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Comparative Genomics of *Mycobacterium*Aminopeptidases

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Abstract

Aminopeptidases are exopeptidases that selectively remove amino acids sequentially from the unblocked N-termini of peptides and play an indispensable role in regulating various cellular activities such as cell division, transcriptional regulation, elimination of misfolded protein and protection of the cell from harsh conditions. However, there is little information about the roles of these enzymes in the physiology and pathogenicity of the *Mycobacteria*.

Here we carried out a comparative genomic analysis of the aminopeptidase coding genes bioinformatically in seven completely sequenced pathogenic and nonpathogenic mycobacterial species. Six genes out of the 10 found in *M tuberculosis* were highly conserved in all mycobacterium species. This set of aminopeptidase is likely indispensable for growth and may serve as a novel target for drug development with high efficacy in the treatment of mycobacterial diseases.

Introduction

Tuberculosis remains a leading cause of death worldwide through afflicting approximately one-third of the world's population and claiming a death toll around 1.2 million annually [1]. *Mycobacterium tuberculosis* is an extraordinary facultative intracellular parasite multiplying within the hostile intracellular environment of macrophage [2, 3] and can survive within granuloma in dormancy state despite the presence of intact immune system [4-7]. However, the bacterial determinants necessary for persistent infection remain enigmatic.

Multidrug-resistant (MDR) *M. tuberculos*is (defined as resistant to at least isoniazid and rifampicin) and extensively drug-resistant (XDR) *M. tuberculosis* (defined as MDR plus resistance to fluoroquinolone and at least one of the injectable drugs such as kanamycin, amikacin) are a daunting obstacle to the control of tuberculosis and are associated with high mortality worldwide [8, 9]. Because of ineffective available antituberculous drugs in the treatment of MDR, there is urgent need to explore new targets and development of new antibiotics that are able to eliminate drug-resistant *M tuberculosis*.

Similarly, leprosy is a chronic disease caused by the *Mleprae*. Its prevalence was 5.2 million including 122 countries, mostly in the developing world, which were endemic for the disease. Inspite of the WHO's efforts to reduce the prevalence of leprosy by the 2005 , this disease remains a major health concern worldwide. Annually, more than 200 000 people with leprosy have been discovered in many areas in Africa, the Americas, and Asia [10]. Additionally, *M. avium* and *M. intracellulare* are nontuberculous mycobacterial (NTM) species are usually grouped in the *M. avium-M. intracellulare* complex (MAIC) for their biochemical similarity. These organisms are most significant NTM species associated with opportunistic infections, causing disseminated infection in patients with AIDS, nodular bronchiectasis and other pulmonary infections, lymphadenitis, and skin infection [11]. Likewise, *M. bovis* is a major cause of bovine tuberculosis with significant economic values. It also causes human TB, particularly in developing countries, and the risk of opportunistic infection with *M.bovis* increased with increasing rate of HIV infections [12]. *M. marinum* is the most common cause of bacterial disease in freshwater and marine fish, what is termed fish tuberculosis [13]. It also causes opportunistic infections in humans such as chronic cutaneous lesions and in some cases systemic infections such as tenosynovitis, septic arthritis and rarely osteomyelitis [14].

Key components of biochemical processes essential for bacterial growth represent an ideal novel drug target for better antibiotics against

drug-resistant mycobacteria. To explore the promise of aminopeptidase for better antibiotic targets, a comparative genomics analysis of the aminopeptidase coding genes predicted in the sequenced mycobacterium genomes was performed using the Basic Local Alignment Search Tool (BLAST) [15].

We downloaded aminopeptidases genes by using “aminopeptidase” and “mycobacterium” as keywords to retrieve aminopeptidase genes on NCBI website. Then the aminopeptidase gene was confirmed at the mycobacterium databases of the Pasteur Institute (Paris, France) using *M tuberculosis* H37Rv gene number (http://www.sanger.ac.uk/Projects). The Phylogenetics were created using MEGA4 software.

