| Mycobacterium tuberculosis | A1H66165 | DHDTWSVGSFFTNPVVTQDVYERLA | IDAA |
| Mycobacterium bovis | ESK77526 |
| Mycobacterium canetti | WP_015289078 |
| Mycobacterium caprae | APU24691 |
| Mycobacterium microti | AWS42565 |
| Mycobacterium mungi | OAQ17379 |
| Mycobacterium orygis | EMT37341 |
| Mycobacterium pinnipedii | PRH92798 |
| Mycobacterium africanum | CCC25558 |
| Mycobacterium angelicum | WP_06835123 |
| Mycobacterium arosiense | WP_083066530 | IAP-A- | AAV-N |
| Mycobacterium asiaticum | WP_03652173 | APE- | EGTG-A |
| Mycobacterium avium | WP_003873728 | AP- | GSV-G |
| Mycobacterium avium subsp. avium | EUA28749 | APE- | ATV-G |
| Mycobacterium avium subsp. paratuberculosis | ETB42591 | AP- | GSV-G |
| Mycobacterium boehmense | WP_05179361 |
| Mycobacterium branderi | WP_083130379 | P-Q- | DQA-R |
| Mycobacterium celatum | WP_062540622 | P-E-Q- | DQA-R |
| Mycobacterium chimaera | WP_089152222 |
| Mycobacterium colombiense | WP_007762823 | AP- | ASV-N |
| Mycobacterium conspicuum | WP_085235610 | AAE- | G-Y-Q |
| Mycobacterium europeum | WP_085240816 | APE- | AGFE |
| Mycobacterium florentinum | WP_085222774 | APE-S- | AAT-G |
| Mycobacterium fragae | WP_065197663 |
| Mycobacterium gastr | WP_007762823 | SP-V- | AAVN-G |
| Mycobacterium gastri ‘Wayne’ | ETW24530 | SP- | AAVN-G |
| Mycobacterium genavense | WP_025737032 | P-O-S- | AAT-N |
| Mycobacterium haemophilum | WP_047313652 | AP-HQ- | KMT-G |
| Mycobacterium heidelbergense | WP_080374492 | PPG- | DSVE |
| Mycobacterium interjectum | WP_066917700 | SPE- | A-VG-I |
| Mycobacterium intermedium | WP_069421516 | PPF-F | A-V |
| Mycobacterium intracellulare | WP_014383357 |
| Mycobacterium kansasi | DCG100214 |
| Mycobacterium koryoxense | WP_065014685 | APE-Q- | DEA-R |
| Mycobacterium lacus | WP_085158024 | ADA-D- | SCS-T |
| Mycobacterium lentiflavum | WP_090599765 | APE-S- | G-T-N |
| Mycobacterium leprae | WP_019098905 | AP-RQ- | KMT-G |
| Mycobacterium lepraemurium | ATAT9689 |
| Mycobacterium liflandii | WP_01554460 | APEI- | AGTGES |
| Mycobacterium malmoense | WP_065434342 | HP- | AAVE |
| Mycobacterium mantenii | WP_083095025 | AP- | AAV |
| Mycobacterium marinum | BB63921 | APEI- | AGTGES |
| Mycobacterium marseillense | WP_095577844 | AP- | ASV-N |
| Mycobacterium montefiorense | WP_109892083 |
| Mycobacterium palustre | WP_085077552 | SP-A- | G-L-A |
| Mycobacterium paraense | WP_085097615 | SPQ- | G-VE |
| Mycobacterium paraffinicum | WP_073874636 | APE-D- | GAGQ |
| Mycobacterium paracelulose | WP_083173737 | APE- | GAA |
| Mycobacterium paradoxum | WP_095591789 | AP-C- | DTV-

**“Tuberculosis” clade (9/9)**

- **Other Mycobacteriaceae (0/62)**

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Figure S1. Partial sequence alignment of a conserved region of the UDP-N-acetylenopyruvylglucosamine reductase (MurB) protein showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade. The alignment shown here is the same as the one in Figure 3 (A), but it contains information for more outgroup species.
| Mycobacterium tuberculosis | A9H9257 | AARGAAELDGATATDMIDIWDETF | - | - |
| Mycobacterium bovis | YP_009359759 | - | - |
| Mycobacterium canetti | WP_042915285 | - | - |
| Mycobacterium caprae | AP036375 | - | - |
| Mycobacterium microti | AOM0081 | - | - |
| Mycobacterium mungi | OAI17023 | - | - |
| Mycobacterium orygis | EMTC3537 | - | - |
| Mycobacterium pinnipedi | PRH92218 | - | - |
| Mycobacterium africanum | CCE27476 | - | - |
| Mycobacterium alsense | WP_083136754 | - | - |
| Mycobacterium anglicum | WP_083114401 | - | - |
| Mycobacterium arosiense | WP_083069243 | - | - |
| Mycobacterium asiaticum | WP_036358582 | - | - |
| Mycobacterium avium | WP_065370704 | - | - |
| Mycobacterium boehmii | WP_085189294 | - | - |
| Mycobacterium branderi | WP_083131585 | - | - |
| Mycobacterium celatum | WP_062541748 | - | - |
| Mycobacterium chimaera | WP_054586211 | - | - |
| Mycobacterium colombiensis | WP_065125779 | - | - |
| Mycobacterium conspicuum | WP_085253420 | - | - |
| Mycobacterium florentinum | WP_08520670 | - | - |
| Mycobacterium fragae | WP_085199625 | - | - |
| Mycobacterium gastri | WP_036417995 | - | - |
| Mycobacterium genavense | WP_025790351 | - | - |
| Mycobacterium gordonae | WP_085044324 | - | - |
| Mycobacterium hekchesornense | WP_048993314 | - | - |
| Mycobacterium heidelbergense | WP_083070276 | - | - |
| Mycobacterium interjectum | WP_036613464 | - | - |
| Mycobacterium intermedium | WP_069420640 | - | - |
| Mycobacterium intracellular | WP_009852914 | - | - |
| Mycobacterium kansasii | OR83169 | - | - |
| Mycobacterium kyorinense | WP_065015558 | - | - |
| Mycobacterium lacus | WP_085162634 | - | - |
| Mycobacterium lentiflavum | WP_090603968 | - | - |
| Mycobacterium lepraemurium | AT28936 | - | - |
| Mycobacterium liflandii | WP_015356308 | - | - |
| Mycobacterium malmoense | WP_065443734 | - | - |
| Mycobacterium marium | WP_012395059 | - | - |
| Mycobacterium montefiorense | WP_018995415 | - | - |
| Mycobacterium nebraskense | WP_046138209 | - | - |
| Mycobacterium noviomagense | WP_083075358 | - | - |
| Mycobacterium palustre | WP_085079959 | - | - |
| Mycobacterium parasouleouli | WP_083168877 | - | - |
| Mycobacterium pamarne | ORM63158 | - | - |
| Mycobacterium pinnipedi | WP_085252566 | - | - |
| Mycobacterium sanguinicolor | WP_085254789 | - | - |
| Mycobacterium secundulense | WP_056329947 | - | - |
| Mycobacterium shigeri | WP_089398943 | - | - |
| Mycobacterium shigaense | WP_083075360 | - | - |
| Mycobacterium sherryi | WP_069593706 | - | - |
| Mycobacterium shemosedi | WP_069349613 | - | - |
| Mycobacterium shinjukense | WP_083046314 | - | - |
| Mycobacterium szulgai | WP_083046701 | - | - |
| Mycobacterium talmoniae | WP_071027060 | - | - |
| Mycobacterium triplex | WP_036430724 | - | - |
| Mycobacterium ulcerans | WP_096370456 | - | - |
| Mycobacterium xenopi | WP_003921093 | - | - |
| Mycolicibacillus trivialis | WP_085108697 | - | - |
| Mycolicibacter algericus | WP_083036313 | - | - |
| Mycolicibacter heraclionensis | WP_085040279 | - | - |
| Mycolicibacter hiberniae | WP_085134780 | - | - |
| Mycolicibacter icosisumamisiliensis | WP_067971646 | - | - |
| Mycolicibacter longobardus | WP_085263510 | - | - |
| Mycolicibacter senensis | WP_085035959 | - | - |
| Mycolicibacter tereus | WP_013828454 | - | - |
| Mycolicibacter terrae | WP_085296646 | - | - |

Figure S2. Partial sequence alignment of a conserved region of the 3'-phosphoadenosine 5'-phosphosulfate reductase CYSH protein showing a seven amino acid insertion that is specific for members of the “Tuberculosis” clade. The alignment shown here is the same as the one in Figure 3 (B), but it contains information for more outgroup species.
### Other Mycobacteriaceae (0/70)

| Mycobacterium species                        | Accession   | Alignment  | Clade (8/8) | Alignment shown here is the same as the one in Figure 4, but it contains information for more outgroup species. |
|----------------------------------------------|-------------|------------|-------------|--------------------------------------------------------------------------------------------------------|
| Mycobacterium tuberculosis                  | CFE24486    | LHLHDHYAEVGSEFADLVDA |            |                                                                                                       |
| Mycobacterium bovis                         | ESK72333    |             |             |                                                                                                       |
| Mycobacterium caprae                        | APR72664    |             |             |                                                                                                       |
| Mycobacterium microti                        | AIC61665    |             |             |                                                                                                       |
| Mycobacterium mungi                         | OA16258     |             |             |                                                                                                       |
| Mycobacterium oryxis                        | EMT3941     |             |             |                                                                                                       |
| Mycobacterium pinnipedii                    | PHR92461    |             |             |                                                                                                       |
| Mycobacterium africana                      | CCG4952     |             |             |                                                                                                       |
| Mycobacterium anglicicum                    | WP_083115022 | R - G - V |            |                                                                                                       |
| Mycobacterium aquatium                      | WP_08317034 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium asiaticum                     | OBI87779    | R - I - A - V |             |                                                                                                       |
| Mycobacterium colombiensis                  | OB143750    | R - I - G - A |             |                                                                                                       |
| Mycobacterium conceptionense               | COG3141665  | R - I - G - E |             |                                                                                                       |
| Mycobacterium conspicuum                    | WP_08535047 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium dioxanotrophicus              | WP_08701487 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium florentinum                   | ORY49668    | R - I - G - V |             |                                                                                                       |
| Mycobacterium gordonea                      | OB87113     | R - I - G - Q |             |                                                                                                       |
| Mycobacterium grosscia                      | WP_070356433| R - I - G - Q |             |                                                                                                       |
| Mycobacterium haemophilum                   | WP_05487909 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium heckeshornease                | KJW21413    | R - I - G - Q |             |                                                                                                       |
| Mycobacterium heidelbergerense              | ORA67140    | R - I - G - Q |             |                                                                                                       |
| Mycobacterium houstonense                   | WP_06690209 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium iranicum                      | WP_06428090 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium krbyneense                    | OB1148681   | R - I - G - Q |             |                                                                                                       |
| Mycobacterium lacus                         | WP_08516696 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium lentiflavum                   | WP_09060824 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium mageritense                   | WP_08140944 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium malmoense                      | WP_08516696 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium malmoense                      | WP_08516696 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium mongoliense                   | WP_08516696 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium montefioriense                | WP_08516696 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium moriokaense                    | WP_08516696 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium nebraskense                    | WP_04618604 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium neoaurum                      | WP_08270300 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium neworleansense                 | CRZ17520    | R - I - G - Q |             |                                                                                                       |
| Mycobacterium novocastrense                  | QAT08746    | R - I - G - Q |             |                                                                                                       |
| Mycobacterium palustre                       | ORW1132     | R - I - G - Q |             |                                                                                                       |
| Mycobacterium paraffinicum                   | WP_07387684 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium parafortuitum                  | WP_08314960 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium paracalculatum                 | WP_08527979 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium peregrinum                     | WP_08648458 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium porcim                        | WP_07592457 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium rhodesiae                      | EHS4895     | R - I - G - Q |             |                                                                                                       |
| Mycobacterium rutinum                       | SEH5726     | R - I - G - Q |             |                                                                                                       |
| Mycobacterium scrofulaceum                   | WP_08628455 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium septicum                       | WP_04451386 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium setense                        | KH20050     | R - I - G - Q |             |                                                                                                       |
| Mycobacterium shinoidei                      | WP_08422628 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium shinjukuense                   | OB143750    | R - I - G - Q |             |                                                                                                       |
| Mycobacterium sengsiasi                      | CKB15625    | R - I - G - Q |             |                                                                                                       |
| Mycobacterium sphagni                        | WP_09498414 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium sulzai                         | WP_08603452 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium vanbanaeii FYR                 | ABM16430    | R - I - G - Q |             |                                                                                                       |
| Mycobacterium vnulseris                      | CD20919     | R - I - G - Q |             |                                                                                                       |
| Mycobacterium wonolyski                      | WP_08514470 | R - I - G - Q |             |                                                                                                       |
| Mycobacterium xenopi                         | ORX10849    | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium aromaticivorans            | WP_09795192 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium austroafricanum            | WP_10538665 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium boenicellii                | WP_07747159 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium brasilense                 | OAS9011     | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium canariense                 | WP_10976269 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium chubuense NB               | AF19703     | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium conceptionense            | WP_06506425 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium confluentis                | ORV24951    | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium domicum                    | WP_08518709 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium alphais                  | WP_02012323 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium farcinogenes               | WP_03692363 | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium flavescens                 | OD85862     | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium fortuitum                  | AL129604    | R - I - G - Q |             |                                                                                                       |
| Mycolicibacterium goodii                     | WP_04974367 | R - I - G - Q |             |                                                                                                       |

