**PhoY2 but not PhoY1 is the PhoU homologue involved in persisters in *Mycobacterium tuberculosis***

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Objectives: Mycobacterial persistence is thought to be the underlying cause of the current lengthy tuberculosis therapy and latent infection. Despite some recent progress, the mechanisms of bacterial persistence are poorly understood. We have recently identified a new persister gene *phoU* from *Escherichia coli* and have shown that the *phoU* mutant has a defect in persisters. The objective of this study is to evaluate the role of two *phoU* homologues *phoY1* and *phoY2* from *Mycobacterium tuberculosis* in mycobacterial persistence.

Methods: *M. tuberculosis phoY1* and *phoY2* mutant strains were constructed. The persister-related phenotypes of the *phoY1* and *phoY2* mutants were assessed in vitro by MIC testing, drug exposure assays and also by survival in the mouse model of tuberculosis infection.

Results: We demonstrated that *M. tuberculosis* PhoY2 is the equivalent of *E. coli* PhoU in that inactivation of *phoY2* but not *phoY1* caused a defect in persistence phenotype as shown by increased susceptibility to rifampicin and pyrazinamide in both MIC testing and drug exposure assays and also reduced persistence in the mouse model.

Conclusions: This study provides further validation that PhoU is involved in persistence not only in *E. coli* but also in *M. tuberculosis* and has implications for the development of new drugs targeting persisters for improved treatment.

Keywords: mycobacterial persistence, mutants, antibiotics

**Introduction**

Tuberculosis (TB) is a leading infectious killer worldwide despite chemotherapy and BCG vaccine. The causative agent *Mycobacterium tuberculosis* is a highly successful pathogen, which has latently infected one-third of the world population and causes 9 million new TB cases and 1.6 million deaths worldwide each year. This global TB situation is expected to be exacerbated by the spread of HIV infection and increasing emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). Although the current TB therapy can cure the disease, it is too long and takes at least 6 months. The lengthy TB therapy makes patient compliance difficult and frequently causes selection of drug-resistant strains. The lengthy TB therapy is thought to be due to the presence of mycobacterial persisters that are not effectively killed by the current TB drugs. Due to the problem of drug-resistant and persister TB, there is currently a great deal of interest in understanding the persister mechanisms and developing new drugs that target persisters to shorten TB therapy.

Persisters were first described by Hobby et al. in 1942 when they found that penicillin killed 99% of a streptococcal culture leaving 1% of the bacterial population intact. This phenomenon was more carefully defined by Bigger in 1944 when he coined the term ‘persisters’ for the 1% surviving bacteria not killed by penicillin. Although persisters were initially defined using log phase cultures that contain a small number of non-growing persisters, the definition of persisters has subsequently been extended to also include non-growing persisters that are enriched in stationary phase cultures. Thus, antibiotic-tolerant bacteria that are not killed by antibiotics but can regrow in the absence of antibiotics and remain susceptible to the same antibiotics are called persisters. Persisters are characterized by slow or no growth with low metabolic activity and phenotypic resistance to antibiotics. The presence of the persister phenomenon has been documented in numerous bacterial species including *M. tuberculosis*. Persisters in biofilms and many bacterial...
infections pose a significant challenge for treatment and disease control.\(^5,11\) Despite the original observation of the persistence phenomenon 60 years ago, the mechanisms of persister formation are poorly understood. Recent studies suggest that toxin–antitoxin (TA) modules may be involved in persister formation.\(^1\)

The first TA module linked to persistence in *Escherichia coli* is HipBA.\(^12\) HipB and HipA, like other TA modules such as RelBE and MazEF, are organized in an operon with the gene hipB encoding the antitoxin, located upstream of the toxin gene hipA.\(^13\) Overexpression of wild-type toxin such as HipA or RelE caused 10- to 1000-fold more persisters.\(^14,15\) HipA and RelE could inhibit macromolecule (protein, RNA and DNA) synthesis and slow down or even stop cell division, raising the possibility that toxins of the TA modules may be involved in persister formation.\(^15\) However, deletion of TA modules such as hipA did not cause a defect in persistence\(^15,16\) presumably due to their redundancy in the genome. A recent study showed that overexpression of unrelated toxic proteins, such as heat shock protein DnaJ and protein PmrC, also caused higher persister formation.\(^17\)

This finding questions the significance of TA modules as a specific and universal mechanism for persister formation.