Aminopeptidases catalyze- the removal of amino acids sequentially from the unblocked N-termini of peptides and proteins. They may degrade the first peptide bond in a polypeptide chain with the liberation of a single amino acid residue (aminopeptidases in a strict sense) or cleave dipeptides or tripeptides (such as dipeptidyl- and tripeptidylpeptidases) from polypeptide substrates [16]. They are ubiquitous in nature being present in animals, plants and microorganisms [17, 18]. The location of these enzymes can be in the subcellular organelles, cytoplasm, membranes, associated with the cell surfaces or secreted [18, 19]. They play an indispensable role on regulating various cellular activities such as cell division, transcriptional regulation, elimination of misfolded or damaged polypeptides, and protection of the cell from harsh conditions [16].

Most aminopeptidases are metallo-enzymes, including some cysteine and serine peptidases [16]. The enzymes are classified on the basis of mechanism of catalysis, the structure of the active site, substrate specificity and molecular properties [16, 20, 21]. MEROPS database (http://merops.sanger.ac.uk/), give a hierarchical, structure-based classification of peptidases. In this database, each peptidase is assigned to a family on the basis of statistically significant similarities in amino acid sequence, and families that are thought to be homologous are grouped together in a clan. A clan represents one or more families that share a single evolutionary origin, evidenced by similar tertiary structures and by the order of catalytic-site residues in the polypeptide chain or often by common sequence motifs around the catalytic residues.

Aminopeptidases were found in eight clans which are designated as MA, MF, MG, MH, MN, MQ (metallo-aminopeptidase), CA (cysteine aminopeptidase) and SC (serine aminopeptidase). The families in clan MA are characterized by the presence of a His-Glu-Xaa-Xaa-His (HEXXH) motif in which two His residues are zinc ligands and the Glu residue has a catalytic role. Clan MF family

contains two zinc ions ligated to the active site (Lys, Asp, Asp, Asp, Glu), clan MG is characterized by two cobalt or manganese ions ligated in the active site (Asp, Asp, His, Glu, Glu); clan MH contains a variety of zinc_dependentexopeptidases , clan MN consists of two zinc ions attached to active site (Asp, Glu, His, His, Glu); and clan MQ contains two cobalt ions chelated by Glu, Glu, His, His, Asp. The remaining two clans CA (cysteine aminopeptidase) and SC (serine aminopeptidase) have no ionic co-factors associated with their structures and their catalysis requires of cysteine or serine residues.

Clan MA has 42 families and the structures of 23 families have been resolved. Clan MF, MG, MN and MQ each consists of a single family with known structure, and clan MH has 4 families with demonstrated structures. Clan CA is comprised of 32 families and the structures of 27 families were determined, whereas clan SC contains 6 families and the structures of 5 families were resolved.

Table 1:- Aminopeptidase-coding genes in *M. tuberculosis, M. Leprae, M. aviumparatuberculosis, M. bovis, M.intracellulare, M. marinum*and *M. smegmatis* genomes.

