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Review article

UNVEILING STRATEGIES FOR SHORTENING TUBERCULOSIS TREATMENT: TARGETING *MYCOBACTERIUM TUBERCULOSIS* STRINGENT RESPONSE AND REVIEWING POLYPHOSPHATE KINASE 2 (PPK2) ENZYMES

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ABSTRACT

Tuberculosis (TB) is one of the oldest infectious diseases known to humankind, with traces of its presence found in remains that are around 17,000 years old. TB is mostly caused by the tiny aerobic non-motile bacillus *Mycobacterium* TB (MTB). The unique shape and chemical content of the mycobacterial cell wall make an efficient TB therapy method challenging. A strict bacterial survival strategy for establishing drug tolerance in the stringent response (SR), MTB is a sophisticated remodeling of metabolism that slows down growth and energy requirements during famine. Recent studies emphasize the need to focus on the SR in MTB as a means of reducing the treatment duration. The MTB genome codes two polyphosphate kinases (PPK-1 and PPK-2), for maintenance of intracellular Inorganic Polyphosphate (Poly P) levels. The identification of a virulence factor of TB growth as well as persistence in host tissues may be helped in MTB using PPK2, which is required to modulate intracellular levels of regulating molecules and to sustain sensitivity to the first-line anti-drug isoniazid. Synthesized and under control by PPK2 enzymes, inorganic polyP is essential in this process since it controls stress reactions. This research, therefore, investigates the significance of PPK2 in the MTB, the chemicals suppressing a bacterial SR in MTB, and the list of PPK2 inhibitors for shortening TB.

Keywords: *Mycobacterium tuberculosis*, Polyphosphate kinase 2, Stringent response, Poly P and Gallein.

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INTRODUCTION

Tuberculosis (TB), which reached epidemic levels in the 19th century across regions such as Europe and North America, remains a major global health threat today. In 2015, TB accounted for approximately 10.4 million new infections and caused 1.8 million deaths worldwide. Currently, the disease continues to pose a significant burden, with around 10.4 million new cases emerging annually [1,2]. Due to *Mycobacterium* TB (MTB)'s significant impact on young people, who are at a higher risk of infection and suffer considerable consequences from the disease, it is known as the "thief of youth." MTB opened the prospect for a prevention program centered on attenuation in the germ laboratory and also for the active search for a pharmacological treatment [3]. For clearing TB infections, factors such as medical non-adherence as well as the rise of multi-drug-resistant (MDR) MTB strains pay to antibiotic therapies' frequent failure. Targeting the SR pathway in MTB is one promising solution. A regulatory mechanism that enables the bacterium to survive under stressful conditions, such as nutrient deprivation, hypoxia, and host immune responses, is the stringent response (SR) in *MTB* [4].

Inorganic polyphosphate (polyP) is involved in many biological processes, from molecular chaperone function to bacterial pathogenicity and phosphorus storage. PolyP kinases (PPK) 1 and 2 in bacteria synthesize polyP. Both groups of enzymes can synthesize polyP, although PPK1s prefer nucleoside triphosphates, whereas PPK2s phosphorylate mono- or diphosphates. The SR is regulated by multiple methods, including PolyP, which is a linear polymer of phosphate residues connected by high-energy phosphoanhydride linkages [5,6]. PPK1 and PPK2 regulate PolyP levels in cells. Fig. 1 shows poly P synthesis and its role in bacteria.

PPK2 is involved in regulating inorganic polyP levels within the bacterium. A significant role is played by PolyP in various cellular processes, and its regulation impacts bacterial survival and virulence [7]. PPK2 impacts virulence phenotypes in bacteria. Pathogenic bacteria such as MTB encode both PPK1 and PPK2, thus making them potential targets for antibacterial drugs [8]. PPK2 deficiency affects susceptibility to the 1st-line anti-TB drug isoniazid [9]. Thus, for full MTB virulence *in vivo*, PPK2 is required. During acute murine infection, a considerably lower lung bacillary burden is exhibited by a PPK2-deficient mutant when analogized to control groups [10].

Structure and mechanism of PPK1 and PPK2

PPK1: Structure

PPK1 typically forms a homodimeric or homotetrameric protein. Its monomers contain a conserved poly P-binding region, magnesium (Mg^{2+}) or manganese (Mn^{2+}) as essential cofactors, and a central catalytic domain that binds nucleoside triphosphates, usually Adenosine Triphosphate (ATP). The ATP-binding pocket is specifically tailored to facilitate phosphate transfer [11,12].

Catalytic mechanism

ATP binding
The substrate ATP enters the enzyme's active site, where specific amino acid residues stabilize its binding.

Transfer reaction
The γ-phosphate of ATP is transferred to a histidine residue in the active site, generating a high-energy, phosphorylated enzyme intermediate.

