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Review Article

Discovery of Powerful Enhancers to Improve the Efficacy of Existing Antibiotics and Antimicrobials - A Strategy to Combat Bacteria Drug Resistance

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Abstract

Ever since antibiotics were first discovered, they have revolutionized modern medicine and saved an enumerable number of lives. However, due to the extensive and indiscriminate use of antibiotics, they have become far less effective. In addition, bacteria are becoming increasingly resistant to our currently available antibiotics, which has created a severe global health crisis. Furthermore, undigested antibiotics, mutated drug-resistant bacterial pathogens, and drug resistant genes have contaminated our environment and food sources. *Mycobacterium tuberculosis* (*M. tb*) – the main causative agent of tuberculosis (TB) – is the deadliest single infectious agent worldwide and has developed numerous drug-resistant strains. The current strategy used to treat drug-resistant TB only further facilitates the development of antibiotic resistance because it involves increasing drug doses and elongating treatment time. Therefore, TB therapy requires a novel and more effective approach.

In our preliminary studies, we tested four cell wall hydrolases and 16 randomly selected drugs on *M. smegmatis* and found synergistic growth inhibition for a majority of the drugs tested. Cell wall hydrolases can potentially act as "enhancers" to augment the efficacy of many antimicrobial agents in pathogenic mycobacteria. Instead of just waiting for expensive new drugs and vaccines to be developed, we propose improving the efficacy of existing antibiotics and anti-TB drugs through the discovery of more powerful enhancers. These enhancers can be peptides, metabolites, antibodies, enzymes, non-toxic small molecules and other natural or synthetic compounds. Overall, more support is needed for studies that involve cell wall hydrolases and other potential enhancers.

Keywords: *Infectious Diseases; Bacterial Infection; Antibiotics; Antimicrobial; Drug Resistance; Mycobacterium; Mycobacterial Diseases; TB; Enhancer; Cell Wall Hydrolase*

Abbreviations

TB: Tuberculosis; *M. tb: Mycobacterium tuberculosis;* WHO: The World Health Organization; CDC: The Center for Disease Control and Prevention; MRSA: Methicillin-resistant *Staphylococcus aureus;* MDR-TB: Multidrug-resistant Tuberculosis; XDR-TB: Extensively or Extreme Drug Resistant TB; Rpf: Resuscitation Promoting Factors

Introduction

General drug resistant issues

Antibiotics and antimicrobial agents have been rightfully viewed as medical wonders. Since their widespread use following World War II, antibiotics ("magic bullets") have saved millions of human lives and provided numerous benefits to public health, agriculture, and the food industry. They have also cured severe infectious diseases, dramatically increased human life expectancy, and ensured the successful growth of domesticated animals and livestock (such as poultry and seafood used in food the industry).

However, over the past several years, antibiotic resistance has grown from a public concern to a severe crisis, which needs to be resolved urgently [1, 6]. The World Health Organization (WHO) has claimed that, "Antibiotics have been a critical public health tool since the discovery of penicillin in 1928, saving the lives of millions of people around the world". Today, however, the emergence of drug resistance in bacteria is reversing the miracles of the past eighty years, with drug choices for the treatment of many bacterial infections becoming increasingly limited, expensive and in some cases, nonexistent [8, 3]. The Centers for Disease Control and Prevention (CDC) estimates that drug-resistant bacteria cause two million illnesses and approximately 23,000 deaths each year in the United States (U.S.) alone [1].

It is commonly known that antibiotic resistant bacteria are developed through four natural mechanisms: 1) permeability, 2) efflux pumps, 3) drug degradation and 4) the alteration of the drug binding targets. The genes conferring drug resistance are transferred in two directions: vertically through gene mutations and horizontally via conjugation (between bacteria), gene transduction (by phages), and gene transformation (the uptake of antibiotic resistance genes from dead organisms by bacteria) [2,4,5,7].