Name	Description	*M.tuberculosis* H37Rv	*M. leprae* TN	*M. avium subsp. paratuberculosis* K-10	*M.bovis* AF2122/97	*M. intracellulare* ATCC 13950	*M. marinum M*	*M. smegmatis* str. MC2 155	*reference*
mapA (*Mt*MetAP1)	Methionine aminopeptidase	Rv0734	83% ML1831	86% MAP4200	100% Mb0755	85% OCU_42990	86% MMAR_1072	78% MSMEG_1485	[22, 23]
mapB (*Mt*MetAP1c)		Rv2861c	90% ML1576	92% MAP2934c	100% Mb2886c	90% OCU_35550	92% MMAR_1842	86% MSMEG_2587	
pepC	Aspartylaminopeptidase	Rv0800	78% ML2213	83% MAP0632	99% Mb0823	82% OCU_06510	79% MMAR_4891	75% MSMEG_5828	[15]
pepE	Putative dipeptidase	Rv2089c	-	84% MAP1823c	100% Mb2116c	85% OCU_24050	85% MMAR_3075	78% MSMEG_3881	[15, 24]
pepB	Leucineaminopeptidase	Rv2213	83% ML0864	83% MAP1953	99% Mb2236	82% OCU_22480	85% MMAR_3258	73% MSMEG_4281	[15, 25]
pepN	Probable aminopeptidase N	Rv2467	83% ML1486	86% MAP2287	99% Mb2494	87% OCU_17850	85% MMAR_3817	78% MSMEG_4690	[15, 26, 27]
pepQ	Putative metallopeptidase	Rv2535c	83% ML0521	80% MAP1096	99% Mb2564c	81% OCU_32460	84% MMAR_2180	73% MSMEG_3034	[15]
PIP	Probable prolineiminopeptidase	Rv0840c	-	72% MAP0684c	99% Mb0863c	69% OCU_07230	75% MMAR_4789	39% MSMEG_2681	[15]
LpqL	Putative lipoprotein aminopeptidase	Rv0418	-	80% MAP3906	100% Mb0426	79% OCU_46220	83% MMAR_0726	65% MSMEG_0806	[28, 29]
lpqM	putative metallo-lipoproteinase	Rv0419	-	77% MAP3908	99% Mb0427	80% OCU_46120	82% MMAR_0728	52% MSMEG_4913	[30]

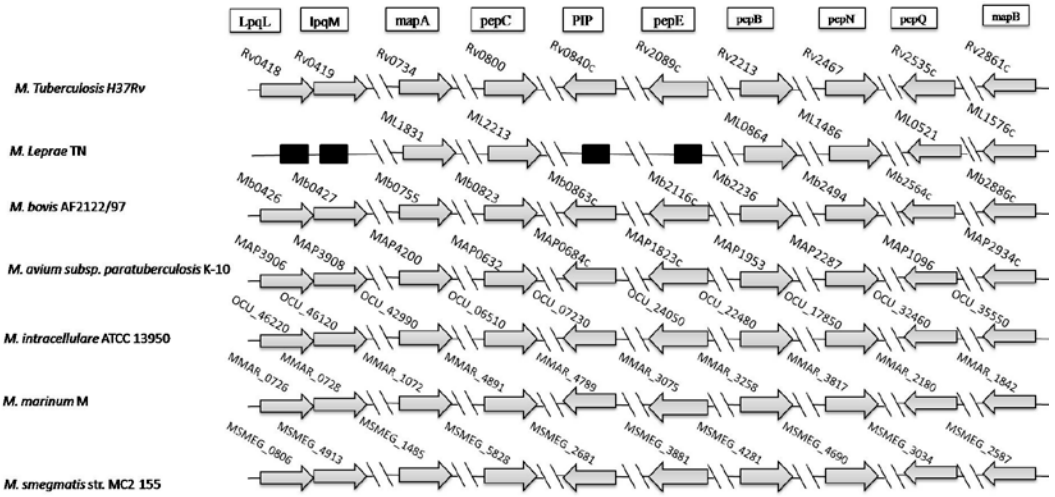


Fig. 1: Comparison of aminopeptidase locus across mycobacterial genomes. Arrows indicate the orientation of Open Reading Frames(ORFs). Black square indicates for pseudogene or deleted gene.

2. The comparative aminopeptidases from mycobacteria

There were 10 aminopeptidases genes that were predicted among mycobacteria table 1. These 10 aminopeptidases have the same evolutionary background and the strains of *M. tuberculosis* complex appear in one cluster that means these proteins have important roles in the life style of *Mycobacterium* (Fig. 2).

Six genes, *mapA*, *mapB*, *pepB*, *pepC*, *pepQ* and *pepN* gene were highly conserved in all mycobacteria species (table 1). In contrast, two genes, *pip* and *lpql* were found deleted in *M. leprae* (Fig. 1).