Figure S3. Partial sequence alignment of a conserved region of the transcriptional regulator protein showing a twelve amino acid deletion that is specific for members of the “Tuberculosis” clade. The alignment shown here is the same as the one in Figure 4, but it contains information for more outgroup species.
Figure S4. Partial sequence alignment of a conserved region of the propionyl-CoA carboxylase beta chain 5 protein showing a one amino acid deletion that is specific for most members of the "Tuberculosis" clade and is absent form most other Mycobacteriaeae.
Figure S5. Partial sequence alignment of a conserved region of the O-succinylbenzoic acid-CoA ligase MenE protein showing a two amino acid insertion that is specific for members of the “Tuberculosis” clade and absent in most other Mycobacteriaceae.
| Mycobacterium tuberculosis        | CKP03712 | VVWAAGGWANDSVSCPRSEVIVR | KAPS | QEDHWYSTGADFKRPAPHHWFDDAT | (9/9) |
| Mycobacterium bovis              | WP_044798715 | - - - | S-S | N- | |
| Mycobacterium canetti            | WP_014001908 | - - - | S- | N- | |
| Mycobacterium caprae             | APU27511 | - - - | S- | N- | |
| Mycobacterium microti            | AMI61521 | - - - | S- | N- | |
| Mycobacterium mungi              | OAI18994 | - - - | S- | N- | |
| Mycobacterium orygis             | EMT3996 | - - - | S- | N- | |
| Mycobacterium pinnipedii         | PHH91614 | - - - | S- | N- | |
| Mycobacterium africanum          | KBG14050 | - - - | S- | N- | |
| Mycobacterium europaeum          | CDG05411 | - - - | S- | N- | |
| Mycobacterium alsense            | WP_083140810 | - - - | S- | N- | |
| Mycobacterium anglicum           | WP_083115754 | - - - | S- | N- | |
| Mycobacterium arosiense          | WP_083069312 | - - - | S- | N- | |
| Mycobacterium asiaticum          | WP_065153828 | - - - | S- | N- | |
| Mycobacterium avium complex      | WP_038534838 | - - - | S- | N- | |
| Mycobacterium bohemicum          | WP_065183286 | - - - | S- | N- | |
| Mycobacterium bohemicum DSM 44   | CPR10993 | - - - | S- | N- | |
| Mycobacterium chimaera           | WP_094080660 | - - - | S- | N- | |
| Mycobacterium colombiense        | WP_00772129 | - - - | S- | N- | |
| Mycobacterium conspicuum         | WP_085232645 | - - - | S- | N- | |
| Mycobacterium florentinum        | WP_085249585 | - - - | S- | N- | |
| Mycobacterium genavense          | WP_025736394 | - - - | S- | N- | |
| Mycobacterium gordonae           | WP_055802821 | - - - | S- | N- | |
| Mycobacterium haemophilum        | WP_04731203 | - - - | S- | N- | |
| Mycobacterium heidelbergense     | WP_083074886 | - - - | S- | N- | |
| Mycobacterium interjectum        | WP_085202284 | - - - | S- | N- | |
| Mycobacterium intracellulare     | WP_064935016 | - - - | S- | N- | |
| Mycobacterium kanssii            | WP_025368714 | - - - | S- | N- | |
| Mycobacterium kanssii 824        | EIA05190 | - - - | S- | N- | |
| Mycobacterium lacus              | WP_085158918 | - - - | S- | N- | |
| Mycobacterium leprae             | WP_010908833 | - - - | S- | N- | |
| Mycobacterium liflandii          | WP_041299754 | - - - | S- | N- | |
| Mycobacterium liflandii 128FXT   | ACC6927 | - - - | S- | N- | |
| Mycobacterium malmoense          | WP_065445100 | - - - | S- | N- | |
| Mycobacterium mantenii           | WP_083099562 | - - - | S- | N- | |
| Mycobacterium marinum            | WP_036450667 | - - - | S- | N- | |
| Mycobacterium marium M            | ACC4634 | - - - | S- | N- | |
| Mycobacterium marium MB2         | EPA085914 | - - - | S- | N- | |
| Mycobacterium marium str. Europe | EPQ70201 | - - - | S- | N- | |
| Mycobacterium nebraskense         | KKB97163 | - - - | S- | N- | |
| Mycobacterium paraense           | WP_085171272 | - - - | S- | N- | |
| Mycobacterium paraffinicum       | WP_073871693 | - - - | S- | N- | |
| Mycobacterium paraintracellulare | WP_014383685 | - - - | S- | N- | |
| Mycobacterium parasaoucoutense   | WP_083176607 | - - - | S- | N- | |
| Mycobacterium parasmense         | WP_085271229 | - - - | S- | N- | |
| Mycobacterium riyadhense         | WP_085258289 | - - - | S- | N- | |
| Mycobacterium saskatchewanense   | WP_085258286 | - - - | S- | N- | |
| Mycobacterium scrofulaceum       | WP_087279052 | - - - | S- | N- | |
| Mycobacterium shinjukuense       | WP_085488682 | - - - | S- | N- | |
| Mycobacterium szulgai            | WP_085670713 | - - - | S- | N- | |
| Mycobacterium talmoniae          | WP_071029146 | - - - | S- | N- | |
| Mycobacterium triplex             | CDK85758 | - - - | S- | N- | |
| Mycobacterium vulneris           | WP_085290696 | - - - | S- | N- | |

Figure S6. Partial sequence alignment of a conserved region of a ligase protein showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade and absent from most other Mycobacteriaceae.
### Mycobacteriaceae

| Species                          | Accession (GenBank) | Sequence | T-SAD-P-N | T-Y-D-S-P | T-Y-D-S-P | T-S-D-P-N | T-S-D-P-N | T-Y-D-S-P | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-S-D-P-N | T-Y-D-S-P | T-Y-D-S-P | T-S-D-P-N | T-S-D-P-N | T-Y-D-S-P | T-Y-D-S-P | T-S-D-P-N | T-S-D-P-N |
|----------------------------------|---------------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Mycobacterium tuberculosis       | AHZ31631            | CGLADDVLLEPVDTINAGFMKPLDGQ | GGS | WGPLGPLGGVNPVGFTPNGVPE |
| Mycobacterium africansum KBS    | EF041456            |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium africansum MAL020 | KBJ26945            |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium bovis             | WP_079293517        |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium bovis AF2122/97   | YP_009361185        |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium bovis BCG str. A | AHM9R9X75           |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium canetti           | WP_080624417        |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium caprae            | CEJ50030            |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium microti           | AMC6113             |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium mungi             | OAQ19140            |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium orygia            | EMT33993            |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium pinnipedi         | WP_107131691        |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium africanum         | KBF93339            |          |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |

#### "Tuberculosis" clade (14/14)

| Species                          | Accession (GenBank) | Sequence | T-SAD-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N | T-Y-D-S-P | T-S-D-P-N |
|----------------------------------|---------------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Mycobacterium bohemicum          | ORU95584            | A-       | S-        | T-        | P-        | P-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        |
| Mycobacterium heidelbergense    | WP_083075946        | A-       | S-        | A-        | PDN-      | P-        | T-        | D-        |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium saskatchewanense  | WP_085258360        | A-       | S-        | T-        | PPE-      | P-        | S-        | D-        |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Mycobacterium shigaense         | WP_096443647        | S-       | P-        | L-        | P-        | P-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        | T-        | S-        |

#### Other Mycobacteriaceae (5/100)

...
| Species                          | GenBank Accession | Sequence Alignment |
|---------------------------------|-------------------|--------------------|
| Mycobacterium peregrinum        | OBB24714          | A - - - - - - - -   |
| Mycobacterium persicum          | WP_06153541       | A - - - - - - - -   |
| Mycobacterium phlei             | WP_061541003      | A - - - - - - - -   |
| Mycobacterium porcinum          | WP_069247544      | P-T-LT-RA         |
| Mycobacterium pseudomoshottisi  | WP_085084433      | T-P                  |
| Mycobacterium rhodesiae         | WP_083112838      | A - - - - - - - -   |
| Mycobacterium riyadhense        | WP_085252439      | S - - - - - - - -   |
| Mycobacterium rufum             | KG171208          | A - - - - - - - -   |
| Mycobacterium scrofulaceum      | WP_065942839      | S - - - - - - - -   |
| Mycobacterium septicum          | WP_044513836      | D - - - - - - - -   |
| Mycobacterium setense           | WP_06876605       | D - - - - - - - -   |
| Mycobacterium shimoidei         | WP_068942839      | E - - - - - - - -   |
| Mycobacterium shinjukuense      | ORB70603          | P - - - - - - - -   |
| Mycobacterium smegmatis         | AAC45273          | E - - - - - - - -   |
| Mycobacterium sphagni           | WP_094824448      | A - - - - - - - -   |
| Mycobacterium szulgai           | WP_068034133      | P - - - - - - - -   |
| Mycobacterium talmoniae         | WP_070106600      | A - - - - - - - -   |
| Mycobacterium triplex           | CDO85942          | S - - - - - - - -   |
| Mycobacterium ulcerans          | WP_011742448      | A - - - - - - - -   |
| Mycobacterium vanbaalenii       | WP_011782749      | S - - - - - - - -   |
| Mycobacterium vulneris          | WP_085137219      | A - - - - - - - -   |
| Mycobacterium wolinskyi         | WP_085144786      | S - - - - - - - -   |
| Mycobacterium xenopi            | WP_085159664      | A - - - - - - - -   |
| Mycobacteroides abscessus       | WP_074254211      | A - - - - - - - -   |
| Mycolicibacillus trivialis      | WP_095109233      | A - - - - - - - -   |
| Mycolicibacter engbaekii        | WP_085126478      | N - - - - - - - -   |
| Mycolicibacter heraklionensis   | WP_074319107      | N - - - - - - - -   |
| Mycolicibacter hibernae         | WP_085134390      | N - - - - - - - -   |
| Mycolicibacter iodosumasiens    | WP_067977023      | N - - - - - - - -   |
| Mycolicibacter kumamotoensis    | WP_065287143      | Q - - - - - - - -   |
| Mycolicibacter minnesotaens     | WP_083027061      | N - - - - - - - -   |
| Mycolicibacter nonchromogenicus | WP_085137219      | N - - - - - - - -   |
| Mycolicibacter senuensis        | WP_085085361      | N - - - - - - - -   |
| Mycolicibacter sinensis         | WP_064854835      | N - - - - - - - -   |
| Mycolicibacter terrae           | WP_085260015      | Q - - - - - - - -   |
| Mycolicibacter agri             | WP_097940589      | N - - - - - - - -   |
| Mycolicibacter aromaticivorans  | WP_085341988      | A - - - - - - - -   |
| Mycolicibacter aurum            | WP_048630679      | S - - - - - - - -   |
| Mycolicibacter austroafricanum  | WP_105389140      | S - - - - - - - -   |
| Mycolicibacter bacteremicus     | WP_079750215      | M - - - - - - - -   |
| Mycolicibacter boenickei        | WP_077740669      | D - - - - - - - -   |
| Mycolicibacter brisbanense      | WP_062872111      | P - - - - - - - -   |
| Mycolicibacter canariasense     | GAS99419          | P - - - - - - - -   |
| Mycolicibacter chlorophenolicum | WP_048472898      | S - - - - - - - -   |
| Mycolicibacter chubuenense      | ORA44304          | S - - - - - - - -   |
| Mycolicibacter conceptionen     | WP_085141702      | D - - - - - - - -   |
| Mycolicibacter confluentis      | WP_085154488      | M - - - - - - - -   |
| Mycolicibacter cosmeticum       | WP_085399014      | P - - - - - - - -   |
| Mycolicibacter doricum          | WP_085119104      | S - - - - - - - -   |
| Mycolicibacter elephants        | WP_083043239      | P - - - - - - - -   |
| Mycolicibacter farcinogens      | WP_0830391028     | D - - - - - - - -   |
| Mycolicibacter flavescens       | WP_069146402      | M - - - - - - - -   |
| Mycolicibacter fortuitum        | WP_064977386      | D - - - - - - - -   |
| Mycolicibacter goodii           | WP_049743638      | S - - - - - - - -   |

Other Mycobacteriaceae (S>100)

Figure S7. Partial sequence alignment of a conserved region of the arabinosyltransferase protein showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade and absent from most other Mycobacteriaceae.
### Mycobacteriaceae

| Species                          | Protein ID          | Note                                      |
|---------------------------------|---------------------|-------------------------------------------|
| Mycobacterium tuberculosis      | AMC68818            | **“Tuberculosis” clade** (9/9)             |
| Mycobacterium bovis             | WP_099719446        |                                           |
| Mycobacterium canetti           | WP_015290613        |                                           |
| Mycobacterium caprae            | AP27937             |                                           |
| Mycobacterium microti           | AMC60048            | E                                         |
| Mycobacterium mungi             | CA17029             |                                           |
| Mycobacterium orygis            | EMJ3209             |                                           |
| Mycobacterium pinnipedi         | PHR9260             | E                                         |
| Mycobacterium africanum         | AMC64655            |                                           |
| Mycobacterium alsense           | WP_083140944        |                                            |
| Mycobacterium arosense          | WP_083062979        | I-A-A                                      |
| Mycobacterium asiaticum         | WP_036558699        | AD-I-VA                                   |
| Mycobacterium avium             | WP_003872521        | I-A-A                                      |
| Mycobacterium bohemicum         | WP_085182922        | I-A-A                                      |
| Mycobacterium colombiense        | WP_007770275        | I-A-A                                      |
| Mycobacterium europeum          | WP_085240324        | D-I-A                                      |
| Mycobacterium florentinum       | WP_085220630        | LD-I-VA                                   |
| Mycobacterium gastris           | WP_036417304        | L-I-VA                                    |
| Mycobacterium genavense         | WP_025735550        | LD-I-VA                                   |
| Mycobacterium gordonia          | WP_055581787        | P-V-ALA                                   |
| Mycobacterium haemophilum       | WP_047315084        | D-I-VA                                   |
| Mycobacterium helvella          | WP_003072097        |                                           |
| Mycobacterium interjectum       | WP_006914039        | D-I-A-V                                   |
| Mycobacterium intermedium       | WP_069420569        | ISAVA                                     |
| Mycobacterium intracellulare    | WP_009953083        | I-A-A                                      |
| Mycobacterium intracellulare A  | AFC54323            | I-A-A                                      |
| Mycobacterium intracellulare s  | AF513947            | I-A-A                                      |
| Mycobacterium kansasi           | WP_025365655        |                                           |
| Mycobacterium lacus             | WP_085161066        |                                           |
| Mycobacterium lentiflavum       | WP_090603912        |                                           |
| Mycobacterium lepra             | WP_010907847        | L-T-N-I-VA                                |
| Mycobacterium leprae 3125609    | Q49768              | L-T-N-I-VA                                |
| Mycobacterium lepraemurium      | AT28905             | I-A-A                                      |
| Mycobacterium lepromatosis      | KJX75569            |                                           |
| Mycobacterium liflandii         | WP_015356470        | S-I-IAA-A                                 |
| Mycobacterium malmöense         | WP_065473899        | D-I-A-A                                   |
| Mycobacterium mantenii          | WP_083095749        | T-I-A-A                                   |
| Mycobacterium marinum           | WP_020703034        | S-I-IAA-A                                 |
| Mycobacterium marsellense       | WP_083020480        | I-A-A                                      |
| Mycobacterium montefioriense    | WP_108925349        | I-V-A                                     |
| Mycobacterium nebraskense       | WP_046186167        | D-I-A-A                                   |
| Mycobacterium palustrum         | WP_085079994        | V-A-V-A                                   |
| Mycobacterium paraenae          | WP_085104172        |                                           |
| Mycobacterium paraffinicum      | WP_085473813        | D-I-A-A                                   |
| Mycobacterium parascholeusen    | WP_083169180        | D-I-A-A                                   |
| Mycobacterium parvum            | WP_085267524        | S-I-A-A                                   |
| Mycobacterium pseudohodshitti J | Q032467             | S-I-IAA-A                                 |
| Mycobacterium riadyphense       | WP_085252570        | D-I-IA-A                                  |
| Mycobacterium saskatchewanense  | WP_085254797        | I-A-A                                     |
| Mycobacterium scrofulaceum      | WP_085276355        | I-A-A                                     |
| Mycobacterium sherrissii        | WP_086639800        |                                           |
| Mycobacterium shigaense         | BAX93495            | I-V-A                                     |
| Mycobacterium sherrisii         | WP_083048336        |                                           |
| Mycobacterium simiae            | WP_061556276        | V-D-I-VA                                  |
| Mycobacterium szulgai           | WP_068156932        | I-AIA-A                                   |
| Mycobacterium triplex           | WP_036470251        | LD-I-VA                                   |
| Mycobacterium ulcerans          | WP_011743588        | S-I-I-IAA-A                               |
| Mycobacterium ulcerans str. Harvey | EUA87706          | S-I-I-IAA-A                               |
| Mycobacterium bovis             | WP_085291629        | I-A-A                                      |
| Mycobacterium xenopi            | WP_003921918        | D-I-IA-A                                  |
| Mycobacterium doricum           | ORV42540            |                                           |
| Mycobacterium elephantis       | WP_083043342        | V-K-C-I-A-A                               |

Figure S8. Partial sequence alignment of a conserved region of the GTPase Era protein showing a one amino acid insertion that is specific for members of the “Tuberculosis” clade.
Figure S9. Partial sequence alignment of a conserved region of the primosome assembly protein PriA showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
Figure S10. Partial sequence alignment of a conserved region of the phospho-sugar mutase / MRSA protein showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade and absent from most other Mycobacteriaceae.
**Figure S11.** Partial sequence alignment of a conserved region of the polyketide synthase Pks8 protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade.