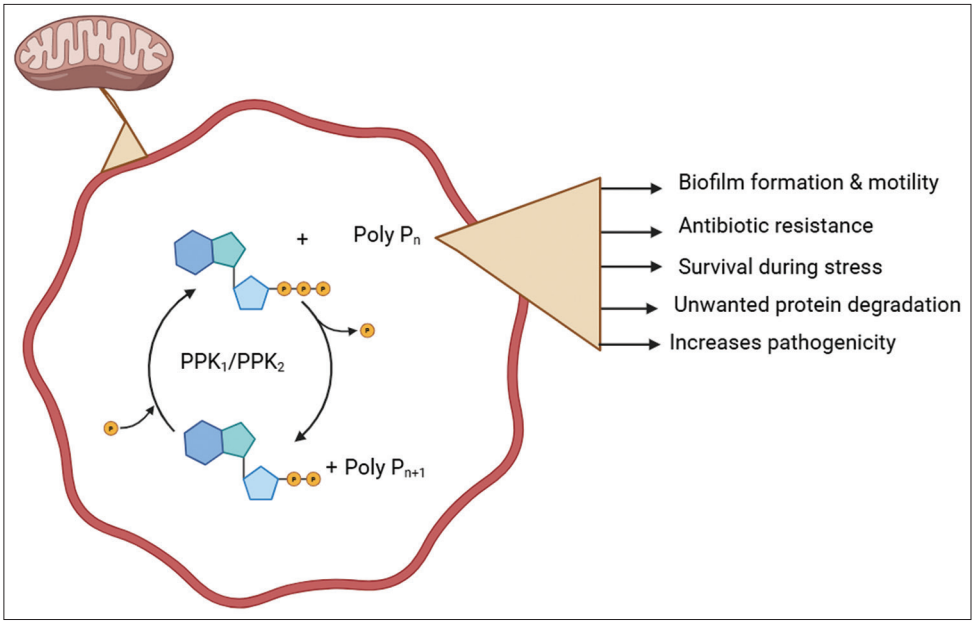


Fig. 1: Synthesis of polyP and its role in bacteria

Poly P synthesis
Through sequential reactions, the enzyme adds phosphate groups from ATP to the growing poly P chain.

Product release
The resulting ADP and the newly elongated poly P are released from the enzyme, concluding the cycle.

Physiological role

PPK1 helps maintain cellular poly P stores, especially under stress or nutrient-limited conditions. This process is vital for survival, acts as an energy buffer, enhances biofilm formation, and increases bacterial resilience within host cells.

PPK2: Structure

PPK2 proteins often form dimers or larger oligomers, with each monomer containing distinct binding sites for poly P and nucleoside diphosphates (NDPs) (typically Guanosine Diphosphate (GDP) Adenosine Diphosphate (ADP)). Their three-dimensional fold supports substrate specificity and the catalytic transfer of phosphate from poly P to NDPs [13].

PPK2 is essential in using polyP for the renewal of NTPs. PPK2 favors polyP for phosphoryl transfer, unlike PPK1, which favors ATP as a phosphate donor. Different bacterial species have different structural organizations of PPK2; it may be a monomer, dimer, or tetramer. For both polyP and NDPs, including GDP and ADP, it features particular binding sites. PPK2's catalytic process starts with long-chain polyP bound to its polyP-binding site. The enzyme then places GDP or ADP close to the active site so that phosphate groups from polyP may be transferred to generate GTP or ATP. Mg^{+} or Mn^{2+} ions stabilize this process. Released once the freshly generated GTP or ATP is used, it is used for vital bacterial functions, including (p)ppGpp production, a major control of bacterial stress responses [14].

Catalytic mechanism

Poly P binding
Poly P binds to the specific site on PPK2, aligning for phosphate transfer.

NDP binding
The enzyme also binds NDPs (e.g., GDP, ADP).

Phosphate transfer
PPK2 catalyzes the transfer of the terminal phosphate group from poly P to the NDP, resulting in the formation of nucleoside triphosphate (GTP or ATP). This differs from PPK1, which uses ATP as the donor for poly P synthesis.

Product release
The generated nucleoside triphosphates are then released, making them available for cellular metabolic processes.

Physiological role

PPK2 is key in regenerating ATP and GTP from their respective diphosphates using poly P as the phosphate donor. This role is crucial during metabolic stress and contributes to energy homeostasis, stress adaptation, bacterial motility, and pathogenicity. In *MTB*, the function of PPK2 supports survival under hostile conditions and is implicated in biofilm formation and antibiotic tolerance.

PPK2 mediates poly P homeostasis, which supports bacterial survival under stress and contributes to virulence and drug tolerance. Experimental knockout of PPK-2 in MTB reduces bacterial loads and pathology in guinea pig models, indicating its direct role in disease progression and stress resistance [15,16].

PPK2 affects isoniazid tolerance

MTB PPK-2 mutants are significantly more tolerant to isoniazid compared to wild-type, highlighting a link between poly P metabolism and frontline drug responsiveness.

Both PPK1 and PPK2 are essential for the physiology and persistence of MTB, making them attractive targets for the development of novel therapeutics. Disrupting these enzymes impairs bacterial energy balance, reduces survival under stress, and enhances susceptibility to antibiotics, providing routes to potentially shorten treatment and combat drug resistance [17,18].

PPK1 and PPK2 are absolutely essential for MTB physiology; hence, these enzymes show great targets for new anti-tubercular treatments. PPK1 inhibition could stop polyP production, therefore lowering bacterial stress tolerance and survival under hostile environments. Blocking PPK2 activity could similarly affect NTP regeneration, hence reducing bacterial virulence and persistence. Designing small-molecule

inhibitors that specifically target the active sites of PPK1 and PPK2 could effectively disrupt bacterial metabolism and the stringent response pathways, offering a promising approach for developing new TB treatment strategies. Targeting these important enzymes will help scientists create fresh approaches to fight MTB, especially those that are either highly drug-resistant or MDR. Understanding the structure, mechanism, and goal of PPK1 and PPK2 enables one to value their participation in bacterial physiology and illness. Fresh antimicrobial medicines aimed to interfere with pathogenicity, biofilm development, and bacterial stress reactions could be created via further study on these enzymes. Targeting PPKs could be a potential future road for the development of next-generation anti-TB medicines, as drug resistance in MTB keeps developing [19,20].

