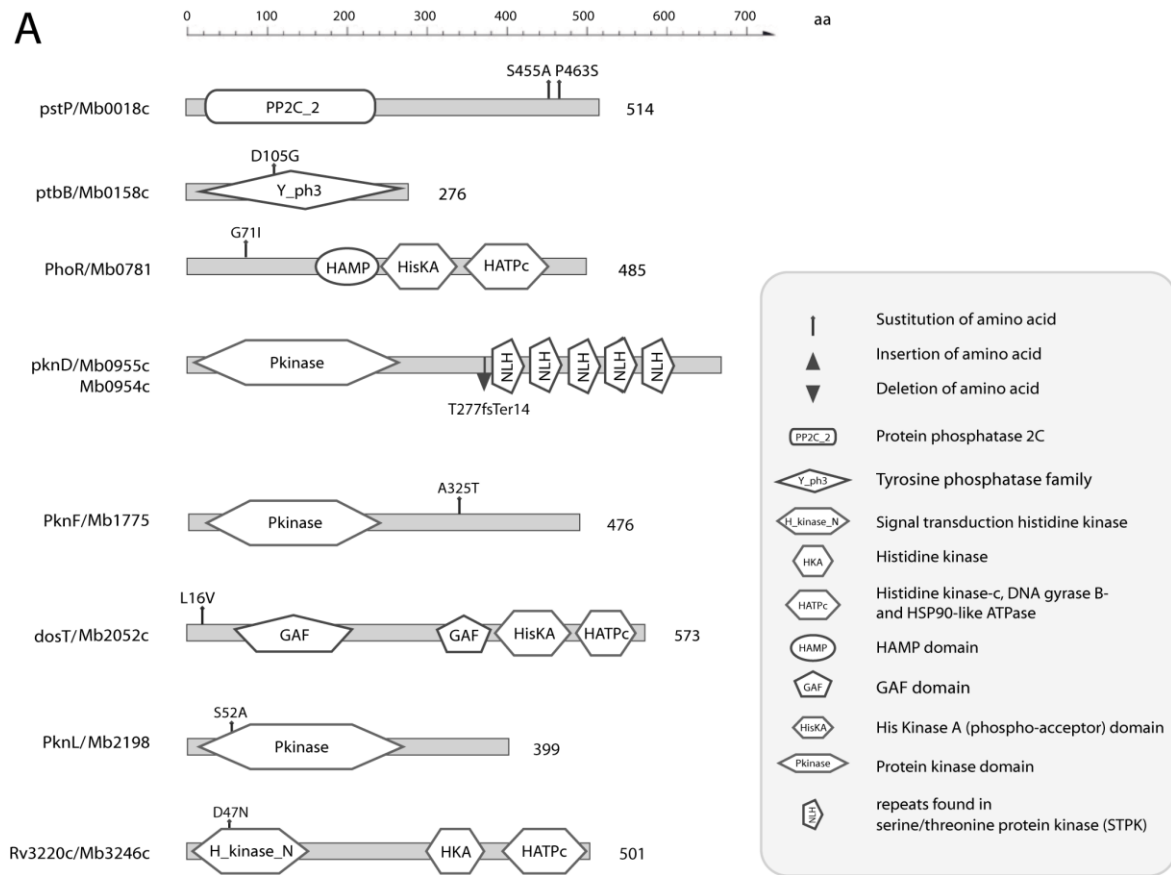


Figure 1A

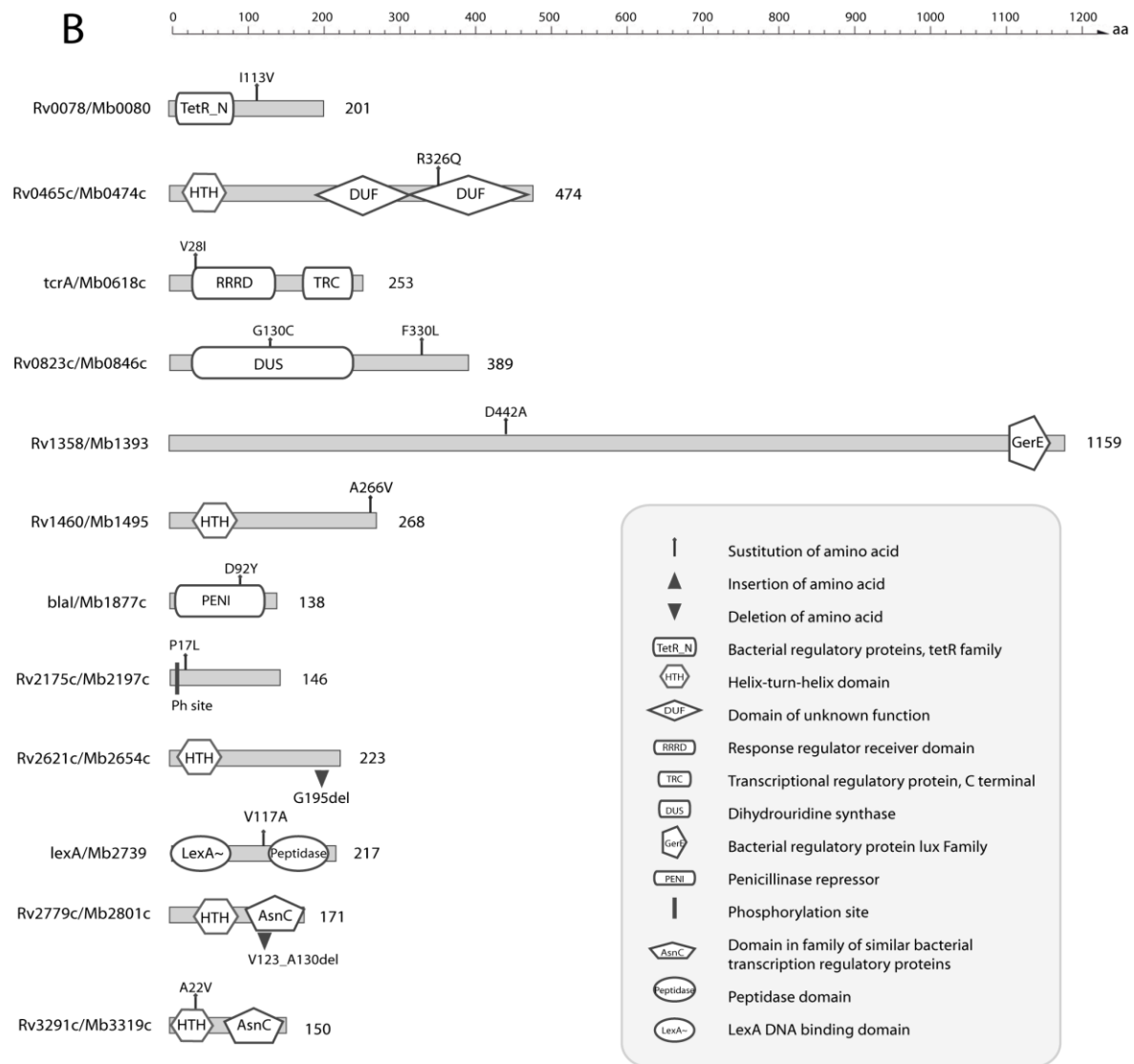


Figure 1B