| Organism                                      | Accession | Sequence |
|-----------------------------------------------|-----------|----------|
| Mycobacterium tuberculosis                    | AIH28258  | G-IDI----LSA--I---AP--L--VA---GA | 539 |
| Mycobacterium africanum MAL010                | KBQ21913  | G-IDI----LSA--I---AP--L--VA---GA | 586 |
| Mycobacterium bovis                           | WP_069253279 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium bovis AF2122/97                 | WP_009359027 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium bovis BCG                      | AMC50496  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium bovis BCG str. P               | CAL71688  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium caprae                          | WP_014000874 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium caprae                          | WP_075744516 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium microti                         | AMC59270  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium mungi                          | WP_064319984 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium orygis                         | WP_003408182 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium pinnipedii                     | WP_105826451 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium africanum                      | KBF66563  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium anglicum                       | WP_083114276 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium avium                         | WP_062886498 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium avium subsp. avium            | EJ066797  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium avium subsp. hominissuis       | WP_058234253 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium avium subsp. paratuberculosis  | ANH28144  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium avium subsp. silvaticus         | ETLB0015  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium colombiense                    | WP_065051421 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium colombiense CECT               | EUA39396  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium haemophilum                    | WP_085607575 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium haemophilum DSM                | ALL56345  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium heidelbergense                 | ORA66842  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium interjectum                    | WP_085200572 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium kansasii                       | WP_063477072 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium kubicae                        | ORRO3439  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium lacus                          | WP_085157733 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium lentiflavum                    | WP_090601745 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium lepraemurium                   | ATA28778  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium malmoense                      | OCB32301  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium marinum                        | WP_103759350 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium palustre                       | WP_085080838 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium parascrofulaceum               | EFG79593  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium pseudohottii                   | WP_106407457 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium pseudohottii j                 | GAG40085  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium riyadhense                     | WP_085249718 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium riyadhense                     | WP_085249719 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium saskatchewanense               | WP_085256883 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium scrofulaceum                   | WP_083175478 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium shigaense                      | WP_096497160 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium shinjukuense                   | WP_083052204 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium szulgai                        | OFS21827  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium timonense                      | WP_083187138 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium triplex                        | WP_036468983 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium xenopi                         | WP_085196881 | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium xenopi 3993                    | EUA44472  | G-IDI----LSA--I---AP--L--VA---GA |
| Mycobacterium xenopi 4042                    | EUA42231  | G-IDI----LSA--I---AP--L--VA---GA |

**“Tuberculosis” clade (13/13)**

**Other Mycobacteraceae (0/36)**
Figure S12. Partial sequence alignment of a conserved region of the Glutamine-dependent NAD(+) synthetase protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade.
Figure S13. Partial sequence alignment of a conserved region of the ribonuclease E protein showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
Figure S14. Partial sequence alignment of a conserved region of the folylpolyglutamate synthase protein FOLC showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | AAC44599 | 392 | 440 |
|---------------------------|-----------|-----|-----|
| Mycobacterium bovis       | WP_008659562 |
| Mycobacterium bovis AF2122/97 | YP_009361030 |
| Mycobacterium bovis BCG   | CU113679  |
| Mycobacterium bovis BCG str. P | CAL73693  |
| Mycobacterium canetti     | WP_015291611 |
| Mycobacterium caprae      | APD73261  |
| Mycobacterium microtuberculosis | PRT06602 |
| Mycobacterium mungi       | OA017167  |
| Mycobacterium oryxis      | WP_003419640 |
| Mycobacterium pinneapelli | PRH15860  |
| Mycobacterium africanum  | OGC87722  |
| Mycobacterium alsense     | WP_083138393 |
| Mycobacterium anglicum    | WP_083115815 |
| Mycobacterium arosiense   | WP_083066595 |
| Mycobacterium asiaticum   | WP_065035927 |
| Mycobacterium avium       | WP_084024827 |
| Mycobacterium avium subsp. avium | EUA25067 |
| Mycobacterium bohemicum   | WP_085182349 |
| Mycobacterium branderi    | WP_083131194 |
| Mycobacterium celatum     | WP_065851537 |
| Mycobacterium chimaera    | WP_095662509 |
| Mycobacterium colombiense | WP_064885253 |
| Mycobacterium colombiense CECT | EJO89075 |
| Mycobacterium conspicum   | WP_085232731 |
| Mycobacterium europeum    | WP_085241416 |
| Mycobacterium florentinum | WP_085231811 |
| Mycobacterium fragae      | WP_085200167 |
| Mycobacterium gastri      | WP_036411215 |
| Mycobacterium gastri 'Wayne' | ETW25367 |
| Mycobacterium genavense   | WP_025736321 |
| Mycobacterium gordanae    | WP_055576781 |
| Mycobacterium haemophilum | WP_047316417 |
| Mycobacterium hassiacum   | WP_018354201 |
| Mycobacterium hassiacum DSM 44 | EKF22101 |
| Mycobacterium heckeshornense | WP_01700308 |
| Mycobacterium heidelbergense | WP_083073758 |
| Mycobacterium holsaticum  | WP_069404806 |
| Mycobacterium insubricum  | WP_083029258 |
| Mycobacterium interjectum | WP_066916583 |
| Mycobacterium intermedium | WP_069419406 |
| Mycobacterium intracellularre | WP_064936671 |
| Mycobacterium intracellularre 1 | EUA07523 |
| Mycobacterium kansaii     | WP_063468661 |
| Mycobacterium kansaii 732 | EUA05593 |
| Mycobacterium kansaii 824 | ETZ96908 |
| Mycobacterium kubicae     | WP_085074662 |
| Mycobacterium kubicae aniporinense | WP_085074674 |
| Mycobacterium lacus       | WP_085158498 |
| Mycobacterium lentiflavum | WP_090607446 |
| Mycobacterium leprae      | WP_010907592 |
| Mycobacterium leprae 3125609 | OAR19947 |
| Mycobacterium lepraeum     | ATA27570 |
| Mycobacterium lepromatosis | KJX75809 |
| Mycobacterium liflandii   | WP_015357483 |
| Mycobacterium malmoense   | WP_065445873 |
| Mycobacterium mantennii   | WP_083098967 |
| Mycobacterium marium      | WP_094360861 |
| Mycobacterium marium str. Europe | EP97053 |
| Mycobacterium marseillense | WP_083019068 |
| Mycobacterium montefiorense | WP_108920271 |
| Mycobacterium nebraskense  | WP_046186104 |
| Mycobacterium novomagense  | WP_083008979 |
| Mycobacterium obuense      | WP_046364506 |
| Mycobacterium palustre     | WP_085079430 |
| Mycobacterium paraense    | WP_085103368 |
| Mycobacterium paraffinicum | WP_073871481 |
| Mycobacterium paraintracellularre | WP_014383732 |
| Mycobacterium paratuberculosis | WP_083178956 |
| Mycobacterium paraseoulense | WP_083178956 |
| Mycobacterium parmesi       | WP_085267343 |
| Mycobacterium persici       | WP_083156050 |
| Mycobacterium pseudohottini | WP_080684796 |

**"Tuberculosis" clade** (12/12)

**Other Mycobacteriaceae (0>100)**
| Mycobacterium pseudoshottsii J GAQ32284 | GAQ32284 | E- |
|-------------------------------|----------------------|----|
| Mycobacterium rhodesiae WP_085120803 | WP_085120803 | -DV- | G-LH-- | T- | E- | - |
| Mycobacterium riyadhense WP_085252094 | WP_085252094 | -N- | E- | V- |
| Mycobacterium saskatchewanense WP_085255252 | WP_085255252 | DA- | - | E- |
| Mycobacterium scrofulaceum WP_067270659 | WP_067270659 | A- | - | E- |
| Mycobacterium sherrisii WP_069400778 | WP_069400778 | - | E- |
| Mycobacterium shigaense BAX04748 | BAX04748 | N- | - | E- |
| Mycobacterium shinjukuense WP_083051286 | WP_083051286 | - | E- |
| Mycobacterium simiae WP_061557414 | WP_061557414 | - | E- |
| Mycobacterium sphagni WP_094479679 | WP_094479679 | D- | G-LH- | N- | E- |
| Mycobacterium szulgai WP_068023088 | WP_068023088 | D- | G-LH- | N- | E- |
| Mycobacterium talmoniae WP_105368780 | WP_105368780 | - | E- |
| Mycobacterium triplex WP_036471856 | WP_036471856 | - | E- |
| Mycobacterium tusciae WP_006243846 | WP_006243846 | DV-S- | GQLHAQ-T | E- |
| Mycobacterium ulcerans WP_096368565 | WP_096368565 | - | E- |
| Mycobacterium ulcerans str. Harvey EUA6576 | EUA6576 | - | E- |
| Mycobacterium vulneris WP_085290802 | WP_085290802 | E- | I- |
| Mycobacterium xenopi WP_039890612 | WP_039890612 | - | E- |
| Mycobacterium xenopi 3993 EUA24298 | EUA24298 | - | E- |
| Mycobacterium xenopi 4042 EUA23439 | EUA23439 | - | E- |
| Mycobacterium xenopi RIVM70036 EID12255 | EID12255 | - | E- |
| Mycobacteroides abscessus WP_062878555 | WP_062878555 | K- | GQLHSS- | N- | E- |
| Mycobacteroides abscessus 1948 EUA64294 | EUA64294 | K- | GQLHSS- | N- | E- |
| Mycobacteroides abscessus MAB_ ETZ87592 | ETZ87592 | K- | GQLHSS- | N- | E- |
| Mycobacteroides abscessus MAB_ ETZ94722 | ETZ94722 | K- | GQLHSS- | N- | E- |
| Mycobacteroides abscessus subsp. abscessus SIL00731 | SIL00731 | K- | GQLHSS- | N- | E- |
| Mycobacteroides immunogenum WP_064630663 | WP_064630663 | K- | GQLHSS- | N- | E- |
| Mycobacteroides saenagens WP_084413958 | WP_084413958 | K- | GQLHSS- | N- | E- |
| Mycolicibacillus koreensis OSC33707 | OSC33707 | D- | G- | S- |
| Mycolicibacillus trivialis WP_085110452 | WP_085110452 | D- | N- | E- |
| Mycolicibacter algericus WP_083040190 | WP_083040190 | - | N- | GS- | E- |
| Mycolicibacter arupensis WP_046189722 | WP_046189722 | - | N- | GS- | E- |
| Mycolicibacter engbaekii WP_085128725 | WP_085128725 | - | N- | GS- | E- |
| Mycolicibacter heraklionensis Q768482 | Q768482 | - | N- | GS- | E- |
| Mycolicibacter hiberniae WP_085134075 | WP_085134075 | - | N- | GS- | E- |
| Mycolicibacter icosiumassiliensis WP_067976690 | WP_067976690 | - | N- | GS- | E- |
| Mycolicibacter kumamotonensis WP_085289399 | WP_085289399 | - | N- | GS- | E- |
| Mycolicibacter longobardus WP_085265466 | WP_085265466 | - | N- | GS- | E- |
| Mycolicibacter minnesotensis WP_083026078 | WP_083026078 | - | N- | GS- | E- |
| Mycolicibacter nonchromogenicus WP_085138940 | WP_085138940 | - | N- | GS- | E- |
| Mycolicibacter senuensis ORW6399 | ORW6399 | - | N- | GS- | E- |
| Mycolicibacter senuensis ORW6399 | ORW6399 | - | N- | GS- | E- |
| Mycolicibacter terreus WP_085260311 | WP_085260311 | - | N- | GS- | E- |
| Mycolicibacterium agri WP_079376117 | WP_079376117 | DV- | GQLHS- | T- | E- |
| Mycolicibacterium aromaticivorans WP_036341814 | WP_036341814 | DV- | G-LH- | T- | E- |
| Mycolicibacterium brunae WP_090588990 | WP_090588990 | S-D- | - | S- | EH- |
| Mycolicibacterium celerflavum WP_083000270 | WP_083000270 | DV-S- | GQLHDO- | T- | E- |
| Mycolicibacterium confuentis WP_085152370 | WP_085152370 | N- | E- |
| Mycolicibacterium duvalii WP_098006287 | WP_098006287 | DV-S- | GQLHSD- | N- | E- |
| Mycolicibacterium fallax WP_085094967 | WP_085094967 | D- | EN- | E- |

Figure S15. Partial sequence alignment of a conserved region of the DNA topoisomerase I protein showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
"Tuberculosis" clade (16/16)

Other Mycobacteriaceae (0/75)

Figure S16. Partial sequence alignment of a conserved region of the metal cation transporting ATPase H protein showing a one amino acid deletion that is specific for members of the "Tuberculosis" clade.
| Mycobacterium tuberculosis | AIH35166 | IWSMSQGFQFYLAFLLLVAGGCYLLRRFL | APYLRMFVULLSTLTLASFYIAVA |
|--------------------------|----------|---------------------------------|--------------------------|
| Mycobacterium bovis      | PHD33000 |                                 |                          |
| Mycobacterium bovis BCG  | AMC50386 |                                 |                          |
| Mycobacterium canetti    | WP_015289972 |                         | G--                     |
| Mycobacterium caprae     | APU25655 |                                 |                          |
| Mycobacterium microti    | AMC59181 |                                 |                          |
| Mycobacterium mungi      | GAQ19790 |                                 |                          |
| Mycobacterium orygis     |          |                                 |                          |
| Mycobacterium pinnipedii | PRH92610 |                                 |                          |
| Mycobacterium africanum  | WP_031670464 |                 | A--                     |
| Mycobacterium shigaense  | WP_096439561 | -IG--M--SO--SRK--H--G--I--QA--V--T--A- |
| Mycobacterium triplex    | CD98416  | -V--I--V--OG--G--L--L--G--V--V--F- |
| Mycobacterium asiaticum  | WP_065034935 | F--S--AVL--GKH--F--T--V--V--F- |
| Mycobacterium asiaticum  | WP_065144193 | -IG--P--G--KH--V--V--V--G--I- |
| Mycobacterium conceptionense | CQD14527 | -IS-F-F-G--GRH--A-I--G-F- |
| Mycobacterium europeum   | WP_085238532 | -V--I--F--L--GAR-L-L-LA-TA-V-V-V-- |
| Mycobacterium florentinum | WP_085225617 | -V-FF-PL--KAH-L-L-I-A-V-V-V-F- |
| Mycobacterium genavense  | WP_025737696 | -V--I--F--PL--KAH-L-L-GA-V-V-V-F- |
| Mycobacterium gordonae   | WP_055576247 | -VI-AV--KPL--GRH--V--A-V-V-V-I- |
| Mycobacterium haemophilum| WP_047313904 | -I--I--F--PL--GTO--A-GA-I-G-F- |
| Mycobacterium heidelbergense | WP_083074796 | -IG--A--F--PL--GAH-L-L-LA-V-V-V-V |
| Mycobacterium interjectum| WP_085204075 | -V--I--AV--GRR--AH-VA-V-V-V- |
| Mycobacterium kansasi    | WP_075542856 | -F--A-VF--PL--G-R-L-L-A-I-G-A |
| Mycobacterium laeus      | WP_085160830 | -F-VSL--SH-T-A-T-V-V-GF- |
| Mycobacterium lentiflavum| CQD13241 | -V--V-VF-L-KTR-L-L-A-I-V-V-F- |
| Mycobacterium lifidii    | WP_015356016 | -IT-TF-F-L--GTH--A-I-V-V-I- |
| Mycobacterium malmoense  | WP_071509519 | -VG--AF--RL--STH--M-A-V-V-V- |
| Mycobacterium malmoense  | WP_083011239 | -VG--AF--RL--STH--M-A-V-V-V- |
| Mycobacterium marinum    | BBG6536  | -I--TF-F-L--GTH--A-I-V-V-I- |
| Mycobacterium paraaffinicum | WP_073877114 | -VG--I--A--FG-PP--G-R-L-L-LA-TA-V-V-V- |
| Mycobacterium paraseoulense | WP_083171883 | -VG--I--F--PL--GAR-L-L-LA-TA-V-V-V- |
| Mycobacterium pseudoshottsii J | GAQ31936 | -IT-TF-F-L--GTH--A-I-V-V-I- |
| Mycobacterium scrofulaceum | WP_067277933 | -VG--VI-A--FG-PL--G-R-L-L-LA-TA-V-V-V- |
| Mycobacterium shinjukenense | WP_083046018 | -A--TA--K-PL--GOD-F--A-V-LC-V- |
| Mycobacterium szulgai     | WP_085673397 | -V--F-I--FKSL--GKH-L-L-G-V-V-VI |
| Mycobacterium ulcerans    | WP_011739687 | -I--TF-F-L--GTH--A-I-V-V-I- |
| Mycobacterium ulcerans str. Harvey EUA90900 | -I--TF-F-L--GTH--A-I-V-V-I- |
| Mycolicibacter longilabulosus | WP_085264210 | -S--IF--FF-PL--RAH-A--TS-V-V-W- |
| Mycolicibacter conceptionense | WP_064899707 | -IS-F-F-G--GRH-A-I-A-I-V-V-I- |
| Mycolicibacter farcinogenes | WP_036387553 | -IS-F-F-G--GRH-A-I-A-I-V-V-I- |