In addition, it has been known for decades that farmers have over used antibiotics in livestock and in the food industry in order to boost growth and prevent infections. In some countries, approximately 80% of total consumption of medically important antibiotics occurs in the animal sector. Thus, unconsumed antibiotics in animals are taken in by humans as food. This provides more opportunities for the development of resistance and contributes to the increased threat of antibiotic resistance. Recently, it has been recommended that both farmers and the food industry stop using antibiotics routinely to promote growth and prevent disease in healthy animals [1,8].

Lastly, environmental pollution of drug-resistant bacteria and antibiotics is another severe issue. It is the major source of antibiotic resistant genes and pollution in the environment. This waste comes from large-scale animal farms, aquaculture and wastewater, antibiotic manufacturing, hospitals, and other contaminated locations. Some of the antibiotics given to humans and animals are also excreted from feces and urine. In the case of waste from animals, manure is rich in nutrients and is often used as fertilizer on crop fields, leading to direct contamination of the environment with both antibiotic residues and resistant bacteria. This has led to severe consequences and represents another challenge for humans to thoroughly eradicate the resistant sources [9,10].

Although continuous and sustainable efforts have been taking place, more is still needed to overcome antibiotic resistance [5, 11, 12, 13, 14, 15]. In theory, as bacterial resistance develops, other existing antibiotics can be used or new antibiotics can be developed before we run out of these "magic bullets". However, in reality, very few new antibiotics have been discovered since the 1980s, for both economic and technical reasons. Therefore, bacteria have become more and more resistant to all of the antibiotics currently at our disposal. Antibiotics – once considered one of the most precious resources – have lost their potency making this one of the most severe threats to human health. For example, the Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is caused by a type of staphylococcal bacteria that has become resistant to many of the antibiotics used to treat ordinary staph infections. MRSA infections also resist the effects of many common antibiotics, so they have become more difficult to treat. This allows the infections to spread, causing complications that can potentially become life-threatening. Staph and MRSA can cause a variety of problems ranging from skin infections, sepsis, pneumonia, all the way to blood stream infections such as bacteremia. Studies have shown that about one in three people (33%) carry staph in their nose – usually without any signs of illness. Approximately two in 100 people carry MRSA, which amounts to several million people affected in the population. Although this number has declined in recent years, MRSA remains an important public health problem [16]. Current efforts to treat infectious diseases caused by bacterial pathogens, have led to a rise in drug resistance to *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Enterococci isolated* from hospitals [1, 17]. In addition, the CDC has reported that antibiotic resistance in *Salmonella* and *Campylobacter* have resulted in 400,000 infections each year in the U.S. [18]. More remains to be done in order to further reduce the risks of these drug resistant infections.

Similarly, antibiotic resistance in *Mycobacterium tuberculosis (M. tb)* has also faced several challenges in terms of drug resistance. Tuberculosis (TB) is an ancient disease with new challenges due to the recent development of MDR-TB and XDR-TB. TB is still the number one life threatening infectious disease in the world. Overall, this article focuses on the issues that drug resistant *M. tb* and other mycobacterial diseases pose.

Mycobacterial diseases

Background: *Mycobacterium* is a bacteria genus that contains many important pathogens including *M. tb and Mycobacterium leprae.* TB remains one of the world's leading causes of death from a single infectious bacterium. It has been part of human society for hundreds

and thousands of years and the pathogen, *M. tb* has developed unique ways to adapt to the human defense system. Up until now, one-third of the world population has been infected with latent *M. tb*. According to a WHO report in 2016, nine million people have the active TB disease and among those patients, 1.7 million die each year including 30% of whom are HIV patients [19,20].

Since most conventional antibiotics cannot treat TB, a dozen efficient anti-TB drugs have been successfully discovered since the 1940s. However, unlike other infectious diseases, these drugs must be administered for a fairly long period of time, at relatively high doses, and in combination with other drugs (or requires multiple drugs in order to be an effective treatment) [21,47].