SR FOR MTB

To adjust the bacteria's physiology, SR regulates various cellular processes, promoting survival in different environments. By inducing metabolic quiescence, bacteria are enabled by the SR to survive host defenses in MTB [21].

Pawełczyk *et al.* [22] inhibited the SR blocks MTB entry into quiescence as well as decreased persistence. A pharmaceutical library of over 2 million compounds was screened for Rel_{MTB} inhibitors. As per the analysis, the lead compound X9 could kill nutrient-starved MTB; also, it augmented isoniazid's killing activity. For small-molecule inhibitors' design against TB, validation of the SR enzyme Rel_{MTB} has been provided as a target.

Rao *et al.* [23] defined the sigma factor SigE role in MTB's SR. By employing RNA sequencing, the transcriptional response's temporal dynamics of a SigE mutant as well as its wild-type progenitor strain to lower phosphate were evaluated. Thus, to cope with the metabolic stress correlated to the adaptation, the SigE was needed to help the bacteria to low phosphate and SR activation.

Giacomo *et al*. [24] elucidated the role of the extracytoplasmic function sigma factor E (SigE) in the stringent response (SR) of Mycobacterium tuberculosis (MTB). Their analysis revealed that although SigE is not directly responsible for initiating the SR, it plays a vital role in safeguarding the cell against stress conditions triggered by low phosphate levels and SR activation.

Dutta *et al.* [25] examined the intranasal SR vaccine, aiming at dendritic cells as an adjunctive therapy against MTB. To enlarge mucosal immune responses, intranasal delivery was appraised. The greatest mycobactericidal activity, along with isoniazid, was exhibited by an incorporated methodology encompassing the DNA MIP-3a/relMtb fusion vaccine's intranasal delivery.

Manganelli *et al.* [26] discovered the SR role in MTB dormancy. By encompassing the molecular cloning methodologies, which lack the ability to hydrolyze (p)ppGpp, a conditional RelMtb overexpression strain was generated. As per the outcome, at the genetic and protein level, the inducible expression was confirmed; however, for characterizing the strain as well as determining the actual effects on the SR, more time and data were needed.

Baruzzo *et al.* [27] estimated the SR mechanism's role in MTB. In supplemented or unsupplemented BHIS medium, macroscopic motility assays were done with 0.3% agar. For the indicated final concentration, ARA, GLU, or NEU5A was added to the molten agar. As per the findings, while the significantly relevant metabolites' plethora was plentiful in the PPK-1 involved niche, R20291 did not gladly respond to a NEU5A gradient.

Karanika *et al*. [28] elucidated the high-throughput dual system for screening PPK mutants for effective ATP regeneration in L-theanine biocatalysis. By converting glutamate to glutamine, an effective ATP regeneration system was built. The foundation was further laid for the catalysis of glutamine to L-theanine by GGT enzyme after adding 6 U/mL GS enzyme as well as 5 U/mL ChPPKD82N-K103E, yielding 13.8±0.2 g/L of glutamine with a conversion rate of 94.4±1.4% in 4 h. This demonstrated that providing the mutant enzyme-driven process with an efficient ATP supply increased the rate of substrate-to-product conversion as well as optimized substrate value.

Ellison *et al*. [29] defined the potential PPK1 inhibitors' discovery and antibacterial study. For bacterial motility, quorum sensing, biofilm formation, and virulence factors, PPK1 was an essential kinase. Via virtual screening together with biological assays, "2" small molecules potentially targeting PPK1 were presented. As per the *in vitro* and *in vivo* outcomes, uropathogenic *Escherichia coli*'s biofilm formation might be disrupted by the interface of those compounds with PPK1, which might minimize invasive ability, together with resistance to oxidative stress.

Pokhrel *et al*. [30] elucidated the inorganic PolyP accumulation that suppressed the dormancy response as well as virulence in MTB. PolyP's intracellular levels were influenced by the activities of PPK1, PPK2, and exopolyphosphates (PPXs). The analysis of MTB single (Dppx2) and double knockout (dkppx) strains revealed that PPX-mediated PolyP degradation was necessary to cause bacterial infection in guinea pigs.

Gao *et al*. [31] defined substrate-binding pockets' rational design in PPK for usage in cost-effective ATP-dependent cascade reactions. By blocking the ADP binding pocket, the short polyP's inhibited PPK. Structural comparisons between PPK from Corynebacterium glutamicum and PPK from Sinorhizobium meliloti revealed that three key amino acid residues lysine, glutamate, and threonine are involved in stabilizing the adenosine group of ADP between the dimeric subunits, thereby influencing enzymatic activity toward short polyphosphate chains.

An interlocked dimer is formed by PPK, with every 80 kDa monomer comprising "4" structural domains. In a tunnel, which encompasses a distinctive ATP-binding pocket, and might put up synthesized polyP's translocation, the PPK active site is also placed. In developing drugs that inhibit PPK enzymes, recent progress has been made, thereby offering a novel strategy to combat bacterial infections. Knowing the structure and mechanism of PPKs provides an improvement in their roles in bacterial homeostasis and potential therapeutic applications. The PPK dimer's structure in the asymmetric unit is explained in Fig. 2.