In *M. tuberculosis*, isocitrate lyase (ICL), required for fatty acid catabolism, was found to be involved in mycobacterial persistence.\(^18\) In addition, inactivation of RelA led to a defect in persistence in the mouse model.\(^19\) We have recently identified a new persister gene *phoU*, involved in persister formation in *E. coli*.\(^16\) PhoU is a negative regulator of the pst operon involved in phosphate uptake but its function is not known. We recently showed that inactivation of *phoU* in *E. coli* leads to a dramatic defect in persister phenotype as demonstrated by reduced persister numbers in persister assays and increased susceptibility to a diverse range of antibiotics and stress conditions (acid pH, starvation, etc.), especially in stationary phase or starved cultures compared with log phase cultures.\(^16\)

Microarray studies indicated that the *E. coli* *phoU* mutant surprisingly expressed high levels of genes involved in energy production and metabolism, efflux/transport, and flagella and chemotaxis synthesis, suggesting that PhoU is a global repressor for cellular metabolism and its inactivation leads to a hyperactive metabolic state as the underlying cause of the persistence defect. This study provides the first evidence of PhoU being a master regulator, beyond its role in phosphate metabolism, being involved in persister formation. We thus proposed a model based on PhoU that serves as a general repressor of cellular metabolism to suppress cellular metabolic activity to facilitate persister formation.\(^16\) PhoU is a ubiquitous protein present in virtually all bacterial species, including *M. tuberculosis*.\(^16\)

*M. tuberculosis*, which is notorious for its ability to persist in vivo despite antibiotic treatment, has two PhoU homologues, PhoY1 and PhoY2,\(^16\) which share 63.4% amino acid identity to each other. PhoY1 and PhoY2 have, respectively, 40% and 44% homology to *E. coli* PhoU. The role of PhoY1 and PhoY2 in the persistence of *M. tuberculosis* is unclear. In this study, we constructed mutants of *PhoU* homologues *phoY1* and *phoY2* and evaluated the role of PhoY1 and PhoY2 in the persistence of *M. tuberculosis*. We show that *M. tuberculosis phoY2* is the equivalent of *E. coli phoU* and that inactivation of *phoY2* but not *phoY1* caused a defect in the persistence phenotype including increased susceptibility to antibiotics and decreased persister formation in vitro, and also reduced persistence in the mouse model, a phenotype that can be complemented by the functional wild-type *phoY2* gene.

**Materials and methods**

**Bacterial growth conditions**

Bacterial strains and plasmids used are shown in Table 1. *E. coli* strains were grown in Luria–Bertani (LB) broth or on LB broth agar. *Mycobacterium smegmatis* mc2155 was grown in LB broth containing 0.5% glycerol, 0.5% dextrose and 0.05% Tween 80. *M. tuberculosis* strain H37Rv was grown in 7H9 liquid medium (Difco) supplemented with 0.05% Tween 80 with 10% bovine serum albumin–dextrose–catalase (ADC) enrichment (Difco) at 37°C for ~2–3 weeks with occasional agitation. When required, the following antibiotics were used at the specified concentrations: kanamycin (25 mg/L) and hygromycin B (150 mg/L for *E. coli* and 50 mg/L for *M. tuberculosis*).

**Knockout mutant construction and complementation**

*phoY1* and *phoY2* genes of *M. tuberculosis* H37Rv were disrupted using specialized transduction as described previously.\(^20\) To create *phoY1::hyg* and *phoY2::hyg*, the hygromycin resistance cassette was used to replace the respective gene’s open reading frames. Successful deletion of the gene was confirmed by junction PCR and DNA sequencing. For complementation of the deletion mutants, wild-type *phoY1* and *phoY2* genes were amplified from H37Rv genomic DNA by PCR and cloned into mycobacterial shuttle vector pMV261 followed by electroporation into the mutants as described previously.\(^21\) Primers used to construct *phoY1* and *phoY2* mutants and complementation are shown in Table 1.