Figure S17. Partial sequence alignment of a conserved region of an acyltransferase protein showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade and absent from most other Mycobacteriaceae.
**Figure S18.** Partial sequence alignment of a conserved region of an alpha-amylase protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium属 | 科学名称 | 可能的氨基酸序列 | 备注 |
|----------------|-----------|-----------------|------|
| tuberculosis   | AIAH3349  | --A--V--E--R--G--I--T--E--A--T--G--D--I | |
| africanum      | WP_105814941 | --T-- | |
| africanaum MALO20 | KB164038  | --T-- | |
| bovis          | WP_024457939 | --T-- | |
| bovis AF2122/97 | WP_00938254 | --T-- | |
| bovis BC05 | AMC49570 | --T-- | |
| canetti        | WP_007004490 | --T-- | |
| microti        | AMC58426 | --T-- | |
| orygi          | WP_003404667 | --T-- | |
| alsense        | WP_008139541 | --A-- | |
| angelicium     | WP_008114869 | --T-- | |
| aquatitcum     | WP_008165026 | --A-- | |
| arosiensense   | WP_008306147 | --A-- | |
| asiaticum      | OB195477 | --D-- | |
| avium          | WP_076220893 | --T-- | |
| bohemicum      | ORV03997 | --T-- | |
| branderi       | WP_008131341 | --T-- | |
| celatum        | WP_006254115 | --Y-- | |
| chimaera       | WP_008195362 | --S-- | |
| colombiense     | WP_006495377 | --T-- | |
| colombiense CECT | EJ06535 | --T-- | |
| europaeum      | WP_008154360 | --E-- | |
| florentium     | WP_008652190 | --I-- | |
| fragae         | WP_008514791 | --T-- | |
| gordonae       | WP_008654989 | --T-- | |
| haemophilum     | WP_005487943 | --T-- | |
| haeumophili     | WP_002511295 | --T-- | |
| heidelbergense  | ORA70482  | --I-- | |
| houstonense    | WP_006698489 | --Y-- | |
| insubricum     | WP_008303151 | --L-- | |
| interjectum    | WP_008691555 | --L-- | |
| intermediate    | WP_008691206 | --V-- | |
| intracellular   | WP_008649292 | --I-- | |
| iranica        | WP_006428698 | --I-- | |
| kansasii       | WP_008347294 | --T-- | |
| komiminense    | WP_009027484 | --T-- | |
| kubicae        | WP_008507677 | --T-- | |
| kyorinense     | WP_004537479 | --T-- | |
| lacus          | WP_008515615 | --I-- | |
| lehmannii      | WP_0084884 | --T-- | |
| lentiflava      | WP_008906299 | --T-- | |
| mageritense    | WP_008643368 | --Y-- | |
| malmoense      | WP_008654426 | --T-- | |
| marium         | WP_009435847 | --T-- | |
| marseillense   | WP_008301179 | --I-- | |
| microti OVS94 | PLV4775 | --T-- | |
| montereinse    | WP_008541545 | --T-- | |
| moriokeana     | WP_008314922 | --A-- | |
| nebraskanse    | WP_004618483 | --I-- | |
| neoaurum       | CD045349 | --T-- | |
| novomagense    | WP_008308666 | --T-- | |
| novocastrense   | WP_008437769 | --T-- | |
| palustre       | WP_008507869 | --T-- | |
| paraense       | WP_008509340 | --T-- | |
| paraffinicum   | WP_007387068 | --T-- | |
| parafortuitum  | WP_008314480 | --T-- | |
| paraschoeflaceum | EF075349 | --T-- | |
| parasitaense   | WP_008526704 | --L-- | |
| peregrinum     | WP_006493613 | --I-- | |
| phlei          | WP_004635217 | --T-- | |
| rhodesiae      | WP_012411052 | --L-- | |
| rutilum        | WP_008341574 | --C-- | |
| saskatchewanense | WP_008525605 | --T-- | |
| scrofulaceum   | WP_008527047 | --T-- | |
| septicum       | WP_004520285 | --T-- | |
| setense        | WP_009524873 | --I-- | |
| sherrillii     | WP_008498415 | --T-- | |
| shigaense       | WP_009442556 | --I-- | |
| shimoidei      | WP_006936757 | --I-- | |
| shinjukuense   | WP_008350187 | --T-- | |

“Tuberculosis” clade (9/9)

Other Mycobacteriaceae (0/>100)
Figure S19. Partial sequence alignment of a conserved region of the hypothetical protein IQ48_14915 showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | AUS50012 | GGRISGRALIRNDRWLAHRLK | NQK | DGKVFEFDNLAPILATHQTVN |
|-----------------------------|---------|------------------------|-----|------------------------|
| Mycobacterium bovis | AMC54246 | - | - | - |
| Mycobacterium canetti | WP_044096082 | - | - | - |
| Mycobacterium caprae | WP_075744483 | - | - | - |
| Mycobacterium microti | PR06705 | - | - | - |
| Mycobacterium mungi | OA018148 | - | - | - |
| Mycobacterium orygis | WP_003404798 | - | - | - |
| Mycobacterium pinnipedii | PRH89837 | - | - | - |
| Mycobacterium africanum | CCC26019 | - | - | - |
| Mycobacterium bohemicum | WP_085183503 | - | - | - |
| Mycobacterium chimaera | WP_089151376 | - | - | - |
| Mycobacterium colombiense | WP_007770353 | - | - | - |
| Mycobacterium florentinum | WP_085219845 | - | - | - |
| Mycobacterium fragae | WP_085290595 | - | - | - |
| Mycobacterium genavense | WP_085290595 | - | - | - |
| Mycobacterium gordonae | WP_085290595 | - | - | - |
| Mycobacterium intracellulare | WP_014379267 | - | - | - |
| Mycobacterium intracellulare 1 | EUA57633 | - | - | - |
| Mycobacterium kansaii | WP_063472356 | - | - | - |
| Mycobacterium kubicae | WP_080513036 | - | - | - |
| Mycobacterium lacus | WP_085161692 | - | - | - |
| Mycobacterium lentiflavum | WP_090606106 | - | - | - |
| Mycobacterium mantenii | WP_080301932 | - | - | - |
| Mycobacterium marinum | WP_014379267 | - | - | - |
| Mycobacterium marinum MB2 | EPQ76271 | - | - | - |
| Mycobacterium marseilense | WP_080301932 | - | - | - |
| Mycobacterium montefioriense | WP_108925544 | - | - | - |
| Mycobacterium paraense | WP_080509884 | - | - | - |
| Mycobacterium riyadhense | WP_085253074 | - | - | - |
| Mycobacterium sherrisii | WP_069398486 | - | - | - |
| Mycobacterium shinjukuense | WP_083050335 | - | - | - |
| Mycobacterium szulgai | WP_083050335 | - | - | - |
| Mycobacterium ulcerans str. Harvey | EUA66423 | - | - | - |
| Mycobacterium ulcerans | WP_085289747 | - | - | - |

**Figure S20.** Partial sequence alignment of a conserved region of the hypothetical protein CAB90_01059 showing a three amino acid insertion that is specific for members of the "Tuberculosis" clade and is absent from most other Mycobacteriaceae.
| Mycobacterium tuberculosi | A1H41577 | VAGLAEVNPVARVDRHLSERHPII | GOVTSLAEARTTYLDEIVTLVGD |  
| Mycobacterium bovis | ESK76721 |  |  |  
| Mycobacterium canetti | WP_014000651 |  |  |  
| Mycobacterium caprae | CEJ50979 |  |  |  
| Mycobacterium microti | WP_105799522 |  |  |  
| Mycobacterium mungi | OA17758 |  |  |  
| Mycobacterium orygis | EMT36547 |  |  |  
| Mycobacterium pinnipedi | PRH92776 |  |  |  
| Mycobacterium africanum | WP_080701602 |  |  |  
| Mycobacterium alsense | WP_083139834 | -P-P-QFAAV-A----D---V-R--IT-D---A- |  |  
| Mycobacterium sphagni | WP_094475828 | I-G-AG-R-I---FD-Q---T---M-AAA |  |  
| Mycobacterium angelicum | WP_083116116 | -G-G-A---D---A---S---V--- |  |  
| Mycobacterium arosiense | WP_083065078 | I-G-AA---D---F---I-G |  |  
| Mycobacterium asiaticum | WP_065144441 | -T---A-A---L---D---G---VA---A-SA |  |  
| Mycobacterium avium | WP_003875481 | -P-SA-AA-A---D---LS---F---R |  |  
| Mycobacterium avium subsp. hominisuis | PBJ38167 | -P-SA-AA-A---D---LS |  |  
| Mycobacterium bohemicum | WP_085160361 | -P-G-AT-A---D---LS-E-EP-S---A- |  |  
| Mycobacterium chimera | WP_089151443 | -TQQ-A---D---S-F---A-V---I-G |  |  
| Mycobacterium colombiense | WP_044487032 | -GIAA-I---D---S-F---G |  |  
| Mycobacterium florentinum | WP_085521266 | -GIAAT-S---D---S-I---TI--- |  |  
| Mycobacterium genavense | WP_025735304 | -GIAE-S---D---VS-I---TI--- |  |  
| Mycobacterium gordonae | WP_055577966 | -H---A-AA-A---D---VS-F---R---I-A--- |  |  
| Mycobacterium haemophilum | WP_094880891 | I-SG-AA---D-VS-I-D-VA-I |  |  
| Mycobacterium interjectum | WP_066914857 | -AA-T---D---VS-I-T---V-A--- |  |  
| Mycobacterium kansasii | WP_036400803 | -RGAAA-T---G---D---V---SI---V---A---H |  |  
| Mycobacterium lacaus | WP_085162108 | -AA-T---D---VS-I-E---A--- |  |  
| Mycobacterium lentiflavum | CQD19472 | -G-AA-S---D---VS-I---TI-A--- |  |  
| Mycobacterium leprae | ATA29984 | -P-SG-AA-A---D---LS-F---RIE |  |  
| Mycobacterium leiflandii | ACG3997 | -DGLAS-D---S---I---R---N- |  |  
| Mycobacterium marinum | WP_103653964 | -DGLAS-I---D---S---I---R---N- |  |  
| Mycobacterium monterlorense | WP_108921694 | -G-AA-S---D---S---I---S- |  |  
| Mycobacterium palustre | WP_085076186 | L-T-AAA-S---D---LS-I---A-RA |  |  
| Mycobacterium paraenae | WP_085096875 | -AV-A---D---VA-I-R-T---A--- |  |  
| Mycobacterium paraaffinicum | WP_07386057 | -GIA-A-L---D---A-A---S-I-P--- |  |  
| Mycobacterium parmensis | WP_085269300 | -G-AD-A---D---G-VS-I-E---S- |  |  
| Mycobacterium pseudohottisilis | WP_086085855 | -DGLAS---D---S---I---R---N- |  |  
| Mycobacterium sherrisi | WP_069399301 | -TG-AA-A---D---A---VS-I-R---G-I-S--- |  |  
| Mycobacterium shinjukuensis | WP_085050757 | -GIAA-G---D---D---L---H- |  |  
| Mycobacterium simiae | WP_083050757 | -SA-EH-I---D---SS-F-R-D---S-AA--- |  |  
| Mycobacterium szulgai | WP_085159784 | AD-G-AA-A---D---A-VA-I-R---I-S--- |  |  
| Mycobacterium timonense | WP_085673230 | I---G-AA-S---D---A---VS-I-R---A-S--- |  |  
| Mycobacterium timonense | WP_083187408 | -P-SG-AA-A---D---LSF---R--- |  |  
| Mycobacterium triplic | CDD09089 | -GIAE-N---D---Q---VS-I---TI--- |  |  
| Mycobacterium ulcers | WP_011739179 | -DGLAS---D---S---I---R---N- |  |  
| Mycobacterium ulcers str. Harvey | EUA91996 | -DGLAS---D---S---I---R---N- |  |  
| Mycobacterium vulneris | WP_085292389 | -GIAA-I---D---S---F---G---IH |  |  
| Mycobacterium xenopi | WP_003921962 | -G-AAA-A---DT---HV-V---V---G-SA--- |  |  