PPKs can vary in structure depending on the organism, but they generally consist of a single polypeptide chain folded into distinct domains. The ATP-binding domain binds ATP, thereby positioning it for catalysis. The polyP-binding domain binds the polyP substrate, typically containing residues that interact with the polyP chain. The phosphate group transfer of ATP to polyP is eased by the catalytic domain. PPKs can

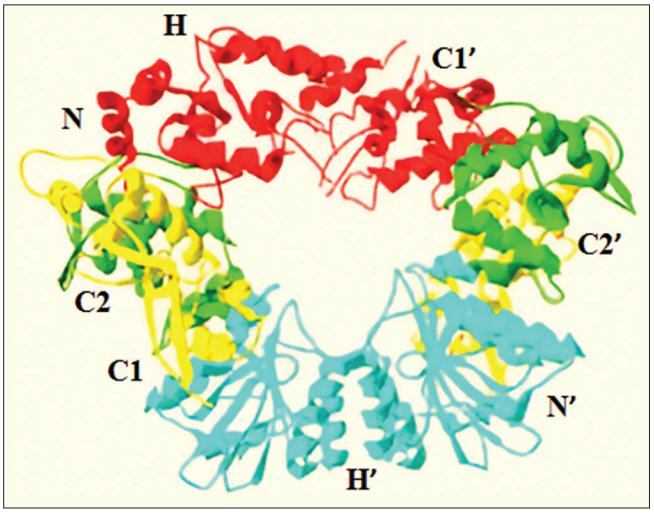


Fig. 2: Structure of the PPK dimer in the asymmetric unit

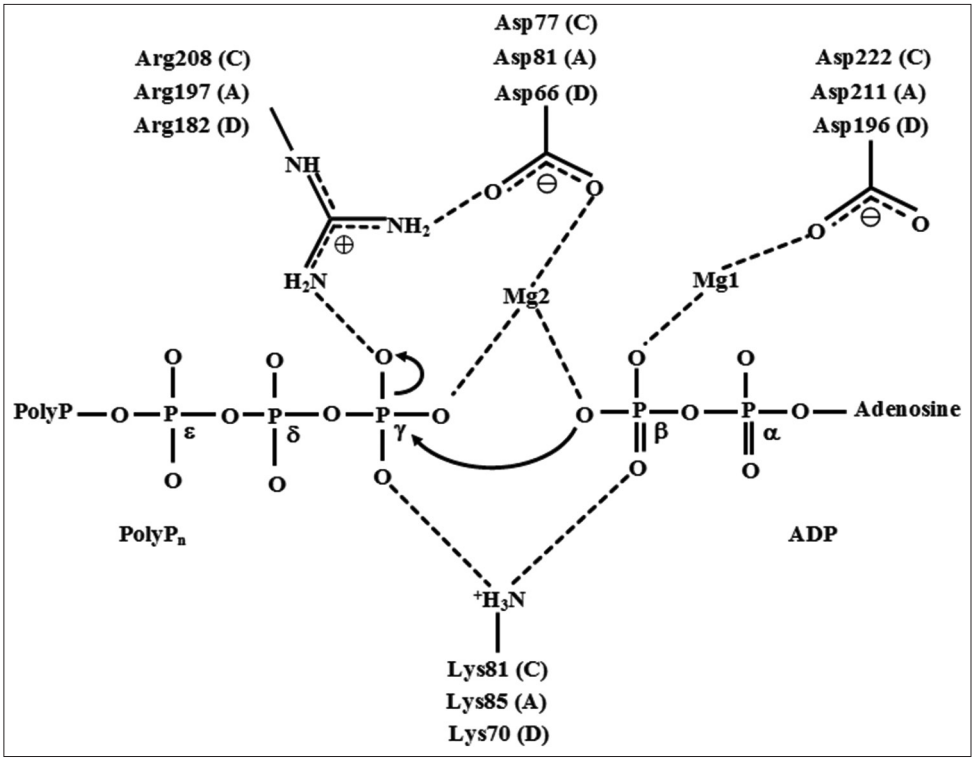


Fig. 3: Catalytic mechanism of polyphosphate kinases 2s: PolyP-dependent phosphorylation of ADP

exist as monomers, dimers, or even higher oligomeric states. Enzyme activity and stability can be influenced by the oligomeric state. Metal ions (such as magnesium or manganese) are required by several PPKs for their catalytic activity. These ions often coordinate with ATP and help to stabilize the transition state during phosphoryl transfer [32].

Moreover, depending on the audience and purpose, explaining the mechanism of PPK can be crucial. The catalytic mechanism of PPK2s: polyP-dependent phosphorylation of ADP is elucidated in Fig. 3.

Usually, the catalytic mechanism of PPKs requires large amounts of movement. Step one is ATP binding. ATP hooks itself to the ATP-binding domain of an enzyme. Particularly, certain residues that interact with ATP's adenine and phosphate groups help to enhance this binding. The second phase is PolyP binding. Inorganic PolyP hooks to its particular enzyme binding site. Usually flexible, the polyP chain fits the binding domain of the enzyme. The third stage is phosphorylation. The terminal phosphate group of ATP is transferred to the PolyP chain to generate ADP and elongates the polyP chain by one phosphate unit. This stage comprises phosphoanhydride bond breaking in ATP and phosphoester bond synthesis with polyP. Product releases mark the last stage. From the enzyme, ADP and the freshly phosphorylated PolyP chain are liberated, therefore completing the catalytic cycle.