**MIC/MBC determination and drug exposure assays**

The MICs of isoniazid and rifampicin were determined by using serial 2-fold dilutions of the compound in 7H9 medium and on 7H11 agar. The MIC of pyrazinamide was determined in 7H9 medium or on 7H11 agar at pH 5.6. The initial cell density was 10⁵ cfu/mL of log phase culture, and the samples were incubated for 15 days at 37°C. The MIC was recorded as the minimum drug concentration that prevented visible growth, and the MBC was recorded as the drug concentration that reduced cfu by 100-fold over the seeded inoculum in the MIC testing.

For drug exposure persister assays, the survival of stationary phase cultures of the *phoY1* and *phoY2* mutants and the wild-type H37Rv to pyrazinamide (200 mg/L) and rifampicin (8 mg/L) was determined. Drug exposure with pyrazinamide was performed in acidic medium at pH 5.5 as described previously.\(^22\) The drug exposure was carried out over a period of 3–9 days at 37°C without shaking. Aliquots of bacterial cultures exposed to the drug were taken at different timepoints and washed in PBS buffer before plating on 7H11 agar plates for cfu count.

**Survival and persistence of Δ*phoY1* and Δ*phoY2* mutants in the mouse model**

Six-week-old female BALB/c mice (NCI, Frederick, MD, USA) were infected via the tail vein with *M. tuberculosis* strain H37Rv. Δ*phoY1* mutant, Δ*phoY2* mutant and the respective mutant complemented strains (all at ~1×10⁷ cfu) in 100 µL of PBS/0.05% Tween 80 using a low dose latent infection model as described previously.\(^23\) Mice (five mice per group) infected with the above different mycobacterial strains were housed in a BSL-3 animal facility. After 8 weeks of infection, mice were sacrificed and the infected organs (lungs and spleens) were homogenized in PBS/0.05% Tween 80, and the homogenates and their appropriate dilutions were plated on 7H11 plates containing 10% ADC and the
PhoU homologue PhoY2 involved in TB persistence

Table 1. Bacterial strains, plasmids, phage and primers used in this study

<table>
<thead>
<tr>
<th>Strains</th>
<th>Description</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli HB101</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; hsdS20 (r&lt;sub&gt;E&lt;/sub&gt;, m&lt;sub&gt;15&lt;/sub&gt;) supE44 ara-14 galK-2 lacY1 proA2 rpsL20 (Sm&lt;sup&gt;´&lt;/sup&gt;) yci-S5 mtl-1–recA13</td>
<td>ATCC</td>
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<td>M. smegmatis mc&lt;sup&gt;2&lt;/sup&gt;155</td>
<td>high-frequency transformation mutant of M. smegmatis ATCC 607</td>
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<td>M. tuberculosis H37Rv</td>
<td>wild-type strain</td>
<td>ATCC</td>
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<td>Plasmids</td>
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<td>p0004s</td>
<td>hygromycin resistance plasmid</td>
<td>W. R. Jacobs Jr</td>
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<tr>
<td>pMV261</td>
<td>E. coli–Mycobacteria shuttle vector</td>
<td>C. K. Stover</td>
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<tr>
<td>Phage</td>
<td></td>
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<tr>
<td>phAE159</td>
<td>temperature-sensitive phage for mycobacteria</td>
<td>W. R. Jacobs Jr</td>
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<tr>
<td>Primers</td>
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<td>Y1KLL</td>
<td>5′-TTTTTTTTTTGAAATTTGGAAGGACACCTATCCAGCTCAACCCGAC-3′ for phoY1 knockout</td>
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<td>Y2KRR</td>
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<td>5′-CGACCCCCCGCTAGCAGCAGAGCCGAC-3′ for junction PCR</td>
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<td>Y1A</td>
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<td>5′-CGACCCCCCGCTAGCAGCAGAGCCGAC-3′ for phoY1 complementation</td>
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<tr>
<td>Y2CA</td>
<td>5′-CGACCCCCCGCTAGCAGCAGAGCCGAC-3′ for phoY1 complementation</td>
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antibiotic cocktail PANTA (Becton Dickinson, Sparks, MD, USA) to prevent contamination. Plates were incubated at 37 °C in a 5% CO<sub>2</sub> environment for 4 weeks before cfu counts were determined. The Johns Hopkins University Animal Care and Use Committee approved all animal procedures.

Statistical treatment
Pairwise comparison of the cfu data for statistical significance was performed using Student’s t-test.