The paralogous of the *map* gene, *mapA* and *mapB* genes encode a methionine aminopeptidase (MAP) that belongs to the peptidase family M24. It has a MetAP1 domain that contains cobalt-binding sites (Fig. 3). It performs the essential post-translational N-terminal methionine excision from nascent polypeptides during protein synthesis and is considered as a potential target to antitubercular drugs [22, 31, 32], which were found conserved among *Mycobacterium species*. *mapA* gene (*Mt*MetAP1a) had been shown more upregulated in log phase, while *mapB* gene (*Mt*MetAP1c) showed a higher level during stationary phase [23]. The finding suggested that the two MetAPs may perform an important function in different growth phases of *M. tuberculosis* [23]. Likewise, *pepC*was found highly conserved among mycobacterium species with an identity of more than (70%). This gene probably encodes for an aspartylaminopeptidase that performs hydrolysis of N-terminal aspartate or glutamate residues from peptides [15]. This enzyme belongs

to the M18 family of metalloproteases and has a Zinc-peptidase domain (Fig. 3).

Similarly,*pepB* was found conserved in all *Mycobacterium species* with high identity. This gene encodes a leucineaminopeptidase that contains a large domain of peptidase_M17 and has zinc-binding sites (Fig. 3). This enzyme catalyzes the removal of amino acids from the N-terminus of a protein and plays a key role in protein degradation and in the metabolism of biologically active peptides [15]. It has been demonstrated in several microorganisms as essential for growth and pathogenesis [33-35]. It was shown to be upregulated in guinea pig lungs infected with *M. tuberculosis* during 30 days of infection [36]. Likewise,*pepN*was present with an identity above 70% in all *Mycobacterium species*. This gene encodes a putative aminopeptidase N, which is a zinc-dependent enzyme that belongs to the peptidase family M1 [15, 26]. It consists of a gluzincin superfamily domain in the N-terminal and an endoplasmic reticulum aminopeptidase domain (ERAP1_C superfamily) in the C-terminal (Fig. 3). *pepN*has been shown upregulated in response to different types of antibacterial compounds and this finding suggests that it may contribute in the drug resistance mechanism of *M. tuberculosis* or may play an important role in the stress response [26]. The *M. tuberculosispepN* mutant strain exhibits hypervirulence and hastens the death of severe combined immune deficiency (SCID) mice [37]. However, *pepE*was found deleted in *M .leprae*, while conserved in other mycobacterium species. This gene encodes for a dipeptidase which belongs to the M24 family and requires co-catalytic ions of cobalt or manganese. The *pepE* consists of a N-terminal non-catalytic domain of creatinase and an aminopeptidase like domain (APP-like) that contains the active site of the enzyme (Fig. 3). It has been showed that the *M tuberculosispepE* mutant strain requires bovine serum albumin (BSA) for enhanced growth [24]. In a similar fashion, *pip* was deleted in *M .leprae*and *M bovis*, whereas the gene was homologous in other pathogenic mycobacterium species. This gene most likely encodes a prolineiminopeptidase that belongs to peptidase family S33 and its enzyme selectively removes N-terminal proline residues from peptides [15]. It has one domain of proline iminopeptidase_1 (Fig. 3).

LpqL was deleted in *M .leprae* and conserved in other *Mycobacterium species*. This gene encodes for a zinc-dependent lipoprotein aminopeptidase that belongs to the peptidase family M28 [28]. This enzyme consists of M28_peptidase *Streptomyces griseus*aminopeptidase and protease-associated domain (PA) interface Zinc binding sites (Fig. 3). *LpqL* was predicted to be localized in the pseudoperiplasm and this suggests that it may be involved in nutrient metabolism [28, 29].

pepQ was found highly conserved in all *Mycobacterium species*. This gene encodes metallopeptidase which belongs to the family M24 and requires co-catalytic ions of cobalt or manganese. The enzyme catalyzes hydrolysis of peptide or polypeptide chains [15]. It has domains similar to the *pepE* domains (Fig. 3).

lpqM gene was found deleted in *M. lepraeand M. avium subsp. paratuberculosis K-10,* whereas, conserved in other mycobacteria species. This gene encodes lipoprotein peptidase (zinc metallopeptidases) of the family M13. It has a zinc peptidase domain similar to *pepC*and *lpqL*(Fig. 2). This enzyme is a membrane-associated protein that has been shown to be essential for efficient DNA transfer by conjugation [38]. It is also involved in the protein secretion pathway of *M. smegmatis*[39].