PPK2 is rather important in bacterial physiology and pathogenicity. For the survival, adaptability, and pathogenicity of bacteria, these roles are absolutely vital. Among the main functions of PPK2 in bacterial physiology are as follows:

1. Phosphate and energy storage: PPK2 synthesizes polyP, which acts as a store of phosphate as well as energy. PolyP could be mobilized in times of environmental stress or nutritional shortage. PPK2's polyP production is engaged in several biochemical reactions, thereby supplying ATP regeneration and other metabolic activities with an energy source [33].
2. Stress response: PPK2 helps bacteria build polyP, which can be used as a phosphate source when outside supplies run out, under phosphate restriction. PPK2 is engaged in the bacterial reaction to oxidative stress, providing security for cells as of damage caused by reactive oxygen species [34].
3. Cellular functions: PolyP has been found to stabilize proteins and DNA, therefore enabling bacteria to retain cellular integrity under demanding circumstances. PolyP produced by PPK2 can function as a signaling molecule, therefore controlling different cellular reactions to environmental changes [35].
4. Motility and biofilm formation: By influencing flagellar function – which is essential for movement toward favorable conditions – PPK2 controls bacterial motility. PPK2 is a fundamental component of bacterial survival in adverse settings and resistance to drugs, as well as helping create biofilm [36].

The PPK also has a significant role in bacterial virulence, such as bacterial physiology. The ability of an organism to infect the host as well as cause a disease is termed virulence. The molecules, which help the bacterium colonize the host at the cellular level, are the factors of virulence.

1. Pathogenicity: Essential for bacterial invasion and colonization of human tissues, virulence factors, including toxins and enzymes, are regulated by PPK2 and, hence, connected to PPK2, which helps bacteria invade host cells and evade host immune responses, therefore improving the capacity of pathogens to cause diseases [37].
2. Host interaction: By controlling polyP levels, PPK2 can alter host immune responses, therefore allowing infections to continue and cause disease. PPK2 enables bacteria to survive in unfavorable environments within the host, including food scarcity, oxidative damage, and acidic pH.
3. Regulation of gene expression: PPK2 controls the expression of genes linked to stress response and virulence, therefore coordinating bacterial adaptation to host conditions and increasing pathogenic potential [38].

PPK2 is thus the name given to a multi-dimensional enzyme that is essential for bacterial physiology and pathogenicity. Its participation in polyP synthesis and control of stress responses, motility, biofilm generation, pathogenicity, and antibiotic resistance emphasizes its

relevance in bacterial survival and adaptation [39], which is shown in Fig. 4.

PPK2 inhibitors: Current status

PPK2 is a key enzyme in MTB that mediates the synthesis and utilization of inorganic poly P, critical for ATP/GTP regeneration, survival under stress, virulence, and drug tolerance. Given its vital role, PPK2 is an attractive target for novel anti-TB therapeutics, and several small-molecule inhibitors have been developed and tested at different stages of validation.

CLASSES OF PPK2 INHIBITORS

Small-molecule organic compounds

Gallein

A synthetic compound found to inhibit PPK2 activity in MTB. *In vitro* studies show that gallein potentiates the antibacterial action of isoniazid, one of the primary anti-TB drugs, leading to significant suppression of bacterial growth. Recent *in vivo* animal model studies (e.g., guinea pig infection models) demonstrate that gallein, when combined with isoniazid, attenuates tissue pathology and reduces bacterial burden. This provides a strong precedent for the clinical relevance of gallein as a PPK2-targeted adjunct therapy [17].

National Service Center (NSC) 9037 and NSC 35676

These compounds are identified as PPK2 inhibitors in biochemical assays. NSC 9037, in particular, has shown >80% inhibition at 100 μM in cell-free systems using MTB PPK2–MBP fusion proteins. However, these compounds remain at the *in vitro* validation stage, with limited or no *in vivo* efficacy data available for MTB.

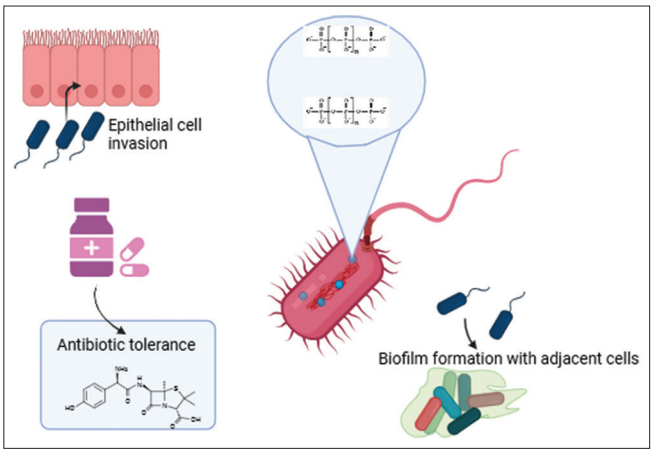


Fig. 4: Schematic of bacterial virulence linked to PPK2 enzymes

Table 1: Key differences between PPK1 and PPK2

Feature	PPK1	PPK2
Preferred substrates	ATP→poly P	poly P→GDP/ADP
Main role	Poly *P* synthesis from nucleoside triphosphates	Nucleoside triphosphate regeneration
Oligomeric state	Dimer/tetramer	Dimer or higher-order oligomer
Mechanistic features	Transfers Pi from ATP to poly P	Transfers Pi from poly P to GDP/ADP

Aptamers: Aptamer g9

Selected for its high binding affinity to MTB PPK2, providing potent inhibition at nanomolar (nM) concentrations *in vitro*. Aptamers can be highly specific, but their application in complex biological systems or *in vivo* TB models has not yet been fully characterized [40,41].