Figure S21. Partial sequence alignment of a conserved region of the transcriptional regulator protein showing one amino acid deletion that is specific for members of the “Tuberculosis” clade and is absent from most other Mycobacteriaceae.
| Mycobacterium tuberculosis     | AIH59242 | TPRERLTRGLAYSAGPVDTRGL | ELEG | VGLGLOSARSTAAGLRRRYREG |
| Mycobacterium bovis            | PHP04760 |                      |       |                        |
| Mycobacterium canetti          | WP_0859696 |             |       |                        |
| Mycobacterium caprae           | WP_003400307 |         |       |                        |
| Mycobacterium microti          | AM57439 |             |       |                        |
| Mycobacterium mungi            | OAA18474 |             |       |                        |
| Mycobacterium orygis           | EM73776 |             |       |                        |
| Mycobacterium pinnipedi        | WP131299  |             |       |                        |
| Mycobacterium africanum        | WP_03169836 |         |       |                        |
| Mycobacterium alsense          | WP_083140941 | -S-A-T-T |       | -Q-S-Q-Q-            |
| Mycobacterium angelicum        | WP_083112583 | -Q-T-V |       | -SV-H-SE-            |
| Mycobacterium arosiene         | WP_083064829 | -Q-A-S-O-L-V |       | -SV-R-Q-Q-            |
| Mycobacterium asiaticum        | WP_083063279 | -T-V |       | -SV-R-Q-Q-            |
| Mycobacterium avium            | WP_003872095 | -A-T-T-L-L-V | A-SV-E |                      |
| Mycobacterium bohemicum        | WP_085179971 | -O-A-T-T-V |       | -AK-Q-O-Q-            |
| Mycobacterium branderi         | WP_083132206 | -O-T-V |       | -SV-GQ-SN-GQ-          |
| Mycobacterium celatum          | WP_062593884 | -O-T-A |       | -SV-GQ-SS-            |
| Mycobacterium chimaera         | WP_08691285 | -A-T-TR |       | -SV-Q-E-            |
| Mycobacterium colombiense      | WP_087771619 | -Q-A-S-O-V |       | SV-Q-SQ-Q-            |
| Mycobacterium conspicuum       | WP_085233853 | S-O-T-V |       | -SV-VQ-SQ-Q-          |
| Mycobacterium europeum         | WP_085239298 | -A-T-T-L-V |       | -V-QA-SQV-KQ-          |
| Mycobacterium florentinum      | WP_085219492 | -O-S-T-L-V | I- AH-VQ-SE-Q |          |
| Mycobacterium fragae           | WP_085199183 | -Q-S-T-V |       | -VD-GQ-N-            |
| Mycobacterium gastri           | WP_085148686 | -V-T-V |       | -SV-Q-SQ-            |
| Mycobacterium genavense        | WP_025738159 | -Q-S-V | I- ADTQAG-E | -Q-            |
| Mycobacterium gordonae         | WP_055578116 | -S-V-T |       | -V-H-EV-            |
| Mycobacterium grossiae         | WP_070351157 | -Q-A-T-L-A |       | -V-GS-WD-S            |
| Mycobacterium haemophilum      | WP_047316866 | A-T-L-V |       | -SV-HS-            |
| Mycobacterium heidelbergense   | WP_083071207 | -O-A-T-V |       | -VHQ-SQH-            |
| Mycobacterium interjectum      | WP_065201846 | -Q-O-T-L-V |       | -SVQ-GAE-            |
| Mycobacterium intermedium      | WP_069421255 | -O-T-V |       | -N-OQAE-H-            |
| Mycobacterium intracellulare   | WP_083457838 | S-A-T-R-V |       | SV-Q-SQ-Q-            |
| Mycobacterium kansassi         | OOK7960 | -S-V-T |       | -V-MQ-Q-            |
| Mycobacterium kubiceae         | WP_085703260 | -T-V |       | -SVQ-SE-            |
| Mycobacterium kyorinense       | WP_065014543 | -Q-N-T-V |       | E-VH-GAQSS-            |
| Mycobacterium lacus            | WP_085163121 | -T-T-V |       | VQ-SQ-            |
| Mycobacterium lentiflavum      | WP_090559796 | -Q-S-S-I-A-A | I-AH-VQ-SE | H |          |
| Mycobacterium lepraemurium     | ATH2710 | A-T-T-L-L-V | A-SV-E |                      |
| Mycobacterium liflandii        | WP_01533887 | -T-V |       | -VH-Q-S-E-            |
| Mycobacterium malmoense        | WP_065444773 | -A-T-T-V |       | -VQ-SQKI-KQ-          |
| Mycobacterium manteni          | WP_083095116 | -O-T-Q-V |       | -V-SQ-SQ-E-            |
| Mycobacterium marismun         | WP_012392018 | -THT-V |       | -SVH-SQ-            |
| Mycobacterium marseilense      | WP_083015749 | -A-T-R-L-V | I-SVE-O-E- |            |
| Mycobacterium montefiorum      | WP_109554672 | -O-S-T-V |       | I-VNTO-QE-            |
| Mycobacterium nebraskense      | KKC05547 | -A-T-T-L-V |       | -VQ-SQKI-KQ-          |
| Mycobacterium noviomagnae      | WP_083080821 | -Q-T-T-V |       | HAG-NV-            |
| Mycobacterium palustrum        | WP_085081011 | -A-NTWV-A | L-V-QA-SE- |          |
| Mycobacterium parahaemochlorum | WP_085243879 | -A-T-T-V |       | -SVQNG-LE-            |
| Mycobacterium parafarzinicum   | WP_085876918 | -A-T-T-L-L-V | A-SV-E |                      |
| Mycobacterium paraclinacellare | WP_041787282 | -A-T-T-V |       | -SVQ-Q-            |
| Mycobacterium paraseudolongum  | WP_083171009 | -T-T-A |       | -VQ-QO-QK-            |
| Mycobacterium parvum           | WP_085270406 | S-A-HA-W | I-ANQAGSO-Q |          |
| Mycobacterium persicum         | WP_083154593 | -T-M-V |       | -VQ-SE-            |
| Mycobacterium riysadhense      | WP_085229991 | -T-I-V |       | -V-H-SQ-            |
| Mycobacterium saskatchewanense | WP_085254176 | -O-V-V- |       | -M-VQ-QE-            |
| Mycobacterium scrofulaceum     | WP_067279752 | S-A-T-T-A-V |       | -A-OQ-SQKI-QK-        |
| Mycobacterium sherrisii        | WP_069401007 | -O-S-T-L-V |       | VDO-Q-SQ-            |
| Mycobacterium shigaense        | WP_096436023 | -Q-S-T-V |       | E-VNQDQ-ER-            |
| Mycobacterium shimpoi         | WP_083493830 | -S-T-V |       | VQAQSS-            |
| Mycobacterium shinjukuense     | WP_085014922 | -T-V |       | VQAQSS-            |
| Mycobacterium smegmatis        | WP_036452144 | -Q-K-T-I-V |       | LIAQTOE-ES-            |
| Mycobacterium szulgai          | WP_085672027 | -T-V |       | SVH-SE-            |
| Mycobacterium talonae          | WP_071207868 | -O-S-T-T-I-V |       | -AHAG-SK-            |
| Mycobacterium triplex          | WP_034653571 | -O-S-V |       | -IAVQAOER-            |
| Mycobacterium ulcerans         | WP_08526415 | -T-V |       | -DHGQ-SE-            |
| Mycobacterium vulneris         | WP_085288267 | -O-A-T-Q-V |       | -SVQ-QSKQ-            |
| Mycobacterium wolinskyi        | WP_067849849 | -HO-S-K-T-I-V |       | NAQ-N-E-            |
| Mycobacterium xenopi           | WP_003919828 | -O-T-T-V |       | HTAQ-NH-            |
| Mycobacterium xilicalculus koreensis | WP_08504210 | -O-T-L-V |       | HTAQ-GT-Q-            |
| Mycobacterium xilicalculus trivalis | WP_06931643 | -O-T-L-V |       | HTAQ-GT-GQ-            |
| Mycobacterium xilicalculus dorimic | WP_08512523 | -S-O-A-K-T-L-V | L-VA-ADTKA-E- | S- |          |

**“Tuberculosis” clade (9/9)**

**Other Mycobacteriaceae (0/65)**

Figure S22. Partial sequence alignment of a conserved region of the hypothetical protein IU12_21070 showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Genus                                | Species            | Accession Number | Sequence                                | Clade | Other Genus                      |
|--------------------------------------|--------------------|------------------|-----------------------------------------|-------|---------------------------------|
| Mycobacterium                       | tuberculosis       | A1H82021         | IAGLSVELGIGATORHQDGLPKTVHARLATATG       | "Tuberculosis" (8/8) | Mycobacteriaceae (0/36) |
| Mycobacterium                       | africanum          | ACM61790         | ---------------------------------------|-------|                                |
| Mycobacterium                       | bovis              | PRI08661         | ---------------------------------------|-------|                                |
| Mycobacterium                       | canetti            | WP_044095667     | ---------------------------------------|-------|                                |
| Mycobacterium                       | microtuberculosis  | PRI02693         | ---------------------------------------|-------|                                |
| Mycobacterium                       | mungi              | OAG18826         | ---------------------------------------|-------|                                |
| Mycobacterium                       | orygis             | EM37799          | ---------------------------------------|-------|                                |
| Mycobacterium                       | pinnipedi          | PRH91345         | ---------------------------------------|-------|                                |
| Mycobacterium                       | alsense            | WP_083136449     | VDDIGW- SQ- -HWR----RM-- T- R-          |       |                                |
| Mycobacterium                       | angelicum          | WP_083126688     | VED-GW- AT- HLR- NM- M- KV-            |       |                                |
| Mycobacterium                       | africanum          | WP_051547711     | VDD-GW- A- HWR- RLA- T- K-           |       |                                |
| Mycobacterium                       | avium              | WP_042792140     | DD-ARV-SG- RA- AE- -TR- D- T-         |       |                                |
| Mycobacterium                       | avium subsp. avium| EUA29469         | DD-ARV- RA- AE- -TR- D- T-           |       |                                |
| Mycobacterium                       | chimaera           | ASL12517         | DD-ARV- RA- AE- -TR- D- T-            |       |                                |
| Mycobacterium                       | colombiense         | WP_064883179     | DD- AGV- SA- R- R- D- -PR- E-         |       |                                |
| Mycobacterium                       | gastri             | WP_051508061     | LDN-LS- SG- RWR- R- L- Q- S           |       |                                |
| Mycobacterium                       | gostri 'Wayne'      | ETN23557         | LDN-LS- SG- RWR- R- L- Q- S           |       |                                |
| Mycobacterium                       | gordoniae          | WP_065047705     | VDD-DR- HVGRE- RLA- N- M- K- V        |       |                                |
| Mycobacterium                       | interjectum        | WP_085201823     | DD-ARV- RA- AE- -TR- D- T-           |       |                                |
| Mycobacterium                       | intracellulare     | WP_014378760     | DD- ARV- RA- AE- -TR- D- T-          |       |                                |
| Mycobacterium                       | intracellulare 1   | EUA54093         | DD- ARV- RA- AE- -TR- D- T-          |       |                                |
| Mycobacterium                       | intracellulare 5   | AF512022         | DD-ARV-SG- RA- AE- -TR- D- T-        |       |                                |
| Mycobacterium                       | kansasii           | ORB00236         | LD- VS- SG- RWR- R- L- TQ-           |       |                                |
| Mycobacterium                       | kansasii 732       | EUA08047         | LD- LP- SG- RWR- R- L- Q-           |       |                                |
| Mycobacterium                       | kansasii ATCC 12   | AGZ51438         | SDN- AS- SGO- RWR- R- L- Q-           |       |                                |
| Mycobacterium                       | liflandii          | WP_01535915      | ADNVAR- CD- RSRV- RMA- V- K-       |       |                                |
| Mycobacterium                       | litorale           | WP_078021462     | VED-GW- AM- HWR- RM- T- K- G          |       |                                |
| Mycobacterium                       | mantenii           | WP_083095136     | -ED-ASV- SA- GOR- D- -PR- Q-         |       |                                |
| Mycobacterium                       | marinum            | WP_020725779     | ADNVAR- CD- RSRV- RMA- V- K-       |       |                                |
| Mycobacterium                       | paraense           | WP_085243871     | VND- GW- HWR- RM- T- R-           |       |                                |
| Mycobacterium                       | paraffinicum       | WP_073879003     | E- GEV- SQ- R- F- -R- GR- D-     |       |                                |
| Mycobacterium                       | paraintracellulare| WP_085318484     | DD- ARV- RA- AE- -TR- D- T-        |       |                                |
| Mycobacterium                       | paraparameus       | WP_085207426     | DH- RSV- ET- HWR- R- YR- N-       |       |                                |
| Mycobacterium                       | persicum           | WP_083154497     | LDD- VS- SG- RWR- R- L- TQ-         |       |                                |
| Mycobacterium                       | rhodesiae          | WP_005142017     | VDDMIKNK- VE- HWR- RM- T- K- G       |       |                                |
| Mycobacterium                       | riyadhense         | WP_085253014     | VDK- GR- N- TTR- RM- T-          |       |                                |
| Mycobacterium                       | shinjukuense       | WP_083052343     | VDD-GSR- HR- H- RLA- II- G-       |       |                                |
| Mycobacterium                       | sphagni            | WP_094483721     | VDE-GIKN- VE- HWR- RM- T- K- G      |       |                                |
| Mycobacterium                       | szulgai            | ORW93167         | VE- GO- A- H- R- M- TM- KV-       |       |                                |
| Mycobacterium                       | vulneris           | WP_085288741     | DD- ASV- SAT- R- R- D- -PR- E-     |       |                                |
| Mycolicibacter                      | arupensis          | WP_046190023     | VRD- GIKN- SE- HWR- RM- NT- K- G    |       |                                |
| Mycolicibacter                      | minnesotensis      | WP_083026308     | VRD- GIKN- SE- HWR- RM- NT- K- G    |       |                                |
| Mycolicibacter                      | sinensis           | WP_064857054     | DD- ASV- SE- HWR- R- ANT- K- G      |       |                                |

Figure S23. Partial sequence alignment of a conserved region of the hypothetical protein IU14_19860 showing a two amino acid deletion that is specific for members of the "Tuberculosis" clade.
| Mycobacterium species                  | Accession             | Amino Acid Sequence                                                                 |
|---------------------------------------|-----------------------|------------------------------------------------------------------------------------|
| *Mycobacterium tuberculosis*          | AIH76484              | LLRDIAVMVLCGLVVWQIYRPGRDLVRTG GPYDNGPVGVDLVLYTAD                                  |
| *Mycobacterium bovis*                 | WP_031702925          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium bovis*                 | AMC48512              | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium canetti*               | WP_015288736          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium caprae*                | WP_075744433          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium microti*               | AMC57487              | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mungi*                 | QA917309              | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium oxytodes*              | EMT37820              | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium pinnipedi*             | PRH91363              | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium africanum*             | WP_031668153          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium aquaticum*             | WP_083164280          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium aurum*                 | WP_048631588          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium boenickei*             | WP_077739677          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium bovis*                 | WP_062288868          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium caprae*                | WP_007771712          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium conceptense*           | CGQ03028              | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium dioxanotrophicus*      | ART73306              | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium doricum*               | WP_085192539          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium elephantis*            | WP_046752337          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium engbaekii*             | WP_085128976          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium felax*                 | WP_085093152          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium fortuitum*             | WP_0653383            | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium fortuitum*             | OB653383             | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium fragae*                | WP_085199226          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium haemophili*            | WP_047316799          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium heraklionense*         | WP_076048367          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium houstonense*           | WP_06901874           | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium intermedium*           | WP_069420698          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium kansasi*               | WP_099225671          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium kubicae*               | WP_085074402          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium leflundii*             | WP_015353934          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_012392075          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_031668153          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_05128976           | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_0653383            | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_085192539          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_012392075          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_0653383            | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_085192539          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_012392075          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_0653383            | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_085192539          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_012392075          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_0653383            | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_085192539          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_012392075          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_0653383            | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_085192539          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium mageritense*           | WP_051758581          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |
| *Mycobacterium marinum*               | WP_012392075          | --------G-A-IIR-----EL----H--RI---T----DR-P                                      |

Figure S24. Partial sequence alignment of a conserved region of a membrane protein showing an eight amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | AUC72874 | TGGGTL.LFADHVRTHSMEY  | SPASHHTY  | GAI.TDQHTPLALYHTKR.LA | 67 |
| Mycobacterium africana GM0411 | CCC28551 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium africanum K85 | KBF47630 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium africanum MAL010 | KBB37364 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis | AKR01299 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis AF2122/97 | YP_009360856 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis AN5 | ESK7591 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis B2 7505 | KAN87758 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis BCG | AAB96960 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis BCG str. A | AMO98807 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis BCG str. P | CAL70112 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis 31/50 | KAN92248 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bovis MAL01093 | KBS51344 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium canetti | WP_015292722 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium caprae | WP_03647839 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium microti | ANC57536 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium mungi | OAK7315 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium oryxis | WP_081608392 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium oryxis 112400015 | EMT33889 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium pinnipedii | PRH91488 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium africanum | AMC61867 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium gordonae | WP_065044389 | --------------- | --------------- | --- | --- | T- ODKT - G - T |
| Mycobacterium alsense | WP_083141168 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium alpense | ORA12304 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium arosense | WP_083066330 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium asiaticum | OBI87458 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium avium | WP_040963252 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium bohemicum | WP_085181641 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium colombiense | WP_064877140 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium conspicum | WP_085233296 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium europeum | CQD22373 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium guttatum | WP_036415427 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium hekeshorumense | WP_048990622 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium heidebergense | ORA65721 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium interjectum | WP_066907899 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium intracellulare | WP_064893654 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium kansasi | K2S64453 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium kubicae | WP_085072951 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium lacus | WP_085155832 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium malmoense | WP_066442061 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium mantenii | WP_083099146 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium marsellense | ORA94115 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium nebraskense | WP_085165693 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium palustre | WP_085079703 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium paraense | ORW52145 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium paraffinicum | OJZ62670 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium parascrofulaceae | WP_007107012 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium parasodulose | WP_063172732 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium parva | WP_085268507 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium scrofulaceum | ORB62829 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium shinjukuense | WP_083046789 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium simiae | WP_061555588 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium szulgai | WP_085669083 | --------------- | --------------- | --- | --- | --- |
| Mycobacterium xenopi | WP_003918915 | --------------- | --------------- | --- | --- | --- |
| Mycolicibacillus trivialis | ODR01013 | --------------- | --------------- | --- | --- | --- |

**“Tuberculosis” clade**

(20/21)

**Other Mycobacteriaceae**

(1/51)