According to the WHO and CDC, the first line drugs used to treat TB are Rifampin, Isoniazid, Pyrazinamide, and Ethambutol (R,I,P,E). However, the recommendations on the treatment of drug resistant TB from the WHO constantly changes each year. The current second line drugs used to treat TB are fluoroquinolones (defined here as high dose levofloxacin, moxifloxacin, and gatifloxacin); Amikacin (Am); Capreomycin (Cm); Kanamycin (Km); Streptomycin (SM); Para-Aminosalicylic Acid (PAS); Ethionamide/Prothionamide (Eto/Pto); Cycloserine/Terizidone (Cs/Trd); Linezolid (Lzd); Clofazimine (Cfz) and other add-on drugs.

Under the leadership of the WHO, CDC and many other public health organizations, there has been an extensive world-wide effort to combat these epidemics and prevent TB that include intensifying diagnoses, isolating active TB patients, treating patients with latent and active TB, and immunization. One example is the use of directly observed treatment (DOT), which significantly increases the efficacy of TB medication[19,22].

Drug resistant issue of *M. tb*

Any treatment that involves multiple medications over several months is challenging for patients who do not have good habits, who are in unstable living environments, and who are part of poorly managed medical systems. As a result, patients do not complete their treatments which leads to the development of drug resistance. Resistance among strains of *M. tb* is a global problem. Drug-resistance against a single drug has increased dramatically. Currently, multi-drug-resistant (MDR) bacteria have become an epidemic. There are 0.5 million MDR cases each year, but only 20% of MDR cases were actually detected. The five countries with the most MDR cases are China, India, Pakistani, Russian and South Africa. Furthermore, extensively or extreme drug resistant TB (XDR-TB) has also been detected, which is MDR-TB that has become resistant to three or more of the six classes of drugs. XDR-TB is a serious global concern because there are very limited treatment options available [21,22].

MDR-TB and XDR-TB pose serious threats to global public health. Drug resistant TB requires a longer treatment times, greater financial costs, increased social burdens to families and communities, and higher mortality rates. This is especially true for immunocompromised individuals such as HIV patients, cancer patients undergoing chemotherapy, and organ transplant patients [20,21,23].

Treating drug resistant TB

Due to technical challenges, the development of a successful TB vaccine has yet to become a reality, and we expect it will take much longer to make a break-though. The current conventional ways to treat drug resistant TB are: 1) Using a combination of drugs (R,I,P,E), 2) Replacing resisted drugs with a second line or add-on drugs, 3) Elongating the treatment period from 6 months to 9 to 12 months, and 4) Developing new drugs. Newer drugs such as Bedaquiline, Delaminid and Linezolid are currently showing better clinical outcomes, but further evaluation is still needed.

Despite all the effort and hard work by the public health community [23, 24], we are still deeply concerned about the current strategies used for treating TB. All of the current treatment strategies involve continuously increasing drug doses and elongating the treatment time of the medication. This direction is risky because it has the potential to induce even more drug resistance for the *M. tb* pathogen. Furthermore, unlike conventional antibiotics, there are a limited number of anti-TB drugs available. Even if there is an effort to produce new drugs – which is a very long and expensive process – what will happen if we still run out of effective drugs?

Indeed, TB therapy needs a newer, more effective strategy. Herein, we propose – while waiting for a new TB vaccine and anti-TB drugs to be developed – improving the efficacy of the existing anti-TB drugs, lowering the dose of medication, shortening the treatment duration,

and decreasing the chance of developing drug-resistant pathogens. We also need to look at using existing conventional antibiotics to cure other bacterial diseases while also making them useful to treat TB. In order to reach this objective, one approach is to discover powerful "enhancers" to boost and optimize the efficacy of existing drugs.

Comments

Strategy of enhancers

Similar to other diseases caused by resistant bacterial pathogens, we need to reevaluate the strategies used to combat drug resistant TB. While waiting for expensive new drugs and vaccines to be developed, we propose improving efficacy of existing antibiotics and anti-TB drugs by discovering more powerful "enhancers".