Results and discussion

Construction of ΔphoY1 and ΔphoY2 mutants of M. tuberculosis H37Rv

To determine the effect of mutation of phoY1 and phoY2 on the persistence phenotype of M. tuberculosis, we first constructed knockout mutant ΔphoY1::hyg and ΔphoY2::hyg alleles using specialized transduction with the temperature-sensitive phage phAE159 as described in the Materials and methods section. Hygromycin-resistant colonies were obtained, which were suggestive of a double-crossover gene replacement event. Junction PCR was used to confirm the mutant genotype for the mutant alleles with the wild-type strain H37Rv as a control. The 666 bp (phoY1) and 642 bp (phoY2) fragments were observed in the wild-type but not in the phoY1 and phoY2 knockout mutants, indicating successful construction of ΔphoY1 and ΔphoY2 mutants of M. tuberculosis.

Inactivation of M. tuberculosis PhoU homologue PhoY2 but not PhoY1 caused increased susceptibility to TB drugs

Since the E. coli phoU mutant has a defect in persister formation as demonstrated by increased susceptibility to antibiotics and a reduced number of persisters, we subjected the phoY1 and phoY2 mutants to antibiotic susceptibility tests and also persister assays as described in the Materials and methods section. Interestingly, inactivation of phoY2 caused increased susceptibility to TB drugs rifampicin and pyrazinamide compared with the wild-type H37Rv in both MIC and MBC experiments (see Table 2). Specifically, the MICs of rifampicin and pyrazinamide for the phoY2 mutant decreased 4-fold and 2-fold, respectively, in 7H9 liquid medium. The MBC of rifampicin for the phoY2 mutant was 4-fold less than that for the parent strain H37Rv but the MBC of pyrazinamide remained unchanged (Table 2). However, inactivation of phoY1 did not significantly alter susceptibility to rifampicin or pyrazinamide in MIC or MBC experiments.
Decreased persisters in \textit{PhoY2} mutant and not \textit{PhoY1} mutant upon pyrazinamide and rifampicin exposure

To determine the effect of pyrazinamide and rifampicin on the persister survival of the \textit{phoY1} and \textit{phoY2} mutants, we subjected stationary phase cultures of the parent strain H37Rv and the above mutants to pyrazinamide (200 mg/L) at pH 5.6 and rifampicin (8 mg/L) at pH 7.0, and determined the cfu values at 0, 3, 9 and 17 days after exposure. No significant difference in survival between the \textit{phoY1} and \textit{phoY2} mutants and the parent strain H37Rv was observed after 3 days exposure to either pyrazinamide or rifampicin \((P>0.05)\). After 9 days pyrazinamide or rifampicin exposure, there was no difference in survival between \textit{phoY1} mutant and the parent strain H37Rv, but the survival of the \textit{phoY2} mutant was greatly decreased and no persisters were detectable (detection limit 100 bacteria) \(\text{(Table 3)}\). However, at day 17, all viable bacteria were killed by pyrazinamide for both the mutants and the parent control strain H37Rv so a comparison to show the defect in persisters of the \textit{phoY2} mutant was not possible \(\text{(data not shown)}\). In the drug-free control, there was no significant decrease in cfu values for the \textit{phoY2} and \textit{phoY1} mutants or the control strain H37Rv, indicating that the loss of persisters after rifampicin or pyrazinamide exposure for the \textit{phoY2} mutant is specific for the defect in \textit{phoY2} and not due to non-specific cell death during the nutrient-limiting conditions during exposure to the drug. These results suggest that \textit{PhoY2}, but not \textit{PhoY1}, is the equivalent of \textit{E. coli} PhoU in \textit{M. tuberculosis}. It is of interest to note that the defect in persister survival of \textit{phoY2} was not obvious at the earlier timepoint of 3 day drug exposure but was seen only after extended exposure up to 9 days. This is consistent with our previous findings for the \textit{E. coli} \textit{phoU} mutant, which, in the drug exposure persister assay, also require a relatively long time for the persistence defect to be demonstrated.\textsuperscript{16} Future studies are needed to determine whether the \textit{PhoY2} mutant also has defective persistence to other TB drugs such as fluoroquinolones and aminoglycosides.