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**Figure S25.** Partial sequence alignment of a conserved region of the hypothetical protein RN11_1864 showing an eight amino acid insertion that is specific for most members of the “Tuberculosis” clade and is absent from most other *Mycobacteriaceae.*
Figure S26. Partial sequence alignment of a conserved region of a transmembrane protein showing a three amino acid deletion that is specific for members of the “Tuberculosis” clade.
Figure S27. Partial sequence alignment of the hypothetical protein ERS181347_00724 showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | CEZ53357 | A0GVE1LVA1APGL | AGL | GLPVINRREVNLVTGPMIALGGSVRAALARLP |
| Mycobacterium bovis | WP_079367471 | | | |
| Mycobacterium bovis 04-303 | ES77813 | | | |
| Mycobacterium bovis BZ 7505 | KAN92334 | | | |
| Mycobacterium canetti | WP_014000118 | | | |
| Mycobacterium caprae | APU24439 | | | |
| Mycobacterium microti | AMG7667 | | | |
| Mycobacterium mungi | QAO17110 | | | |
| Mycobacterium orgys | EM73555 | | | |
| Mycobacterium pinnipedi | PRH90420 | | | |
| Mycobacterium africanum | WP_031670740 | | | |
| Mycobacterium alvei | WP_083139375 | | | |
| Mycobacterium angelicum | WP_083115428 | | | |
| Mycobacterium asiaticum | OBK20320 | | | |
| Mycobacterium branderi | WP_083133151 | | | |
| Mycobacterium celatum | WP_085168233 | | | |
| Mycobacterium europeum | COD08726 | | | |
| Mycobacterium florentinum | WP_085222998 | | | |
| Mycobacterium fragae | WP_085199132 | | | |
| Mycobacterium gastrin 'Wayne' | ETW25013 | | | |
| Mycobacterium genavense | WP_025736453 | | | |
| Mycobacterium gordonae | WP_082658447 | | | |
| Mycobacterium haemophilum | WP_047314440 | | | |
| Mycobacterium heckeshornense | KVM23493 | | | |
| Mycobacterium intermedium | WP_069421459 | | | |
| Mycobacterium kansaii | KZ568912 | | | |
| Mycobacterium komykense | WP_090277595 | | | |
| Mycobacterium koryouense | WP_092455665 | | | |
| Mycobacterium lacus | ORW00162 | | | |
| Mycobacterium lentiflavum | WP_090608336 | | | |
| Mycobacterium malmoense | WP_065442438 | | | |
| Mycobacterium marium | BBC63542 | | | |
| Mycobacterium marium M | ACC38917 | | | |
| Mycobacterium montefiorens | QB638016 | | | |
| Mycobacterium palustre | WP_085078342 | | | |
| Mycobacterium paraffinicum | WP_073877657 | | | |
| Mycobacterium paraschoenou | WP_083174383 | | | |
| Mycobacterium pseudodshottsi | WP_086085135 | | | |
| Mycobacterium pseudoschottisi J | BBA86270 | | | |
| Mycobacterium riijadense | WP_085248927 | | | |
| Mycobacterium saskatchewanense | WP_085258063 | | | |
| Mycobacterium scrobiculaceum | WP_067298277 | | | |
| Mycobacterium senouense | WP_085082032 | | | |
| Mycobacterium shimoidai | WP_069394259 | | | |
| Mycobacterium shinjukuense | ORB72323 | | | |
| Mycobacterium simiae | WP_06557986 | | | |
| Mycobacterium szulgi | WP_085670321 | | | |
| Mycobacterium talmoniae | ODH05957 | | | |
| Mycobacterium triplex | WP_036472013 | | | |
| Mycobacterium ulcerans | WP_071497524 | | | |
| Mycobacterium ulcerans Agys | ABL03689 | | | |
| Mycobacterium ulcerans str. Harvey | EUA91645 | | | |
| Mycobacterium ulcerans subsp. shinshuense | BVA43493 | | | |
| Mycobacterium xenopi | WP_004571681 | | | |

"Tuberculosis" clade

(11/11)

Other

Mycobacteriaceae

(0/45)

Figure S28. Partial sequence alignment of a conserved membrane protein showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | A1H34285 | C303005A1P4FLAWYD | D2YSR | RLSKRAEGDIDWITLDDIPETHLY | 144 | 193 |
| Mycobacterium bovis | WP_079293488 | | | | |
| Mycobacterium bovis BCG | AMC48903 | | | | |
| Mycobacterium canetti | WP_015288989 | | | | |
| Mycobacterium caprae | APU24584 | | | | |
| Mycobacterium microti | AMC57837 | | | | |
| Mycobacterium mungi | OAT93653 | | | | |
| Mycobacterium oryis | EMT37363 | | | | |
| Mycobacterium pinnipedii | WP_105826294 | | | | |
| Mycobacterium africanum | C65523 | | | | |
| Mycobacterium literale | WP_078017669 | I - LT SQ - E | V - R - Q | L - E - | D - |
| Mycobacterium parsmone | WP_085271685 | I - S - DR - N | - | - | |
| Mycobacterium vaccae | WP_003922468 | | | | |
| Mycolicibacterium aurum | WP_055110674 | | | | |
| Mycolicibacterium aureum | WP_078030337 | I - LT SH - D | V - R - Q | - | M - E - D - |
| Mycolicibacterium aquaticum | WP_083165348 | I - D - | | | |
| Mycolicibacterium arosegense | WP_063046206 | I - G - | | | |
| Mycolicibacterium avium | WP_00873851 | I - G - | H - | | N - E - |
| Mycolicibacterium branderi | WP_083133853 | I - G - | - | - | |
| Mycolicibacterium celatum | WP_062539905 | I - G - | | | |
| Mycolicibacterium colombiensis | WP_064808705 | I - G - | H - | S - | |
| Mycolicibacterium conspicuum | WP_085263610 | I - G - | | | |
| Mycolicibacterium dioxanotrophicus | WP_057080095 | I - D - | | | |
| Mycolicibacterium fragae | WP_081597892 | I - G - | | | |
| Mycolicibacterium genavense | WP_025736934 | I - G - | H - A - G - | |
| Mycolicibacterium haemophilum | WP_047313750 | I - G - | - | | |
| Mycolicibacterium heckshornense | WP_048888442 | | | | |
| Mycolicibacterium heidelbergerense | WP_085071460 | I - G - | | | |
| Mycolicibacterium holstaeum | WP_069404626 | I - D - | - | R - Q - | E - |
| Mycolicibacterium kansasii | ORB5236 | I - D - | W - G - | Q - |
| Mycolicibacterium kyotinense | WP_045374037 | I - G - | | | |
| Mycolicibacterium lacus | WP_085160062 | I - G - | S - G | | E - D - |
| Mycolicibacterium lentiflavum | WP_090599306 | I - G - | A - G - | |
| Mycolicibacterium mageritense | WP_06433503 | I - D - | R - | E - Q - |
| Mycolicibacterium malmoense | WP_065477819 | I - SE | KMR - | Q - E - |
| Mycolicibacterium marinum | WP_103654799 | I - D - | A - | G - |
| Mycolicibacterium marseillense | WP_083019135 | | K | I - G - | H - | N - Q - V - |
| Mycolicibacterium nebraskense | WP_046184827 | | | | |
| Mycolicibacterium neworleansense | WP_090516231 | | | | |
| Mycolicibacterium noviomagenense | WP_083086813 | | A | I - G - | | |
| Mycolicibacterium palustris | WP_085077826 | I - G - | | | |
| Mycolicibacterium paraense | WP_085093052 | | K | I - G | H - Q - D - | S - |
| Mycolicibacterium paraaffliforme | WP_073874446 | | I - G | | | |
| Mycolicibacterium percorensis | WP_055110674 | I - D - | | | |
| Mycolicibacterium porcinum | WP_056892323 | I - D - | | | |
| Mycolicibacterium rhodesiae | WP_083117990 | I - S - D - | | | |
| Mycolicibacterium saskatchewanense | WP_056257856 | I - G - | | | |
| Mycolicibacterium scrobulaeum | WP_067268722 | | | | |
| Mycolicibacterium septicum | WP_045453989 | | I - D - | | | |
| Mycolicibacterium shimoidei | WP_006938966 | I - G - | E - O - K - | | |
| Mycolicibacterium shinjukuense | WP_083052071 | | R - | | | |
| Mycolicibacterium simiae | WP_061558126 | E - | I - G - | | H - A - G - | Q - |
| Mycolicibacterium smegmatis | WP_085125219 | | I - D - | | | |
| Mycolicibacterium sphagmus | WP_09448727 | | I - G | | | |
| Mycolicibacterium talmoniae | WP_071024725 | I - G - | | | |
| Mycolicibacterium timonensis | WP_063197512 | I - G - | | | |
| Mycolicibacterium ulerae | WP_071497704 | I - D - | | | |
| Mycolicibacterium ulerae Agy99 | ABL0976 | I - D - | | | |
| Mycolicibacterium vulneris | WP_066462411 | I - D - | R - Q - | D - |
| Mycolicibacterium wolinskyi | WP_006154447 | I - | | | |
| Mycolicibacterium xenopi | WP_006622468 | I - G - | | | |
| Mycolicibacterium aromaticivorans | WP_036324979 | I - T - E | | | |
| Mycolicibacterium boenickei | WP_077740206 | I - D - | | | |
| Mycolicibacterium brisbanense | WP_062895997 | I - D - | | | |
| Mycolicibacterium chubuense | WP_014813742 | I - E - | | | |
| Mycolicibacterium confluentis | WP_088151664 | | | | |
| Mycolicibacterium fortuitum | WP_054600105 | E | I - D - | | | |
| Mycolicibacterium goodii | WP_049473404 | I - D - | | | |

Figure S29. Partial sequence alignment of the fructose-bisphosphate aldolase protein showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade and is absent from most other Mycobacteriaceae.
Figure S30. Partial sequence alignment of the anti-sigma K factor protein showing a one amino acid insertion that is specific for members of the “Tuberculosis” clade.
### Mycobacterium tuberculosis
CFR80796

### Mycobacterium bovis
WP_086449525

### Mycobacterium canettii
WP_014000269

### Mycobacterium caprae
WP_075744462

### Mycobacterium microti
AMC8006

### Mycobacterium mungi
OAI5855

### Mycobacterium oryxis
EM72158

### Mycobacterium pinnipedi
PRH91716

### Mycobacterium africanum
CC25597

### Mycobacterium abscessus
AMU19729

### Mycobacterium abscessus
WP_083136232

### Mycobacterium angelicum
WP_083113177

### Mycobacterium arosense
WP_083066974

### Mycobacterium asiaticum
OBK25681

### Mycobacterium avium
WP_099180031

### Mycobacterium bohemicum
WP_085179499

### Mycobacterium branderi
WP_083132158

### Mycobacterium celatum
ORV18499

### Mycobacterium chelonae
WP_078323055

### Mycobacterium colombiense
WP_044483695

### Mycobacterium colombiense CECT
EJO90713

### Mycobacterium conspicuum
WP_054728754

### Mycobacterium europeum
CQD1023

### Mycobacterium florentinum
WP_085222805

### Mycobacterium gastri
WP_036415996

### Mycobacterium gastri 'Wayne'
ETR23170

### Mycobacterium genavense
WP_025738082

### Mycobacterium gordoniae
WP_055678894

### Mycobacterium heckeshornense
WP_099863499

### Mycobacterium heidelbergense
WP_083074522

### Mycobacterium immunogenum
WP_081273231

### Mycobacterium interjectum
WP_066917754

### Mycobacterium intermedium
WP_069421895

### Mycobacterium intracellulare
OBH4494

### Mycobacterium intracellulare M
ETT27230

### Mycobacterium kansasi
ORB85383

### Mycobacterium kansasi ATCC 12
AGZ52059

### Mycobacterium kubicae
WP_085075091

### Mycobacterium kyorinense
ORW05915

### Mycobacterium lacus
WP_085175202

### Mycobacterium lentiflavum
WP_090598874

### Mycobacterium malmense
OCB1307

### Mycobacterium marinum str. Europe
EP070038

### Mycobacterium microti
WP_101528209

### Mycobacterium montefiorensis
GB40606

### Mycobacterium nebraskense
WP_046185623

### Mycobacterium noviomagensis
WP_083087044

### Mycobacterium palustris
WP_085077568

### Mycobacterium paraaeus
WP_085087250

### Mycobacterium paraffinicum
WP_073871019

### Mycobacterium paraseoulensii
WP_083172469

### Mycobacterium parvum
WP_085721815

### Mycobacterium percum
WP_075546990

### Mycobacterium pseudothrixtis J
BBA66600

### Mycobacterium riadyense
WP_085250406

### Mycobacterium salmoniphilum
WP_078330540

### Mycobacterium saskatchewanense
WP_085258683

### Mycobacterium scrofaeascomum
WP_067223246

### Mycobacterium sherrisi
WP_085188007

### Mycobacterium shigaense
WP_085517819

### Mycobacterium shinjukuense
WP_085157202

### Mycobacterium simiae
WP_044509708

### Mycobacterium szulgai
WP_085870065

### Mycobacterium troplix
WP_036472085

### Mycobacterium ulcercans
WP_071497893

### Mycobacterium vulnifera
WP_085288067

### Mycobacterium xenopi
WP_081485287

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**“Tuberculosis” clade (9/9)**

**Other Mycobacteria (0/73)**

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Figure S31. Partial sequence alignment of a conserved protein showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium species                  | Accession Number | Information                                      |
|---------------------------------------|------------------|-------------------------------------------------|
| Mycobacterium tuberculosis            | CFR63098         | 16S rDNA for identification                      |
| Mycobacterium africanum K65          | EF042240         |                                                  |
| Mycobacterium bovis                   | WP_024457966     |                                                  |
| Mycobacterium bovis AF2120/97         | YP_009375978     |                                                  |
| Mycobacterium canetti                 | WP_01400331      |                                                  |
| Mycobacterium caprae                  | APU24819         |                                                  |
| Mycobacterium microti                 | AM558179         |                                                  |
| Mycobacterium mungi                   | AO17243          |                                                  |
| Mycobacterium oryis                   | WP_003403250     |                                                  |
| Mycobacterium pinnipedi               | PRN91813         |                                                  |
| Mycobacterium africanum               | WP_031669274     |                                                  |
| Mycobacterium alsense                 | OQZ93823         |                                                  |
| Mycobacterium angelicum               | WP_08312899      |                                                  |
| Mycobacterium aquaticum               | WP_08316823      |                                                  |
| Mycobacterium asiaticum               | OBK14580         |                                                  |
| Mycobacterium bovis                   | WP_083172961     |                                                  |
| Mycobacterium branderi                | WP_083130215     |                                                  |
| Mycobacterium conspicuum               | WP_085233365     |                                                  |
| Mycobacterium dioxanotrophicum        | WP_087074515     |                                                  |
| Mycobacterium euraceae                | WP_085240572     |                                                  |
| Mycobacterium gastrae                 | WP_036408766     |                                                  |
| Mycobacterium gordiae                 | WP_069522298     |                                                  |
| Mycobacterium grossiae                | WP_070351321     |                                                  |
| Mycobacterium haemophilum             | KL03035          |                                                  |
| Mycobacterium haemophilum DSM          | AKN18209         |                                                  |
| Mycobacterium hassiacum               | WP_018354877     |                                                  |
| Mycobacterium haemophilum DSM 44      | EKF24870         |                                                  |
| Mycobacterium heckeshornense          | WP_040903025     |                                                  |
| Mycobacterium heidelbergense          | WP_083074006     |                                                  |
| Mycobacterium holstactic              | WP_084223007     |                                                  |
| Mycobacterium insubricum              | ORA65210         |                                                  |
| Mycobacterium intermedium             | WP_069421482     |                                                  |
| Mycobacterium iranicum                | WP_064364762     |                                                  |
| Mycobacterium kansasii                | ORB85511         |                                                  |
| Mycobacterium konicanci               | WP_090276792     |                                                  |
| Mycobacterium kubicae                 | WP_085072688     |                                                  |
| Mycobacterium kyorinense              | WP_065015356     |                                                  |
| Mycobacterium lacus                   | WP_085157428     |                                                  |
| Mycobacterium lehmannii               | WP_094289799     |                                                  |
| Mycobacterium lentiflandii            | WP_015354635     |                                                  |
| Mycobacterium litorale                | AQT82457         |                                                  |
| Mycobacterium litarideren            | WP_052506286     |                                                  |
| Mycobacterium mageritense             | WP_036429256     |                                                  |
| Mycobacterium malmoense               | WP_095943739     |                                                  |
| Mycobacterium malmoense               | WP_071513547     |                                                  |
| Mycobacterium marinum                 | WP_020371883     |                                                  |
| Mycobacterium monacense               | WP_083044910     |                                                  |
| Mycobacterium moriockaense            | WP_083166994     |                                                  |
| Mycobacterium musagiense              | WP_064886359     |                                                  |
| Mycobacterium neaurum                 | CDG06673         |                                                  |
| Mycobacterium neumannii               | WP_094293426     |                                                  |
| Mycobacterium neworleansense          | WP_090515180     |                                                  |
| Mycobacterium novomagdese             | WP_030862999     |                                                  |
| Mycobacterium novocastricense         | WP_067392028     |                                                  |
| Mycobacterium obuense                 | WP_046361607     |                                                  |
| Mycobacterium palustre                | WP_05801053      |                                                  |
| Mycobacterium parafortunatum          | WP_083145991     |                                                  |
| Mycobacterium paraseoulsense          | WP_083171635     |                                                  |
| Mycobacterium peregrinum              | WP_064886359     |                                                  |
| Mycobacterium perlubimense            | WP_051682689     |                                                  |
| Mycobacterium porcinum                | WP_069426551     |                                                  |
| Mycobacterium rhodesiae               | ORB56199         |                                                  |
| Mycobacterium riyadhense              | WP_08524176      |                                                  |
| Mycobacterium rufulum                 | KG166926         |                                                  |
| Mycobacterium rutulum                 | WP_083405530     |                                                  |
| Mycobacterium saskatchewanense        | WP_085258140     |                                                  |
| Mycobacterium septicum                | WP_044515982     |                                                  |
| Mycobacterium setense                 | WP_064871624     |                                                  |
| Mycobacterium sberiae                 | WP_083402775     |                                                  |
| Mycobacterium shigaei                 | WP_06935222      |                                                  |
| Mycobacterium shinjukuense            | WP_083050879     |                                                  |
| Mycobacterium smegmatis               | WP_003389271     |                                                  |
| Mycobacterium sphagni                 | WP_094480564     |                                                  |