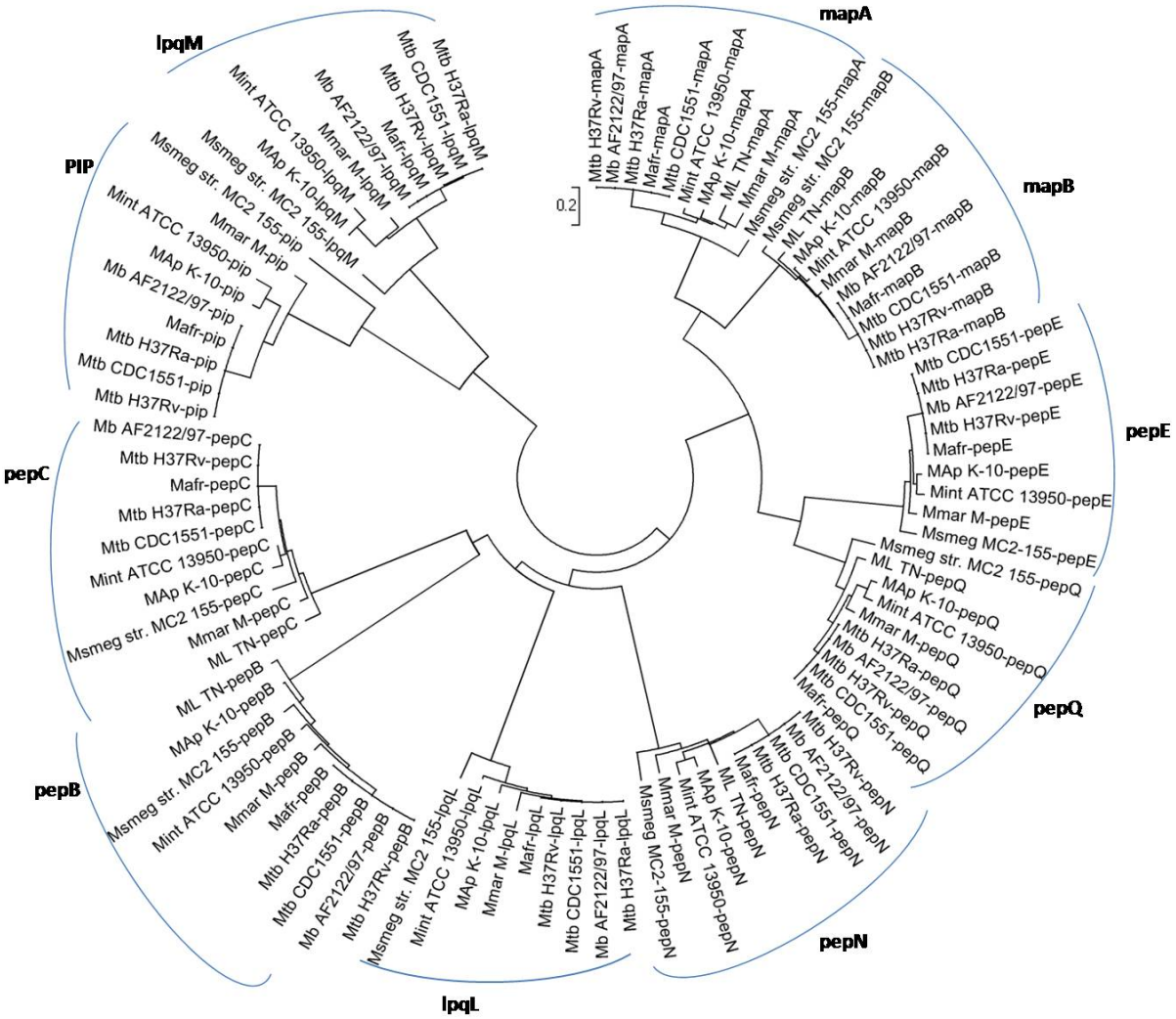


Fig.2: Phylogenetic analysis of aminopeptidases from 10 completely sequenced mycobacterial genome: The analysis was performed by the neighbor-joining method of Molecular Evolutionary Genetics Analysis Software Version 4.0 (MEGA4). The phylogenetic trees show that all

aminopeptidases have same evolutionary background. The strains of *M. tuberculosis* complex appear in one cluster that means those proteins have important roles