\textbf{Table 2. MIC and MBC values (mg/L) of \textit{phoY1} and \textit{phoY2} mutants compared with parent strain H37Rv}

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC H37Rv</th>
<th>MBC H37Rv</th>
<th>MIC \textit{phoY1}</th>
<th>MBC \textit{phoY1}</th>
<th>MIC \textit{phoY2}</th>
<th>MBC \textit{phoY2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PZA pH 5.9</td>
<td>200</td>
<td>400</td>
<td>200</td>
<td>400</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>Rifampicin (pH 7.0, 7H9 no ADC)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.05–0.1</td>
<td>0.2</td>
<td>0.025</td>
<td>0.05</td>
</tr>
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</table>

PZA, pyrazinamide; Rif, rifampicin.

The starting inocula used for H37Rv, \textit{phoY1} and \textit{phoY2} mutants were: \(5\times10^5\), \(6.3\times10^5\) and \(6.3\times10^5\), respectively. MIC/MBC testing was performed in 7H9 liquid medium as described in the Materials and methods section.

\textbf{Table 3. \textit{PhoY2} mutant but not \textit{PhoY1} mutant has reduced persisters in vitro}

\begin{tabular}{|c|c|c|c|c|c|}
\hline
Drug concentration & Strain & Bacterial count (cfu/mL) & \textbf{start} & \textbf{day 3} & \textbf{day 9} \\
\hline
\textbf{Pyrazinamide (pH 5.6, 7H9 no ADC)} & H37Rv & 3.1±0.10\times10^6 & 3.1±0.45\times10^5 & 5.3±0.15\times10^5 \\
200 mg/L & H37Rv \textit{ΔphoY1} & 3.2±0.17\times10^6 & 4.3±0.50\times10^5 & 3.3±0.15\times10^5 \\
200 mg/L & H37Rv \textit{ΔphoY2} & 2.4±0.79\times10^6 & 4.83±0.06\times10^5 & 0a \\
0 mg/L & H37Rv & 3.1±0.10\times10^6 & 6.9±0.46\times10^5 & 1.33±0.15\times10^5 \\
0 mg/L & H37Rv \textit{ΔphoY1} & 3.2±0.17\times10^6 & 2.07±0.47\times10^6 & 3.97±0.25\times10^5 \\
0 mg/L & H37Rv \textit{ΔphoY2} & 2.4±0.79\times10^6 & 4.77±1.05\times10^5 & 2.77±0.15\times10^5 \\
\hline
\textbf{Rifampicin (pH 7.0, 7H9 no ADC)} & H37Rv & 3.1±0.10\times10^6 & 3.37±0.51\times10^5 & 1.67±0.25\times10^6 \\
200 mg/L & H37Rv \textit{ΔphoY1} & 3.2±0.17\times10^6 & 4.97±0.70\times10^5 & 3.13±0.31\times10^5 \\
200 mg/L & H37Rv \textit{ΔphoY2} & 2.4±0.79\times10^6 & 2.27±0.57\times10^5 & 0a \\
0 mg/L & H37Rv & 3.1±0.10\times10^6 & 1.33±0.40\times10^9 & 3.5±0.26\times10^9 \\
0 mg/L & H37Rv \textit{ΔphoY1} & 3.2±0.17\times10^6 & 2.0±0.35\times10^9 & 3.57±0.32\times10^9 \\
0 mg/L & H37Rv \textit{ΔphoY2} & 2.4±0.79\times10^6 & 1.37±0.32\times10^9 & 3.0±0.40\times10^9 \\
\hline
\end{tabular}

\(a\)Below detection limit of 100 bacteria.
parent strain H37Rv (P<0.05), whereas the PhoY1 mutant survived and persisted as well as the H37Rv control strain. Interestingly, complementation of the PhoY2 mutant with the wild-type phoY2 gene restored the persistence phenotype of the PhoY2 mutant to the wild-type level of H37Rv in both spleens and lungs (Table 4). This experiment validates that the PhoY2 mutant not only has an in vitro defect in persister survival in drug exposure assays as shown above, but also exhibited an in vivo persistence defect in the mouse model (Table 4). Our data suggest that PhoY2 mutation does alter persistence as shown by the persister drug exposure assay, the mouse study and also the MIC/MBC values for the PhoY2 mutant. There is significant debate regarding whether persister mutants have any alteration in MIC/MBC values. It has been proposed that persister mutants should not alter the MIC but only affect persistence.24 However, this may not necessarily be true as mutation in persister genes could well affect antibiotic susceptibility not only in persisters as shown in persister assays but also in growing bacteria as shown in MIC/MBC tests in the case of PhoU mutation16 and also SucB and UbiF mutations.25 In addition, we have found that mutants with a defect in persistence may or may not have altered MIC/MBC values (J. Chen and Y. Zhang, unpublished data).