**Tuberculosis clade (11/11)**

**Other Mycobacteriaceae (0/100)**
| Mycobacterium szulgai          | OBF24477   | -Q--Q-VSG-R-T--D-SR-D-TS-V--HH-G--D-D-          |
| Mycobacterium thermoresistibile| WP_003925971 | -LV-R-SG-Q-T--PR-D-SSS-HH-A--D-D-          |
| Mycobacterium tuscae           | WP_006246889 | -V--D-PK-K-S--RTR-NSS-HH-E--D-D-          |
| Mycobacterium ulcerans         | OIN1799    | -I-QQ-VRG-Q-T--SR-D-TSS-HH-G--D-I-          |
| Mycobacterium vaccae           | WP_003929667 | -PV-T-AG-R-T--SR-TSSS-HH-D--D-D-          |
| Mycobacterium vanbaalenii      | WP_011778477 | -P-V-DG-AG-R-T--SR-D-TSSS-HH-D--D-D-          |
| Mycobacterium vulneris         | WP_065461665 | -V--E-ISG-Q-T--PR-D-TSS-HH-A--D-D-          |
| Mycobacterium wolinskyi        | WP_085144452 | -V--S-SG-Q-T--PR-D-TSSS-HH-A--D-D-          |
| Mycobacterium xenopi           | WP_003921185 | -A--E-EQ-M--SR-TSSS-KH-G--D-D-          |
| Mycobacteroides abscessus      | WP_062878541 | -V--S-AG-Q-T--SR-D-TSSS-HH-D--D-D-          |
| Mycobacteroides abscessus 6G-0 | EIUS1479  | -V--S-AG-Q-T--SR-D-TSSS-HH-D--D-D-          |
| Mycobacteroides abscessus WAB  | ET295059  | -V--S-AG-Q-T--SR-D-TSSS-HH-D--D-D-          |
| Mycobacteroides abscessus subsp. bolletii | SRAM2964 | -V--S-AG-Q-T--SR-D-TSSS-HH-D--D-D-          |
| Mycobacteroides franklinii     | WP_070937364 | -PV-QS-AG-Q-T--SR-D-TSSS-HH-D--D-D-          |
| Mycobacteroides immunogenum    | WP_043078480 | -V--S-AG-Q-T--SR-D-TSSS-HH-D--D-D-          |
| Mycolicibacillus koreensis     | WP_085301948 | -SA-AG-Q-T-VP-A-WRSS-VHTD-YYID-I          |
| Mycolicibacillus trivialis     | ODRS0896   | -SA-AG-Q-T-VP-A-WRSS-VHTD-YYID-I          |
| Mycolicibacter algericus       | WP_083037378 | -EPKQ-AG-Q-S--SR-NSSS-KHNG-D-D          |
| Mycolicibacter arupensis       | WP_046187553 | -PV--AG-Q-T--T--SR-D-TVS--HH-A--D-D-          |
| Mycolicibacter heraklionensis  | OBG40431   | -EPVQ--AG-Q-T--T--SR-D-DSS-V--HH-A--D-D-          |
| Mycolicibacter kumamotoensis   | WP_084014048 | -PVQ-AG-Q-S-S-SR-RNSSS-KHNG-D-D          |
| Mycolicibacter longobardus     | ORN08555   | -PVQ-AG-Q-S-S-SR-RNSSS-KHNG-D-D          |
| Mycolicibacter minnesotensis   | WP_083022762 | -EPVQ-AG-Q-T--T--SR-D-DSSV--HH-A--D-D-          |
| Mycolicibacter senuensis       | ORV66931   | -EPVQ-AG-Q-S-S-SR-NSSS-KHNG-D-D          |
| Mycolicibacter sinensis        | WP_013827584 | -EPVK-AG-Q-S-S-SR-NSSS-KHNG-D-D          |
| Mycolicibacter virginiensis    | WP_105294735 | -EPVQ--AG-Q-T--T--SR-D-DSSV--HH-A--D-D-          |
| Mycolicibacterium agri         | WP_097939130 | -EA-QRA-AE-M--SR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium aromaticivorans | WP_036342341 | -T-A-D-AGQ-T--SR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium aurum        | WP_087031251 | -EQ--TG-Q-T--R-D-TSSS-HHNG-D-D          |
| Mycolicibacterium austroafricanum | WP_036372019 | -P-V-QQ-AG-R-T--SR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium berceniucum  | WP_083055450 | -GP-G-TG-Q-T--SR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium boenickii    | WP_097262321 | -V--IESG-Q-T--PR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium brisbanense  | WP_062829260 | -LV--AG-R-T--PR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium brumae       | WP_090567927 | -PV--PP-PQFFSPT-AHNG-D-D--V-V          |
| Mycolicibacterium canariense   | WP_062658133 | -EV--SGST-V--PR-D-TSSS-HHNG-D-D--A-A          |
| Mycolicibacterium celeriflavum | ORA45241    | -P-AEAAG-T-M--SR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium chloropenilicum | WP_048471748 | -Q-D-PETSS--RPR-DNASSS-HHNG-D-D          |
| Mycolicibacterium chubuense     | WP_014814166 | -A--PESSV--RPR-DNNSSS-HHNG-D-D          |
| Mycolicibacterium conceptionense | WP_085142680 | -V--EISG-Q-T--PR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium confluentis  | ORV31237   | -DGSS-GQQL-S-SDSSTSSS-HHNG-DLVL          |
| Mycolicibacterium cosmeticum   | WP_036401504 | -EV-T-GST-T--PR-D-TSSS-HHNG-D-D--A-A          |
| Mycolicibacterium diernhoferi  | WP_073856988 | -PDGAAGRT--R-D-TSSS-HHNG-D-D          |
| Mycolicibacterium duvallii     | WP_098002401 | -E-D-EETSS--QPR-SSSSHHSSEHNG-D-V          |
| Mycolicibacterium elephantis   | WP_046751223 | -SA-SEAEGQ-T--SR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium fallax       | ORV00389    | -SA-DEPE-I-FRSAHTSSHNG-D-V          |
| Mycolicibacterium farcinogenes | WP_036398134 | -V--EISGQT--PR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium flavescens   | WP_069413236 | -GAEEGSM-T-M--SL-ETSSS-HHNG-D-D          |
| Mycolicibacterium fortuitum    | WP_061262392 | -V--DISGQT--PR-D-TSSS-HHNG-D-D          |
| Mycolicibacterium goodii       | WP_049748265 | -ELVR-TGQRT--PR-D-TSSS-HHNG-D-D          |

Figure S32. Partial sequence alignment of the exonuclease V subunit alpha protein showing a two amino acid deletion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | WP_08136683 | ENL-TLDQKA-AT-VP---AV(PR)---LP  | AGP-SGG-GT-TT-GV |
| Mycobacterium bovis | WP_083113736 | I-VLO-DS-R-VP---M-ARAL  | DPE-PON-MR- | TA-LV-V |
| Mycobacterium canetti | WP_03706144 | EL-AHQA-CTPQ-AI-|SL  | AGP-WGNV  | TA-V |
| Mycobacterium caprae | WP_085249617 | QORW59825  | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium microti | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium mungi | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium orygis | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium pinnipedii | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium alsense | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium bohemicum | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium genavense | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium haemophilum | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium interjectum | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium kansasi | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium kansasii 862 | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium kansasii 824 | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium kansasii ATCC 12 | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium kubicae | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium lacus | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium malmoense | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium nebraskense | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium parvum | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium parvum | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium riyadhense | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium sherrisii | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium shinjukuense | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium szulgai | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |
| Mycobacterium triplex | WP_085302737 | ORW30813  | OIN80796  | WP_05826372  |

**Tuberculosis** clade

(9/9)

Other **Mycobacterium**  
(0/35)

Figure S33. Partial sequence alignment of the multidrug resistance protein EmrB showing a three amino acid deletion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | CRK96409 | 39 | NLDNHEYTAIVGSSDPHRGQVAERSL | IDRIIFDDATFVDVNAVSATELF |
|---------------------------|----------|----|----------------------------|--------------------------|
| Mycobacterium bovis        | WP_09711734 | 39 | -                      | -                     |
| Mycobacterium canetti     | WP_014000421 | 36 | -                      | -                     |
| Mycobacterium caprae      | CEJ50081 | 31 | -                      | -                     |
| Mycobacterium microti     | AMC58309 | 31 | -                      | -                     |
| Mycobacterium mungi       | WP_003403998 | 32 | -                      | -                     |
| Mycobacterium orygis      | EMT37003 | 31 | -                      | -                     |
| Mycobacterium pinnipedi   | PRH91966 | 31 | -                      | -                     |
| Mycobacterium africanum  | CCC25873 | 32 | -                      | -                     |
| Mycobacterium aroliense   | WP_083049222 | 38 | -                      | -                     |
| Mycobacterium asiaticum   | WP_085198299 | 36 | -                      | -                     |
| Mycobacterium avium       | WP_003875788 | 39 | -                      | -                     |
| Mycobacterium avium 104   | ASK65322 | 35 | -                      | -                     |
| Mycobacterium avium XTB13-223 | KSR84903 | 34 | -                      | -                     |
| Mycobacterium avium subsp. avium | ETB14229 | 35 | -                      | -                     |
| Mycobacterium avium subsp. hominis | BN42719 | 34 | -                      | -                     |
| Mycobacterium avium subsp. silvaticum | ETB67290 | 35 | -                      | -                     |
| Mycobacterium bohemicum   | WP_085182626 | 39 | -                      | -                     |
| Mycobacterium colombiense | WP_007772616 | 39 | -                      | -                     |
| Mycobacterium conspicuum   | WP_085234712 | 35 | -                      | -                     |
| Mycobacterium europeum    | WP_085243013 | 35 | -                      | -                     |
| Mycobacterium flavescens  | WP_085225586 | 35 | -                      | -                     |
| Mycobacterium gastrin      | WP_084295352 | 35 | -                      | -                     |
| Mycobacterium gastric ‘Wayne’ | ETW24487 | 34 | -                      | -                     |
| Mycobacterium genavense    | WP_025736905 | 35 | -                      | -                     |
| Mycobacterium gordonae    | OBS00484 | 35 | -                      | -                     |
| Mycobacterium haemophilum  | WP_082129372 | 34 | -                      | -                     |
| Mycobacterium interjectum  | WP_086910927 | 34 | -                      | -                     |
| Mycobacterium intermedium  | WP_069419766 | 35 | -                      | -                     |
| Mycobacterium intracellulare | WP_014379089 | 35 | -                      | -                     |
| Mycobacterium intracellulare M | ET329401 | 35 | -                      | -                     |
| Mycobacterium kansasi     | KZ63551 | 34 | -                      | -                     |
| Mycobacterium kansasi 732 | EUA00975 | 34 | -                      | -                     |
| Mycobacterium kansasii ATCC 12 | AGZ250746 | 35 | -                      | -                     |
| Mycobacterium kubaei      | WP_085074999 | 35 | -                      | -                     |
| Mycobacterium kubaei      | WP_085159265 | 35 | -                      | -                     |
| Mycobacterium lentiflavum | WP_090660894 | 35 | -                      | -                     |
| Mycobacterium lepraeumurium | ATG27729 | 35 | -                      | -                     |
| Mycobacterium malmense    | WP_085009929 | 35 | -                      | -                     |
| Mycobacterium mantoni      | WP_083039128 | 35 | -                      | -                     |
| Mycobacterium marinum      | WP_020730751 | 33 | -                      | -                     |
| Mycobacterium marseillense | WP_083014775 | 33 | -                      | -                     |
| Mycobacterium montefioriense | GBG36281 | 35 | -                      | -                     |
| Mycobacterium nebrascense  | WP_045184403 | 35 | -                      | -                     |
| Mycobacterium palustrum    | WP_085078568 | 33 | -                      | -                     |
| Mycobacterium paraense     | WP_085096529 | 33 | -                      | -                     |
| Mycobacterium paraffinicum | WP_073810161 | 33 | -                      | -                     |
| Mycobacterium parascrofulaceum | EFG77779 | 33 | -                      | -                     |
| Mycobacterium parasequolens | WP_063156081 | 33 | -                      | -                     |
| Mycobacterium parvum       | WP_085269545 | 33 | -                      | -                     |
| Mycobacterium riyadhense   | ORW84112 | 33 | -                      | -                     |
| Mycobacterium saskatchewanense | WP_085255275 | 32 | -                      | -                     |
| Mycobacterium scrofulaceum | WP_087269440 | 32 | -                      | -                     |
| Mycobacterium sherrisi     | WP_089401920 | 32 | -                      | -                     |
| Mycobacterium shigense     | WP_096442961 | 32 | -                      | -                     |
| Mycobacterium shinjukuenense | WP_083048132 | 32 | -                      | -                     |
| Mycobacterium simiae       | WP_061557220 | 32 | -                      | -                     |
| Mycobacterium szulgai      | ORX19116 | 32 | -                      | -                     |
| Mycobacterium Sylvaticus   | C009904 | 32 | -                      | -                     |
| Mycobacterium ulcerans     | WP_011738744 | 32 | -                      | -                     |
| Mycobacterium ulcerans str. Harvey | EUA92729 | 32 | -                      | -                     |
| Mycobacterium vulneris     | WP_085288893 | 32 | -                      | -                     |

Figure S34. Partial sequence alignment of the Hypothetical protein ERS024123_05484 showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade.
Figure S35. Partial sequence alignment of the LuxR family transcriptional regulator protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade.
**Mycobacterium tuberculosis**