Other notable inhibitors

Pyrazinoic acid

Binds directly to the aspartate residue (Asp67) of Rv2783 (PPK2) in MTB, inducing conformational changes that inhibit catalytic activity. This mechanism has been biochemically validated, but *in vivo* data remain preliminary.

Acetylated benzoylated relacin (AB), X9

Relacin derivatives inhibit PPK2 activity in biochemical assays by binding active sites, resulting in impaired biofilm formation and altered cell morphology. X9's precise interaction with MTB PPK2 is still under study, particularly regarding its safety and efficacy in biological systems.

In MTB virulence and drug tolerance, a key role is played by PPK-2. In microbial stress adaptation, virulence, as well as drug tolerance, PPK-2 serves as an essential molecule [42]. The PPK-2 mutant strain is also more lenient to the 1st-line anti-TB drug isoniazid and impaired survival in Tumor-differentiated Human Peripheral blood (THP-1) macrophages [43]. Another enzyme encompassed in polyP metabolism, which drives the synthesis of GTP as well as ATP employing polyP as a phosphate donor, is PPK-2 [44]. PPK-2 is a promising drug target for combating MTB infections [45].

A comparative overview of PPK1 and PPK2 highlighting their structural and functional distinctions is presented in Table 1, while Table 2 summarizes the reported PPK2 inhibitors that show potential as novel anti-tubercular agents. Collectively, these findings position PPK2 as a promising therapeutic target for developing new strategies to combat M. tuberculosis infections

Sharma *et al.* [46] elucidated the universal PPK with the PPK2c of Ralstonia eutropha that accepts purine together with pyrimidine nucleotides for MTB. The PPK2-type PPK's properties were analyzed. As per the evaluation, nucleoside-tetraphosphates, namely AT(4)P, GT(4)P, CT(4)P, dTT(4)P, along with UT(4)P, were spotted in substantial amounts. For replacing ATP and fuelling the hexokinase-catalyzed phosphorylation of glucose, PPK2c and polyP were wielded.

Bowlin *et al.* [47] examined the PPK2 encompassed in the biofilm of MTB's formation, morphology, as well as ultra-microstructure, as well as survivability in macrophages. Advanced levels of lipid oxidation, together with citrate cycle activity, are involved in strains accumulating polyP that were deficient in ppx1, PPK2, or ppx2. Moreover, malonyl-coenzyme A levels compared to the wild strain significantly decreased.

Sgaragli *et al.* [48] elucidated the PPK2 as an extracellular signal, which could ease bacterial existence in eukaryotic cells. In disodium together with macrophages, phagosome acidification and lysosome activity were

Table 2: PPK2 inhibitors

Inhibitor	Validation	Mode of action	*In vivo* data	Notes
Gallein	*In vitro*, *in vivo*	Blocks PPK2, potentiates isoniazid	Yes	Effective as an adjunct to first-line TB therapy in models
NSC 9037, 35676	*In vitro*	PPK2 inhibition	No	Structural analogs, limited to biochemical validation
Aptamer g9	*In vitro*	High-affinity PPK2 binding	No	Nanomolar potency, lacks animal data
Pyrazinoic acid	*In vitro*	Conformational change in PPK2	Early/Unknown	Targets Asp67 on PPK2; animal data limited
Relacin derivatives	*In vitro*	Binds active site, impairs biofilm	No	Potential as multifactorial inhibitors

inhibited by PolyP. In macrophages, early endosomal markers were minimized. As per the outcome, pathogenicity was potentiated by acting as an extracellular signal, which constrains phagosome maturation.

Hildenbrand *et al.* [49] defined the substrate recognition as well as appliance revealed by ligand-bound PPK2 structures for MTB. For catalyzing nucleotide phosphates' reversible phosphorylation, PolyP was deployed by PPK2. For new pharmaceutical compounds, it was highly associated with targets. The technique's importance for bacterial pathogens' virulence has resulted in the technique as a possible goal for antibacterial discovery.

He *et al.* [50] examined the gallein as well as isoniazid act synergistically for attenuating MTB growth in human macrophages along with the PPK2 enzymes. By augmenting the accumulation of (i) cellular, (ii) extracellular, together with (iii) surface polyp, Mtb was adapted to the TB frontline antibiotic isoniazid (INH). As per the evaluation, levels of Mtb ppk1 together with PPK2 mRNAs were not considerably affected; thus, the effects on polyP levels weren't mediated by changes in mRNA levels.

PPK2 Inhibitors for MTB

PPK2 enzymes especially devour polyP to phosphorylate nucleoside mono-or diphosphates [51]. Reduced virulence in vi was shown by PPK2-deficient mutants, indicating its importance for Mtb survival [52]. The remaining polyP's source was found to be a novel class of enzyme termed PPK2 [53,54]. Some of the significant PPK2 inhibitors for MTB are NSC 35676, NSC 30205, NSC 345647, NSC 9037, 11f, 11g, 11i, gallein, and aptamer g9 [55,56]. The studies of different PPK2 inhibitors of MTB with their structure, inhibition potency, and tested reaction are elucidated in Table 3.