An "Enhancer" refers to a substance that can help a drug overcome resistance, cause a synergistic effect with one of the current anti-TB drugs, or assist the immune system to control the pathogen. We also need to extensively understand the mechanisms that confer drug resistance in order to develop detailed strategies. We expect "enhancers" to have the following features:

1. Substances or biological agents that are ready to be used and minimally toxic.
2. Can assist drugs to induce fast killing of bacterial pathogens, which would minimize the opportunities of the pathogen to develop drug resistance.
3. The enhancer(s) is from an abundant resource (can be replaced if the pathogen develops drug resistance to the enhancer).
4. The enhancer(s) can maintain permeability of the bacterial cells to the other drugs.
5. The enhancer(s) can facilitate latency of the bacterial pathogen and block the reactivation process of *M. tuberculosis.*
6. The enhancer(s) can counteract any known mechanisms of drug resistance such as: blocking the degradation of antibiotics by resistant pathogens, inhibiting the efflux pump, and providing additional binding targets for the anti-TB drugs and antibiotics [12, 14, 55].

These enhancers can be peptides, metabolites, antibodies, enzymes, specific non-toxic small molecules and other natural or synthetic compounds [25]. The enhancers can be delivered with liposomes, nano-particles, and be injected and/or administered together with oral medicine.

In our *in vitro* study of cell wall hydrolases, we found that they were able to synergize with multiple drugs to inhibit the growth of *Mycobactrium smegmatis* and *M. bovis* BCG. Although it was only a preliminary study using "non-pathogenic mycobacterial strains" and not *M. tb*, we believe that cell wall hydrolases can potentially act as an "enhancer" to augment the efficacy of many antimicrobial agents in pathogenic mycobacteria.

Preliminary studies and discussions for cell wall hydrolases, a potential enhancer to inhibit the growth of *Mycobacteria*

Mycobacterial cell wall and cell envelope

The mycobacterial cell wall is different from that of any other bacteria and has long been recognized as a potential drug target, making it the focus of intensive studies. Mycobacteria possess a unique region that is comprised of up to 60% lipids (only ~10% for the other Gram-positive bacteria) and constitutes up to 40% of the dry weight of the bacterial cell. The *M. tb* envelope is thick, waxy, impermeable and highly complex, which presents one of the greatest challenges in its treatment. There are at least 8 motifs in the cell wall/envelope region linked covalently or non-covalently to each other, which includes peptidoglycan, oligosaccharide linkers, Arabinogalactan, mycolic acids covalently linked to branched Arabinan, free surface lipids, surface glycans (Capsules), surface channels, and lipoarabinomannan (LAM) [27, 28, 29, 30, 31]. Many genes and gene products involved in the biosynthesis and metabolism of this structure have been proven to be essential to the survival of the pathogen [32]. As a whole, the uniquely organized structural integrity is crucial to bacterial survival and pathogenesis. The highly hydrophobic cell envelope of *M. tb* enables it to survive in hostile host environments and renders many drugs ineffective.

Cell wall hydrolases

Bacterial cell wall hydrolases (cell wall cleaving enzymes) are a group of enzymes that cleave specific bonds within the cell wall ne-

twork. These unusual enzymes are capable of hydrolyzing and thus disrupting the unique bacterial outer envelope using the insoluble cell wall/envelope as a substrate, which could trigger the bacterial cell to burst. Many organisms possess cell wall hydrolases that specifically target bacterial pathogens as a mechanism to eliminate infections such as fungi, birds, insects, mammals and humans [33,34].

