**Supplemental Figure 1**: Relative expression levels of the *kdpABC* and *kdpDE* genes of *kdpD* and *kdpE* mutants in varying K+ concentrations. Total RNA was extracted and cDNA was used for quantitative analysis, with the expression of *sigA* as a reference for determining the relative expression levels. The graphs show the average of three biological replicates. Error bars indicate Std.Dev. The differences were analyzed by multiple t tests: ns indicate p＞0.05; \*\*\* indicates p < 0.001.

**Supplemental Figure 2**. Survival fraction of the wild type (W.T.), the *kdpA* mutant (*kdpA*::Tn), and the complemented strain(*kdpA*::Tn-*pSMT3L*-*kdpA*) after streptomycin exposure. The three strains were exposed to 250 μg/mL streptomycin in 7 mM K+ (A and B), or 140 mM K+ (C and D), and survival was determined by spot plating. The colonies were counted after 7 days of growth. The graphs show the average of at least three independent experiments. Error bars indicate Std.Dev. One-Way ANOVA was used for the statistical analysis: ns indicate p＞0.05 ,\* indicates p < 0.05.

**Supplemental Table** 1: The 15 mutant strains whose growth was inhibited in the low rifampicin concentration.

**Supplemental Table 2**: MICs of *M. marinum* wild type and mutant strains.

**Supplemental Table 3:** MIC for CCCP of *M. marinum* wild type, *kdpA* mutant, and complemented strains.

**Supplemental Table 4**: List of primers used in this study.



Supplemental Figure 1

 Supplemental Figure 2

Supplemental Table 1

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Gene Locus  | Transposon insertion sites  | Product |
| *arsC* | MMAR\_2055 | +131nt | arsenic-transport integral membrane protein ArsC |
| *umaA* | MMAR\_0794 | -11nt | mycolic acid synthase UmaA |
| *MMAR\_4275* | MMAR\_4275 | -80nt | PadR-like transcriptional regulatory protein |
| *MMAR\_0777* | MMAR\_0777 | +78nt | conserved secreted protein |
| *oppB* | MMAR\_4136 | +581nt | oligopeptide-transport integral membrane protein ABC transporter OppB |
| *udgA* | MMAR\_0603 | +505nt | UDP-glucose dehydrogenase UdgA |
| *MMAR\_0496* | MMAR\_0496 | +574nt | conserved transmembrane protein |
| *MMAR\_0976* | MMAR\_0976 | +231nt | conserved hypothetical protein |
| *MMAR\_3984* | MMAR\_3984 | +8nt | PPE family protein |
| *MMAR\_0183* | MMAR\_0183 | +742nt | PE-PGRS family protein |
| *mas* | MMAR\_1767 | +1298nt | multifunctional mycocerosic acid synthase membrane-associated Mas |
| *ftsW* | MMAR\_3194 | +652nt | FtsW-like protein FtsW |
| *MMAR\_0806* | MMAR\_0806 | +635nt | PE-PGRS family protein |
| *kdpA* | MMAR\_0631 | +75nt | potassium-transporting ATPase a subunit, KdpA |
| *MMAR\_0461* | MMAR\_0461 | +346nt | conserved hypothetical transmembrane protein |

“+131nt” indicates that the transposon insertion site is 131 nt after the transcription start codon. “-11nt” indicates that the transposon insertion site is 11 nt before the transcription start codon.Supplemental Table 2

|  |  |
| --- | --- |
| Strains | MIC (μg/mL ) |
| RIF | STR | INH | CFX | GEN |
| W.T. | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *arsC*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *umaA*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *MMAR\_4275*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *MMAR\_0776*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *oppB*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *udgA*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *MMAR\_0496*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *MMAR\_0976*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *MMAR\_3984*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *MMAR\_0183* | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *mas*::Tn | 0.03125 | 2.5 | 0.25 | 0.3125 | 3.125 |
| *fstW*::Tn | 0.0625 | 2.5 | 0.25 | 0.625 | 12.5 |
| *MMAR\_0806*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *kdpA*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |
| *MMAR\_0461*::Tn | 0.25 | 2.5 | 0.25 | 1.25 | 12.5 |

RIF, rifampin; STR, streptomycin; INH, isoniazid; CFX, ciprofloxacin; GEN, gentamicin.

Supplemental Table 3

|  |  |
| --- | --- |
| Strains | MIC of CCCP (mM) |
| 7 mM K+ | 70 mM K+ | 140 mM K+ |
| W.T. | 0.125 | 0.125 | 0.125 |
| *kdpA*::Tn | 0.375 | 0.1875 | 0.125 |
| *kdpA*::Tn-pSMT3L-*kdpA* | 0.125 | 0.125 | 0.125 |

Supplemental Table 4

|  |  |  |
| --- | --- | --- |
| Gene | Primer sequence | Comments |
| MycoMarT7 | CGGGGACTTATCAGCCAACCTG | MycoMar-specific primers |
| *sigA* | FW-CTCAAGCAGATCGGCAAGGT | For qRT-PCR |
| RV-CGGTCTGTCAACTCAGCCAT |
| *kdpA* | FW- TTCCAGTTGATGCAGGGCAA |
| RV- GTTCGTGTTGGTGACGAAGC |
| *kdpB* | FW- GAGATCGCGCTCAACATCCT |
| RV- GGCCTTTGAGTAGATCGCCA |
| *kdpC* | FW- CTACCCGCTGTTGGTCTGG |
| RV- ACAGGTTTCCCGTCGACTTC |
| *kdpD* | FW- CAGATAACCGGCATCGAGCA |
| RV- AACGCTTCTGGGGTGATGTC |
| *kdpE* | FW- GACATGTCCGGCATCGAAGT |
| RV- TGCACCTTGTCCGACGAATC |