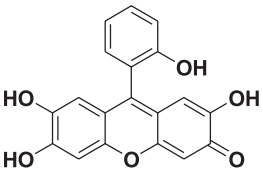
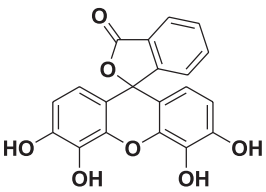
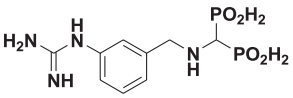
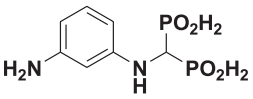
As the SR is vital for MTB stress adaptation, virulence, and antibiotic tolerance, targeting this pathway depicts an attractive technique to enhance TB results [62,63].

The SR, which plays a vital role in bacterial survival, virulence, and antibiotic tolerance, is a conserved bacterial adaptation that occurs in reference to stress conditions, namely nutrient starvation [64-66]. The SR is induced throughout chronic TB infection in MTB [67,68]. Pharmacologically targeting the SR or enhancing host immunity against it holds promise for more effective TB treatments [69,70]. Some of the significant inhibitors targeting PPK2 enzymes with the SR as the treatment of MTB are elucidated in Table 4.

Effect of targeting PPK2 on TB treatment

In different analyses, targeting PPK2 in the MTB treatment duration has shown better outcomes. A key enzyme involved in polyP metabolism is PPK [74]. Dysregulation of PolyP levels affects MTB survival. PolyP deficiency is related to augmented susceptibility to frontline TB drugs [14,75]. There are a few studies that explain the effects of targeting PPK2 on TB treatment duration.

Table 3: Studies of different PPK2 inhibitors of MTB with its structure, importance, and inhibition potency

Inhibitors	Structure	Importance	Inhibition potency	References
NSC 9037		NSC 9037 holds promise as a potential therapeutic agent for shortening TB treatment by targeting the SR pathway.	Less than 80% Inhibition at 100 μM of MTB PPK2–MBP fusion	[57]
Aptamer g9	Not available	Aptamers were found to be important in the fight against TB, offering targeted approaches for early detection and potential treatments.	Better nM values for MTB in PPK2	[58]
Gallein		Gallein represents a promising way to enhance TB treatment by potentiating the effects of existing antibiotics.	Attained better values for MTB in PPK2	[59]
11 i		Both 11i and 11g come under the order when analyzing the PPK2 enzymes for the MTB. These inhibitors were less suitable for MTB.	Not mentioned	[60]
11g			Not mentioned	[61]

PPK: Polyphosphate kinases, MTB: *Mycobacterium* tuberculosis

Table 4: Some of the significant inhibitors targeting PPK2 enzymes with the SR as the treatment of MTB

Inhibitor name	Target	Mode of action	References
Pyrazinoic acid	PPK2	Binding to Asp67 of Rv2783 caused conformational changes in the protein, thus leading to the inhibition of its catalytic activities.	[33]
Acetylated and acetylated (AC) benzoylated Relacin (AB) compound		As recommended by enzyme kinetics, it might be inhibited by binding to active sites, which results in impaired biofilm formation as well as elongated cell's emergence.	[71]
X9		The compound binds to the active site of the protein, but it is unclear whether it interacts specifically with amino acid D265.	[72]
NSC9037 and NSC35676		The mechanism was not clear to identify	[73]

PPK: Polyphosphate kinases

The TB treatment is very lengthy and burdensome. The following are some of the significant outcomes attained in different studies regarding the treatment of MTB duration.

Potent inhibitors of MTB PPK could potentially reduce treatment duration when combined with standard anti-TB regimens [8,76].

A small population of bacteria's presence characterized by antibiotic tolerance is reflected by the long time requisite for curative TB treatment [77,78].

Hence, it is clear that targeting PPK-2 could lead to more effective TB treatment regimens, potentially shortening the duration of therapy.

CHALLENGES IN TARGETING PPK2 ENZYMES

Structural homology and off-target effects

Although PPK2 is absent in higher eukaryotes, it shares certain structural features and catalytic motifs with other nucleoside-binding and kinase enzymes. Some PPK2 proteins, for example, have structural and functional similarities with thymidylate kinases, which are present in both bacteria and eukaryotes. This poses the following challenges:

Risk of off-target effects

Inhibitors designed for PPK2 may unintentionally inhibit human kinases with overlapping binding sites or conserved nucleotide-binding loops, potentially resulting in unwanted toxicity or side effects [79,80].

Selectivity requirements

Effective drug development must engineer small molecules that bind specifically to structural determinants unique to bacterial PPK2, thereby sparing human proteins [81,82].

Bacterial resistance mechanisms

Target site mutations

Bacteria have the capacity to develop resistance to PPK2 inhibitors via point mutations in the PPK2 active site, altering drug binding and rendering inhibitors less effective [83].

Redundancy in polyP metabolism

Many pathogenic bacteria encode multiple PPK enzymes, including both PPK1 and multiple PPK2 isoforms. Partial inhibition of PPK2 could be compensated for by other kinases, maintaining polyP homeostasis and undermining the efficacy of single-target inhibitors [84].

Compensatory pathways

MTB can repurpose accumulated polyP as a phosphate donor under certain metabolic conditions, possibly bypassing PPK2 inhibition and sustaining ATP synthesis and stress adaptation [85].