Bacteria also possess cell wall hydrolases, which are found around living bacterial cells, and are presumably associated with cell wall modification and cell wall fragment recycling during normal cell division and separation. There are two sub-groups of hydrolases: one group acts on the outer envelope that include glycohydrolases (or glycosidases), esterases/lipases (cutinases), proteases/peptidases/proteinases, phosphoesterases, and etc. The other group acts on the inner cell wall of peptidoglycan and are generally named peptidoglycan hydrolases or autolysins that include glycohydrolases (N-acetyl muramidases and N-acetyl glucosaminidase), amidases, and endopeptidases. In fact, cell wall degradation or cleaving enzymes can be further divided into two other subclasses: one uses H_2O to break bonds within the cell wall network (hydrolases), while the other class is able to cleave the cell wall conjugates without using H_2O (lytic enzymes such as lytic autolysins). The study of cell wall hydrolases and other lytic enzymes in *M. tuberculosis* or other *Mycobacterium* species started much later than that of other bacterial strains [35]. In an early study of *M. smegmatis* cell lysates, we detected natural enzymatic activity capable of cleaving cell wall polysaccharides to release oligosaccharides and glycolipids [36]. In 2005, *cwlM* (encoded from *M. tb* gene Rv3915) was successfully cloned, purified, and its gene product was partially characterized [37, 38]. At the same time, resuscitation promoting factors (Rpf) genes were also identified in *Micrococcus luteus* [39]. Using an informatics approach, the homology of five resuscitation promoting factors (Rpf A-E) was identified within the *M. tuberculosis* genome [39] and cloned [40]. In 2007, two proteins that interacted with RpfB were identified and isolated using the yeast two-hybrid screening method. The protein, RipA, which is encoded by the Rv1477 gene of *M. tuberculosis* displayed strong cell wall hydrolase activity [41, 42]. Previously, its gene was also identified as essential, virulent and containing an NlpC/P60 endopeptidase domain [32, 44].

Since then, more cell wall hydrolase genes have been identified, the recombinant enzymes have been cloned and the important biological roles of this class of enzymes have been elucidated and discovered both in vivo and in vitro [43, 64, 65]. Such genes would include: Rv2917c, Rv3717, Rv2911, Rv3330, Rv0050, Rv1478, Rv2566c, Rv1288, Rv0774c, Rv1169c and Rv2869c [45, 46, 48, 49, 50, 51, 52, 53]. Certain proteins such as AmiB, RpfB, and RpfE were characterized using cell-free assays, or through protein domain/structure analysis. Additionally, their cell wall cleaving activity was also confirmed [54,55,60].

In theory, the number of cell wall hydrolases should be limited and their gene expression highly regulated because of the potential suicidal abilities of these enzymes. However, we have found that each bacterial genome (including mycobacteria) contains many (more than 20) putative hydrolase genes. This not only demonstrates the importance of those cell wall cleaving enzymes, but also provides a large pool of enzymes that can be used in future therapeutic studies.

In addition to bacterial hydrolases, there has also been an effort to identify cell wall hydrolases in viruses or bacteriophages. Recently endolysins derived from mycobacteriophages were isolated and used as both analytical tools and as inhibitors of mycobacterium growth [56, 57, 58, 59].

Synergistic effect of the cell wall hydrolases with the drugs tested

In our preliminary study using non-pathogenic strains of *M. smegmatis* and *M. bovis* (BCG), we tried to determine whether cell wall hydrolases could be employed as an exogenous reagent to improve the efficacy of antimicrobial agents against mycobacteria. First, we found that co-application of low dose (4 -20 uM) drugs with cell wall hydrolases was equally as effective to using high dose drugs (100 uM) alone (for Isoniazid, Bacitracin, Rifabutin and Azithromycin) [55].

Since some drugs can kill the bacteria at high doses, we chose very low doses (7 uM) to test our hypothesis. Ten conventional antibiotics and six anti-tuberculosis drugs were randomly selected and used to treat *M. smegmatis* cultures alone or in combination with cell wall hydrolases. Cell growth was monitored using a turbidity assay at OD650 nm (OD_{650}). The four cell wall hydrolases respectively used were lysozyme (a commercial enzyme), Hydrolase-30 (a native enzyme purified from *M. smegmatis*) [60], RipA-His6, and RpfE-His6 (two recombinant enzymes encoded by *M. tuberculosis* genes Rv1477 and Rv2450c) [41, 42].