in the life style of *Mycobacterium*. Abbreviations are as follows: Mtb, *M. tuberculosis*; Mafr, *M. africanum*; Mmar, *M. marinum*; MAp, *M. avium subsp. paratuberculosis*; Mint, *M. intracellulare*; Msmeg, *M. smegmatis*.

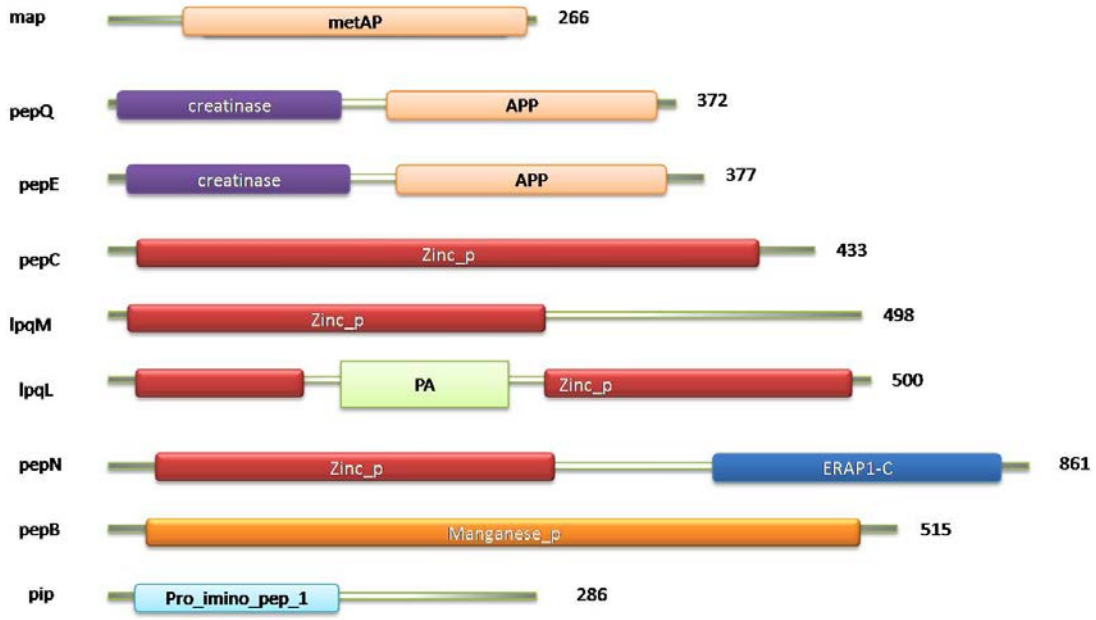
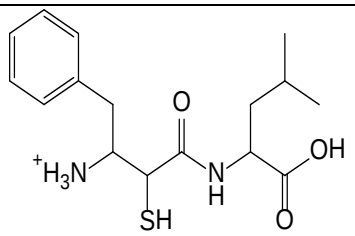
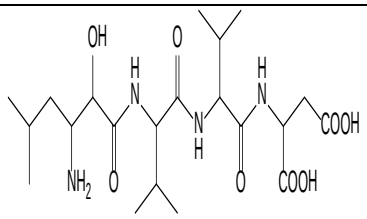
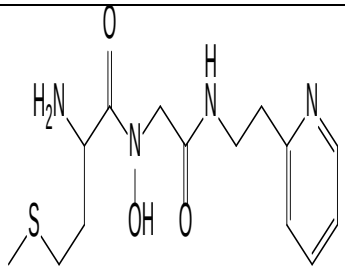
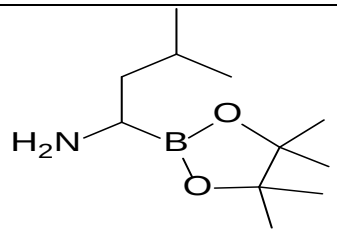
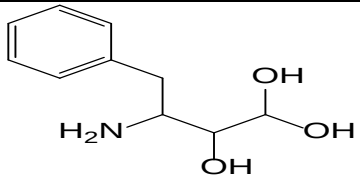