Complex role in antibiotic sensitivity

While PPK2 inhibition can sensitize some bacteria to antibiotics, MTB mutants lacking PPK2 have displayed increased tolerance to isoniazid (a first-line anti-TB drug), implying that PPK2-targeted therapy may not universally enhance the efficacy of all antibiotics and may even promote resistance in certain scenarios.

Thorough pre-clinical assessment of drug interactions with existing antibiotics is therefore crucial before clinical trials [86,87].

Drug development hurdles

Designing dual-specificity or highly selective inhibitors

Since both PPK1 and PPK2 may cooperate to regulate polyP cycling in bacteria, targeting both enzymes may be required for complete pathway disruption. However, achieving potent inhibition against both PPK classes without affecting unrelated human enzymes increases complexity in medicinal chemistry [88,89].

Absence of eukaryotic homologs

While the lack of direct PPK2 homologs in human cells is an advantage, it can also complicate pre-clinical toxicity and off-target effect studies, as standard human cell-line screening may miss subtle cross-reactions with structurally similar human kinases or other nucleotide-binding proteins [90,91].

Pharmacokinetics and drug efflux

Bacterial multidrug efflux pumps and cell envelope impermeability, especially in MTB, can limit intracellular drug concentrations, reducing the therapeutic impact of PPK2 inhibitors [71,92,93].

Targeting PPK2 for antibacterial therapeutic development faces several scientific and translational challenges: ensuring selectivity to avoid off-target effects, combating bacterial compensatory and resistance mechanisms, understanding complex interactions with existing antibiotics, and overcoming the pharmacokinetic and efflux barriers of pathogenic bacteria. Strategic inhibitor design and meticulous pre-clinical testing are critical for advancing PPK2 inhibitors toward clinical utility [94,95].

RESULTS AND DISCUSSION

Recently, more than 10 million adults and 1 million children have been affected by TB, as per the World Health Organization. When infected people cough, sneeze, or spit, it spreads through the air.

A species of pathogenic bacteria in the family Mycobacteriaceae, along with the causative agent of TB, is termed MTB. The SR allows the bacteria to contribute to longer-term mycobacterial survival. PPK, which catalyzes phosphate molecules' reversible transfer between ATP and inorganic PolyP, is one of the most useful enzymes for the enzymatic regeneration of ATP. Shortening TB treatment involves targeting various aspects of bacterial metabolism and stress responses to enhance the effectiveness of antibiotic treatment regimens. An extensive review of the unveiled strategies for shortening TB treatment that target SR and PPK2 enzymes is provided in this paper. Therefore, this review focuses on innovative approaches to shorten the treatment duration for TB. It explores two main strategies, such as targeting MTBSR mechanisms and examining the role of PPK2 enzymes in TB treatment.

MTB SR

The study focuses on understanding and targeting the stringent response (SR) in Mycobacterium tuberculosis (MTB) as a potential strategy to inhibit bacterial persistence and enhance the overall effectiveness of tuberculosis treatment.

PPK2 enzymes

Examined closely is the function of PPK2 enzymes in MTB metabolism and survival. Different therapeutic approaches based on perceptions of these enzymes could interfere with bacterial metabolism, thereby perhaps reducing the length of TB treatment courses.

Thus, the complicated interaction between the SR and PolyP metabolism in MTB is investigated; moreover, the function of PPK2 enzymes is underlined. Furthermore, highlighted are creative medicinal approaches meant to disrupt these pathways, including the development and effectiveness of PPK2 inhibitors. Furthermore , the possible influence of these approaches on TB treatment plans, therefore implying that focusing on PolyP and the SR could drastically shorten treatment times and improve patient adherence.

CONCLUSION

Transforming TB treatment by targeting the SR and PolyP metabolism in MTB presents significant potential. This review has examined the possibilities of targeting SR and PolyP metabolism in MT to reduce treatment durations and enhance patient outcomes. The SR, mediated by (p)ppGpp and regulated by PPK2 enzymes, is essential for MT's

capacity to endure stress and remain in a latent state. Altering these pathways is a possible avenue for therapeutic intervention. In diminishing the robustness of MT under unfavorable conditions, inhibitors of PPK2 enzymes, essential for PolyP production and breakdown, have demonstrated promise. The use of PPK2 inhibitors in TB treatment protocols represents a significant advancement in the fight against this enduring illness. However, an intricate network of regulatory mechanisms is implicated in the SR. A comprehensive understanding and accurate targeting, which are challenging to attain, are necessary for inhibiting this response without compromising other essential bacterial processes. The development pipeline for PPK2 inhibitors is advancing, with gallein leading due to positive results in animal models. NSC 9037 and related molecules are promising as biochemical tools, though they have yet to progress to *in vivo* validation. Aptamer-based and relacin-type approaches remain experimental. Ongoing research focuses on optimizing potency, selectivity, safety, and clinical applicability for next-generation TB treatment regimens. Consequently, the researchers must acknowledge this limitation and seek an optimal resolution in the future. Certain encoded and innovative genes and proteins may provide new bacterial targets, which could be utilized for the development of vaccines, pharmaceuticals, and more selective diagnostic reagents in the future.

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CONFLICTS OF INTEREST

The authors assert that no competing financial interests exist.

AUTHOR CONTRIBUTIONS

Conceptualization, Formal analysis, Dr. Mallela Vijaya Jyothi, Investigation, resources, data curation, writing original draft, Singirisetty Triveni, data collection, Dr. Chilamakuru Naresh Babu, writing review editing, supervision, Dr. K. Vijaya Bhaskar.

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