Figure 1 provides a summary of *M. smegmatis* culture turbidity, which was measured under four different conditions: 1) Control (no treatment), 2) Four enzymes only, 3) 16 drugs alone, and 4) the combination of enzyme and drugs. All four hydrolases showed some

growth inhibition, but only for a short period of time. After 32 hours of incubation, there was no significant difference between the enzyme only treatment and control bacteria. *M. smegmatis* bacterial cultures were treated with 7 μM of low dose drugs that included six common anti-TB drugs (pyrazinamide, isoniazid, ethambutol, rifampicin, rifabutin, and streptomycin) and ten conventional antibiotics (penicillin, ampicillin, tetracycline, cephalosporin c, kanamycin, novobiocin, azithromycin, polymyxin B, bacitracin and vancomycin) alone, and in combination with either (a) lysozyme, (b) Hydrolase-30, (c) RipA-His6, or (d) RpfE-His6, respectively. The results (OD_{650}) revealed a significant synergistic effect on growth inhibition for all of the drugs when used in combination with the enzymes tested.

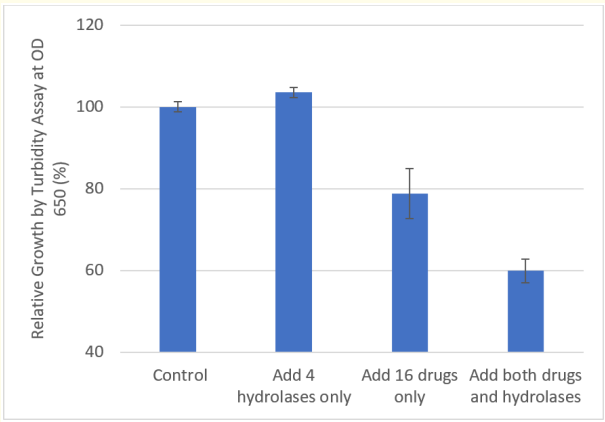

Figure 1: *M. smegmatis culture turbidity after 32 hours of growth when treated respectively with 16 drugs alone, enzymes alone and in combination with enzymes and drugs. The OD_{650nm} of the control group is listed on the left.*

It was also found that at low dose levels, only four drugs (Rifampicin, Novobiocin, Vancomycin and Kanamycin) were strong enough to inhibit growth when used alone and apparently do not need a hydrolase enhancer to improve the their efficacies. However, the rest of the drugs were considered to be "weak drugs", because when used alone there were no significant differences with the controls and enzyme only treatments (Figure 2).

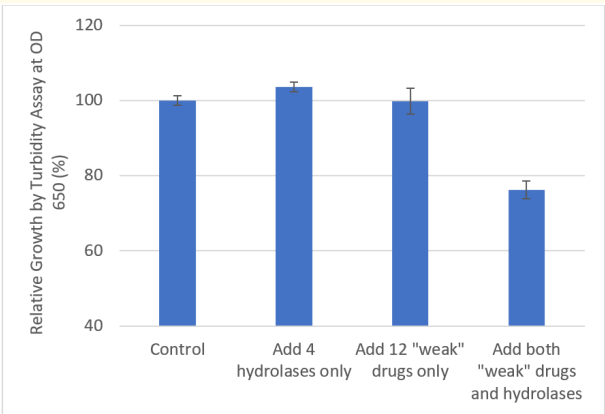

Figure 2: *M. smegmatis culture turbidity after 32 hours of growth which treated respectively with 12 weak drugs alone, enzymes alone and in combination with enzyme and drugs. A significant growth inhibition or a synergy can be found for the group treated with a combination of both weak drugs and cell wall hydrolases.*

Figure 2 utilizes the same set of data as Fig.1, except the four strong drugs (rifampicin, novobiocin, vancomycin and kanamycin) were removed, leaving only the 12 weak drugs. The results indicated significant inhibition of mycobacterial growth after the addition of the hydrolases, which seemed to improve the efficacy of the weak drugs at the lower dose level.