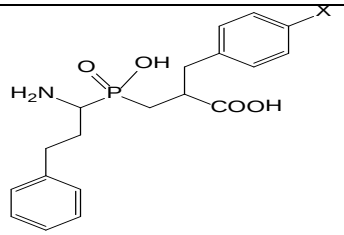
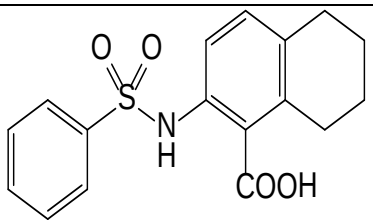
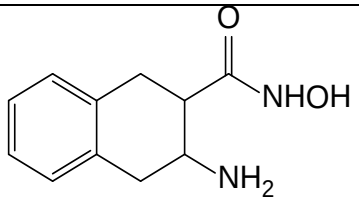
Fig. 3: Representative schematic domain compositions of aminopeptidases:
Almost all Mycobacteria aminopeptidases are metallopeptidases except pip is a serine aminopeptidase. pepC, lpqM, lpqL and pepN have zinc_binding domain. However, pepE, pepQ and map are cobalt or manganese_dependent enzyme. Numbers on the right side indicate the number of amino acids. metAP, methionine aminopeptidase; APP, aminopeptidase P; zinc_p, zinc_peptidase; PA, protease associated; ERAP1_C, endoplasmic reticulum aminopeptidase domain.

3. The inhibitors of aminopeptidase

The methionine aminopeptidase, leucineaminopeptidase and aminopeptidase N are playing an essential role in physiological processes, clinical disorders and pathogenesis of several microbes that are considered important targets for designing antimicrobial agents. The most common aminopeptidase inhibitors and their relevant targets were summarized in Table 2.

Table 2: aminopeptidase inhibitors

Name	Inhibitory concentration and Aminopeptidase target	Chemical structure	References
Bestatin compound	4.4 microM (APN); 0.55 microM (LAP)		[40]
Amastatin	IC_{50} = 0.5 Mm (APN)		[41]
Hydroxamic acids	2.5µM(EcMetAP1); 48-91µM (hMetAP1 and hMetAP2)		[42]
Boronic acid	Ki = 0.13 µM (LAP); IC50= 25 nM (APN)		[43]
Aminoaldehydes	Ki = 3 µM (APN); Ki = 100 µM (LAP)		[44]

Phosphonic acid	Ki= 36 nM (APN)		[45]
Sulfonamides	IC50= 9.0nM (hMethAP II)		[46]
Tetralone derivatives	IC50= 4 μM (APN) ; IC50= 10 μM (LAP)		[47]

4. Concluding remarks:

Aminopeptidase play an important role in protein metabolism by post-translation modification and/or elimination of misfolded or damaged protein. Mycobacterium genome has 10 genes that encode for aminopeptidases and six of those were found highly conserved among Mycobacterium species. These aminopeptidase may be essential for growth and may serve as potential target for eradication of tuberculosis.

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