It has been demonstrated that PhoU expression is correlated with persister formation as it is only expressed in stationary phase or aged cultures under nutrient-limiting conditions16,26 or overexpressed in biofilms of E. coli.27 In our PhoU overexpression study using arabinose-inducible pBAD vector, we found that overexpression of phoU caused increased persister formation in log phase E. coli cultures, but overexpression of control genes furA, fur and rcsB involved in L-form formation28 in the same vector did not (W. Shi and Y. Zhang, unpublished observation). Studies are underway to assess whether controlled overexpression of phoY2 also leads to increased persister formation in M. tuberculosis. More recently, consistent with our previous finding that PhoU is important for persister formation in E. coli,16 Lee et al.29 found that PhoU played a critical role in persister phenotype in Pseudomonas putida as shown by higher susceptibility and decreased persisters in the phoU mutant. It is of interest to note that phoY2 (Rv0821c) was significantly up-regulated in a 96 h nutrient starvation persistence model of M. tuberculosis,30 suggesting that phoY2 may be important for mycobacterial persistence. Our findings in this study provide further evidence that phoY2 is involved in persistence in M. tuberculosis. Future studies are needed to determine the persister phenotype to other TB drugs such as aminoglycosides and quinolones, long-term persistence of the phoY2 mutant in the mouse model and also to elucidate the mechanism by which phoY2 mediates persistence in M. tuberculosis.

In conclusion, we have shown that the M. tuberculosis PhoU homologue PhoY2 but not PhoY1 is the equivalent of E. coli PhoU as demonstrated by increased susceptibility to TB drugs and a defect in persistence in the mouse model. This is another study that confirms the role of PhoU in persistence in a different organism (i.e. M. tuberculosis) besides E. coli. Studies are currently underway to address the detailed mechanisms of how PhoU or PhoY2 mediates persister formation. The identification of PhoY2 as being involved in mycobacterial persistence is a significant finding that has implications on not only understanding the mechanism of persistence but also development of new drugs and vaccines that target persister organisms for improved control of TB.

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We are grateful to Bill Jacobs for providing mycobacterial plasmids and phages.

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Transparency declarations
None to declare.

References

| Table 4. Survival of phoY1 and phoY2 mutants and their complemented strains in mice |
|------------------------------------------|------------------|------------------|
| PhoY1 mutant                          | Beginning cfu    | cfu/spleen       | cfu/lung         |
| H37Rv                                  | 0.84 ± 0.11 x 10^4 | 3.03 ± 0.19 x 10^3 | 1.20 ± 0.10 x 10^2 |
| H37Rv ΔphoY1                           | 1.18 ± 0.09 x 10^4 | 7.39 ± 0.48 x 10^3 | 3.73 ± 0.27 x 10^2 |
| H37Rv ΔphoY1 complemented             | 1.25 ± 0.35 x 10^4 | 4.38 ± 0.85 x 10^3 | 3.81 ± 0.92 x 10^2 |
| H37Rv ΔphoY2                           | 1.13 ± 0.27 x 10^4 | 1.71 ± 1.16 x 10^4 | 1.6 ± 1.0 x 10^4   |
| H37Rv ΔphoY2 complemented             | 1.35 ± 0.40 x 10^6 | 5.82 ± 0.62 x 10^3 | 4.89 ± 0.40 x 10^2 |

The phoY2 mutant had 10- to 30-fold fewer cfu in both lungs and spleen than the control parent strain H37Rv (*P<0.05 compared with the cfu of control H37Rv). Complementation of the phoY2 mutant restored the level of persistence to that of the parent strain H37Rv. There was no significant difference in survival or persistence in lungs and spleen between H37Rv, the phoY2 mutant and the complemented strains (P>0.05).
Escherichia coli formation and tolerance to multiple antibiotics and stresses in Li Y, Zhang Y. PhoU is a persistence switch involved in persister survival and tolerance to multiple antibiotics and stresses in K-12 that affects frequency of persistence after inhibition of murein biosynthesis. Front Biosci 2004; 9: 1059–72.