Viable plate count assays with colony-forming units (CFUs), vital staining assays, and oxygen consumption tests were also performed and supported the above observations. Four cell wall hydrolases from different sources were used and all showed a synergistic trend. Among the four different hydrolases tested, both lysozyme and Hydrolase-30 appeared to be the strongest. However, the enzyme solutions used were at different concentrations and not well standardized.

The mechanism of enzymatic action for each hydrolytic enzyme is different and is not well understood. However, it is presumed that the permeability of the cell wall and envelope increased, which resulted in easier access of the drugs and antimicrobials. Each hydrolase tested may only enhance the activity of certain drugs due to the specificity of cleavage sites for each enzyme on the bacterial cell wall. Additionally, the change in turgor pressure and metabolic activity induced by the hydrolytic enzymes on the cell envelope may have altered the physiological condition (such as respiration) of the bacteria[55].

It should be noted that with the help of the cell wall hydrolases (the enhancers), some of the conventional antibiotics that were not generally effective on mycobacteria became effective. These findings demonstrate that the cell wall hydrolytic enzymes – as a group of biological agents – have the ability to improve the potency of many current antimicrobial drugs by weakening the unique rigid mycobacterial cell wall and rendering ineffective antibiotics effective in killing *mycobacteria* [55, 60].

Enhancer idea fits to mycobacterial diseases because hydrolase can counter the permeability blockage caused by drug resistance

The enhancer idea is not new, as similar synergistic effects between lytic or hydrolytic enzymes and antibiotics have been reported in *Listeria monocytogenes, Streptococcus pneumoniae,* and *Staphylococcus aureus* [63, 61, 62]. However, our earlier study expands upon these findings by focusing on waxy mycobacteria, which have a thick and impermeable cell envelopes. We also randomly tested multiple drugs and enzymes (cell wall hydrolases) in combination.

One of the reasons that make hydrolases good candidates to be an "enhancer" is because of how abundant they are. Different organisms (including humans, birds, plants, fungi, bacteria and viruses) all contain cell wall hydrolases and cell wall lytic enzymes. The *M. tuberculosis* genome alone contains more than 20 putative cell wall hydrolase genes, which presumably play important roles in cell division, cell separation, and pathogenesis. *M. smegmatis* express nearly 30 cell wall hydrolases. The putative genes of cell wall hydrolases can be easily found from various genomes. However, in order to utilize these precious resources, we need a greater understanding, strategic planning, state of the art design, and better organization. The enzymatic assays will be optimized and then standardized. Only after this has been done will the most powerful cell wall hydrolases be selected as therapeutic agent (or enhancer) candidates. After all, not every cell wall hydrolase can be used as an "enhancer" or exogenously. The success of this enhancer idea depends on a better understanding of the process of mycobacterial infection, cell biology, the mechanism of bacterial resistance at the molecular level and the modern trends in antimicrobial design for other bacterial infections. Specifically, we need to improve our knowledge of hydrolytic enzymes, find the genes encoding each enzyme, characterize their physical and chemical properties, identify cleavage sites on the mycobacterial envelope, and better understand the regulation of gene expression and catalytic activities.

Conclusions and Perspectives

1. TB is an ongoing worldwide health problem. New strategies for therapeutic intervention are needed to combat drug-resistant strains of *M. tuberculosis*.
2. Cell wall hydrolases are an example of a unique group of potential enhancers, which are presumed to increase permeability of the cell wall and envelope and allows for easier access of the drugs to mycobacterial pathogen.
3. More research is needed to further explore the mechanism of hydrolase augmentation when coupled with conventional antibiotics and anti-TB drugs. The synergetic effect of the enzymes and the drugs in our earlier study needs to be validated using pathogenic strains of *M. tb.*
4. Discovery of more powerful "enhancers" is a practical and economic way to improve the efficacy of existing anti-TB drugs and conventional antibiotics. In addition to the cell wall hydrolytic enzymes, we believe that there are more "enhancers" that exist which can induce a synergistic effect and improve the efficiency of the existing drugs.

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