**Mycobacterium bovis**

**Mycobacterium canetti**

**Mycobacterium caprae**

**Mycobacterium microti**

**Mycobacterium mungi**

**Mycobacterium oryzae**

**Mycobacterium pinnipedi**

**Mycobacterium africanum**

**Mycobacterium angeli**

**Mycobacterium avium**

**Mycobacterium branderi**

**Mycobacterium chimaera**

**Mycobacterium conspicuum**

**Mycobacterium fragae**

**Mycobacterium gilvum PYR-GCK**

**Mycobacterium hasiacum DSM 44**

**Mycobacterium holsaticum**

**Mycobacterium houstonense**

**Mycobacterium insubricum**

**Mycobacterium intracellulare**

**Mycobacterium leprae**

**Mycobacterium kumamotoensis**

**Mycobacterium wolinskyi**

**Mycobacterium vaccae**

**Mycobacterium shimoidei**

**Mycobacterium setense**

**Mycobacterium rhodesiae JS60**

**Mycobacterium rhodesiae**

**Mycobacterium parafortuitum**

**Mycobacterium paraffinicum**

**Mycobacterium neworleansense**

**Mycobacterium neumannii**

**Mycobacterium insubricum**

**Mycobacterium kubicae**

**Mycobacterium litorale**

**Mycobacterium lepraemurium**

**Mycobacterium malmoense**

**Mycobacterium montefioreense**

**Mycobacterium moriokense**

**Mycobacterium neumannii**

**Mycobacterium neworleansense**

**Mycobacterium obuense**

**Mycobacterium paraffinicum**

**Mycobacterium peregrinum**

**Mycobacterium phlei**

**Mycobacterium phlei RIVM601174**

**Mycobacterium porcinum**

**Mycobacterium rhodesiae**

**Mycobacterium tuberculosis**

**Mycobacterium avium**

**Mycobacterium bovis**

**Mycobacterium canetti**

**Mycobacterium caprae**

**Mycobacterium microti**

**Mycobacterium mungi**

**Mycobacterium oryzae**

**Mycobacterium pinnipedi**

**Mycobacterium africanum**

**Mycobacterium angeli**

**Mycobacterium avium**

**Mycobacterium branderi**

**Mycobacterium chimaera**

**Mycobacterium conspicuum**

**Mycobacterium fragae**

**Mycobacterium gilvum PYR-GCK**

**Mycobacterium hasiacum DSM 44**

**Mycobacterium holsaticum**

**Mycobacterium houstonense**

**Mycobacterium insubricum**

**Mycobacterium intracellulare**

**Mycobacterium leprae**

**Mycobacterium kumamotoensis**

**Mycobacterium wolinskyi**

**Mycobacterium vaccae**

**Mycobacterium shimoidei**

**Mycobacterium setense**

**Mycobacterium rhodesiae JS60**

**Mycobacterium rufum**

**Mycobacterium rutilum**

**Mycobacterium scrofulaceum**

**Mycobacterium septicum**

**Mycobacterium setense**

**Mycobacterium shimoidei**

**Mycobacterium smegmatis**

**Mycobacterium szulgai**

**Mycobacterium thermoresistibilis**

**Mycobacterium tubae**

**Mycobacterium vaccae**

**Mycobacterium wolinskyi**

**Mycobacterium flavescens**

**Mycobacterium fortuitum**

**Mycobacterium fortuitum**

**Mycobacterium tuberculosis**

**Mycobacterium avium**

**Mycobacterium bovis**

**Mycobacterium canetti**

**Mycobacterium caprae**

**Mycobacterium microti**

**Mycobacterium mungi**

**Mycobacterium oryzae**

**Mycobacterium pinnipedi**

**Mycobacterium africanum**

**Mycobacterium angeli**

**Mycobacterium avium**

**Mycobacterium branderi**

**Mycobacterium chimaera**

**Mycobacterium conspicuum**

**Mycobacterium fragae**

**Mycobacterium gilvum PYR-GCK**

**Mycobacterium hasiacum DSM 44**

**Mycobacterium holsaticum**

**Mycobacterium houstonense**

**Mycobacterium insubricum**

**Mycobacterium intracellulare**

**Mycobacterium leprae**

**Mycobacterium kumamotoensis**

**Mycobacterium wolinskyi**

**Mycobacterium vaccae**

**Mycobacterium shimoidei**

**Mycobacterium setense**

**Mycobacterium rhodesiae JS60**

**Mycobacterium rufum**

**Mycobacterium rutilum**

**Mycobacterium scrofulaceum**

**Mycobacterium septicum**

**Mycobacterium setense**

**Mycobacterium shimoidei**

**Mycobacterium smegmatis**

**Mycobacterium szulgai**

**Mycobacterium thermoresistibilis**

**Mycobacterium tubae**

**Mycobacterium vaccae**

**Mycobacterium wolinskyi**

**Mycobacterium flavescens**

**Mycobacterium fortuitum**

Figure S36. Partial sequence alignment of the polypropenyl-diphosphate synthase GrC protein showing a three amino acid deletion that is specific for members of the “Tuberculosis” clade.
**Mycobacterium tuberculosis**
**Mycobacterium bovis**
**Mycobacterium canetti**
**Mycobacterium caprae**
**Mycobacterium microti**
**Mycobacterium mungi**
**Mycobacterium orygis**
**Mycobacterium pinnipedii**
**Mycobacterium africanum**
**Mycobacterium heckeshornense**
**Mycobacterium alsense**
**Mycobacterium angelicum**
**Mycobacterium alsense**
**Mycobacterium pinnipedii**
**Mycobacterium bovigen**
**Mycobacterium avium**
**Mycobacterium celatum**
**Mycobacterium conceptionense**
**Mycobacterium conspicuum**
**Mycobacterium flavescens**
**Mycobacterium goodii**
**Mycobacterium heidelbergense**
**Mycobacterium interjectum**
**Mycobacterium bohemicum DSM 44**
**Mycobacterium branderi**
**Mycobacterium canetti**
**Mycobacterium celatum**
**Mycobacterium conceptionense**
**Mycobacterium conspicuum**
**Mycobacterium flavescens**
**Mycobacterium goodii**
**Mycobacterium heidelbergense**
**Mycobacterium interjectum**
**Mycobacterium bohemicum**
**Mycobacterium kansasi DSM 44**
**Mycobacterium kansasi 662**
**Mycobacterium komansoni**
**Mycobacterium kubitschekii**
**Mycobacterium kyorinense**
**Mycobacterium malmoense**
**Mycobacterium mantoni**
**Mycobacterium moriokaeense**
**Mycobacterium noviomagensis**
**Mycobacterium palustre**
**Mycobacterium paraense**
**Mycobacterium paraseoulense**
**Mycobacterium phlei**
**Mycobacterium phlei RIVM01174**
**Mycobacterium pinnipedii**
**Mycobacterium rhodesiae**
**Mycobacterium riadytense**
**Mycobacterium sromulacum**
**Mycobacterium shinjukiense**
**Mycobacterium smegmatis**
**Mycobacterium smegmatis str. W**
**Mycobacterium szulgai**
**Mycobacterium wohloni**

**Tuberculosis** clade (9/9)

| Mycobacterium | Accession |
|---------------|-----------|
| *M. tuberculosis* | AMCT2161 |
| *M. bovis* | WP_046026275 |
| *M. canetti* | WP_014000545 |
| *M. caprae* | APU25148 |
| *M. microti* | AMC68531 |
| *M. mungi* | OAQ12823 |
| *M. orygis* | EMT8698 |
| *M. pinnipedii* | WP_105826514 |
| *M. africanum* | AMCT2161 |
| *M. heckeshornense* | WP_09868739 |
| *M. alsense* | WP_083136113 |
| *M. angelicum* | WP_083115663 |
| *M. avium* | WP_042906693 |
| *M. avium* | WP_062895358 |
| *M. avium subsp. hominissuis* | KDP03581 |
| *M. avium subsp. paratuberculosis* | EG095428 |
| *M. bohemicum* | WP_085179490 |
| *M. branderi* | WP_083130528 |
| *M. canetti* | WP_015289146 |
| *M. celatum* | WP_062504037 |
| *M. conceptionense* | CDD18494 |
| *M. conspicuum* | WP_085235531 |
| *M. flavescens* | WP_069415848 |
| *M. goodii* | WP_049748383 |
| *M. heidelbergense* | WP_083074558 |
| *M. interjectum* | WP_068618997 |
| *M. bohemicum* | WP_068621126 |
| *M. kansasi* | OOK67047 |
| *M. kansasi DSM 44* | WP_083130528 |
| *M. kubicae* | WP_085072853 |
| *M. kyorinense* | WP_085076639 |
| *M. malmoense* | WP_090345003 |
| *M. manitoni* | WP_083093173 |
| *M. moriokaeense* | WP_083150488 |
| *M. noviomagensis* | WP_083089911 |
| *M. palustre* | WP_085080870 |
| *M. paraense* | WP_085097394 |
| *M. paraseoulense* | WP_083171678 |
| *M. phlei* | WP_040632604 |
| *M. phlei RIVM01174* | EID18229 |
| *M. pinnipedii* | WP_107131609 |
| *M. rhodesiae* | WP_014209449 |
| *M. riadytense* | WP_085251554 |
| *M. sromulacum* | WP_062752820 |
| *M. shinjukiense* | WP_083047968 |
| *M. smegmatis* | WP_011727438 |
| *M. smegmatis str. W* | AFQ37579 |
| *M. szulgai* | WP_085670070 |
| *M. wohloni* | WP_067859049 |

**Other Mycobacteriaceae** (1/43)

| Mycobacterium | Accession |
|---------------|-----------|

**Figure S37.** Partial sequence alignment of the polyphosphate synthase GrcC protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade and is absent from most other Mycobacteriaceae.
| Mycobacterium tuberculosis | Mycobacterium bovis | Mycobacterium canetti | Mycobacterium caprae | Mycobacterium microti |
|-----------------------------|---------------------|-----------------------|----------------------|----------------------|
| AHI44962 | WP_024457259 | WP_044096226 | APU25380 | ACG58828 |

**"Tuberculosis" clade (9/9)**

| Other Mycobacteriaceae (0/31) |
|-----------------------------|
| Mycobacterium ulcerans | Mycobacterium scrofulaceum | Mycobacterium triplex |
| OAI019120 | WP_065297856 | WP_036470807 |

**Figure S38.** Partial sequence alignment of a cold-shock protein showing a two amino acid deletion that is specific for members of the "Tuberculosis" clade.
**Mycobacterium tuberculosis**

**Mycobacterium africanum MAL010**

**Mycobacterium bovis**

**Mycobacterium bovis B 72/05**

**Mycobacterium bovis BCG str. A**

**Mycobacterium bovis BCG str. P**

**Mycobacterium canettii**

**Mycobacterium caprae**

**Mycobacterium microti**

**Mycobacterium oryzae 112400015**

**Mycobacterium pinnipedi**

**Mycobacterium africanum**

**Mycobacterium alsenense**

**Mycobacterium angelicum**

**Mycobacterium caelebium**

**Mycobacterium canettii CIPT 14**

**Mycobacterium celatum**

**Mycobacterium chimera**

**Mycobacterium colombiense**

**Mycobacterium florentinum**

**Mycobacterium kansasii**

**Mycobacterium intermedium**

**Mycobacterium japonicum**

**Mycobacterium jiangsuense**

**Mycobacterium koreense**

**Mycobacterium leprae**

**Mycobacterium leprae intracellularle**

**Mycobacterium lutzerei**

**Mycobacterium marmoratum**

**Mycobacterium marmori**

**Mycobacterium marmoreum**

**Mycobacterium marmoreum**

**Mycobacterium melitense**

**Mycobacterium mintermedium**

**Mycobacterium mungi**

**Mycobacterium naviforme**

**Mycobacterium novocastrense**

**Mycobacterium paraense**

**Mycobacterium paraffinicum**

**Mycobacterium paraintracellulare**

**Mycobacterium paracrosfulaceum**

**Mycobacterium parasaoulese**

**Mycobacterium perdemic**

**Mycobacterium percomum**

**Mycobacterium rhodesiae**

**Mycobacterium rhyadense**

**Mycobacterium scrofulaceum**

**Mycobacterium shiwaii**

**Mycobacterium shinjukuense**

**Mycobacterium szulgai**

**Mycobacterium trehalose**

**Mycobacterium wulnaris**

**Mycobacterium arupensis**

**Mycobacterium heraklionenis**

**Mycobacterium icosaosmamiliensis**

**Mycobacterium sinensis**

**Mycobacterium ternei**

**Mycobacterium virginiensis**

**Mycobacterium celerflavum**

**Mycobacterium chlorophenolicum**

**Mycobacterium chubuense**

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**Figure S39. Partial sequence alignment of a transcriptional regulator protein showing a one amino acid insertion that is specific for members of the “Tuberculosis” clade.**
**Tuberculosis**

(clade (9/9))

| Mycobacterium tuberculosis | AIH45031 | \(\text{GHGGTVMGATEDAVLRMPMRLGLP} \cdots \text{QPMEGSPEGHNFPOSQRIOLCHP} \cdots \text{LNTTF} \) |
| Mycobacterium bovis | WP_024457230 | \(\cdots \) |
| Mycobacterium canetti | WP_015289796 | \(\cdots \) |
| Mycobacterium caprae | APU25476 | \(\cdots \) |
| Mycobacterium microtuli | AM58949 | \(\cdots \) |
| Mycobacterium mungi | OA217721 | \(\cdots \) |
| Mycobacterium orygis | EMY36366 | \(\cdots \) |
| Mycobacterium pinnipedi | PRH95354 | \(\cdots \) |
| Mycobacterium africanum | CCC26455 | \(\cdots \) |
| Mycobacterium asiaticum | OB19167 | \(\cdots \) |
| Mycobacterium avium subsp. avium | EUA25771 | \(\cdots \) |
| Mycobacterium boehmicum DSM 44 | CPG13160 | \(\cdots \) |
| Mycobacterium celatum | ORV15424 | \(\cdots \) |
| Mycobacterium celerifiant | WP_083006896 | \(\cdots \) |
| Mycobacterium chimera | ASL13824 | \(\cdots \) |
| Mycobacterium chlorophenolicum | WP_082168977 | \(\cdots \) |
| Mycobacterium colombiense | OMZ22620 | \(\cdots \) |
| Mycobacterium florentinum | WP_085219889 | \(\cdots \) |
| Mycobacterium fraga | WP_085191413 | \(\cdots \) |
| Mycobacterium franklinii | WP_070936847 | \(\cdots \) |
| Mycobacterium gastrichromogenes | WP_036411119 | \(\cdots \) |
| Mycobacterium gastr 'Wayne' | ETW25423 | \(\cdots \) |
| Mycobacterium gordiae | OBJ80230 | \(\cdots \) |
| Mycobacterium intermedium | WP_069406565 | \(\cdots \) |
| Mycobacterium intracellulare | OBQ03636 | \(\cdots \) |
| Mycobacterium intracellulare 1 | EUA25771 | \(\cdots \) |
| Mycobacterium intracellulare A | AFC42261 | \(\cdots \) |
| Mycobacterium intracellulare M | AFC47404 | \(\cdots \) |
| Mycobacterium intracellulare s | OCB23789 | \(\cdots \) |
| Mycobacterium kansaii | OOK67750 | \(\cdots \) |
| Mycobacterium kansaii 824 | EUA01622 | \(\cdots \) |
| Mycobacterium kansaii A7CC 12 | AGZ52470 | \(\cdots \) |
| Mycobacterium kolumbien | CRL66992 | \(\cdots \) |
| Mycobacterium lacus | WP_085161939 | \(\cdots \) |
| Mycobacterium lehmannii | WP_094286928 | \(\cdots \) |
| Mycobacterium lutzneri | KIU15640 | \(\cdots \) |
| Mycobacterium malmesburensense | WP_090344856 | \(\cdots \) |
| Mycobacterium moriokaense | WP_083140908 | \(\cdots \) |
| Mycobacterium neumanni | WP_094294587 | \(\cdots \) |
| Mycobacterium novomagense | WP_080388940 | \(\cdots \) |
| Mycobacterium novocastrense | WP_067389187 | \(\cdots \) |
| Mycobacterium paraenae | ORR340175 | \(\cdots \) |
| Mycobacterium parastratocellare | WP_014383957 | \(\cdots \) |
| Mycobacterium parasrofulaceum | EF075144 | \(\cdots \) |
| Mycobacterium paraseoulense | WP_083172274 | \(\cdots \) |
| Mycobacterium persicum | WP_083156214 | \(\cdots \) |
| Mycobacterium scrofulaceum | WP_083047999 | \(\cdots \) |
| Mycobacterium shimoidei | WP_069368374 | \(\cdots \) |
| Mycobacterium shinjukuense | WP_083046157 | \(\cdots \) |
| Mycobacterium sinense | OBQ04837 | \(\cdots \) |
| Mycobacterium tusciae | WP_040538944 | \(\cdots \) |

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Other Mycobacteriaceae (0/43)

Figure S40. Partial sequence alignment of the hypothetical protein IQ40_04435 showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade.
Mycobacterium tuberculosis
Mycobacterium bovis
Mycobacterium bovis BCG str. A
Mycobacterium canetti
Mycobacterium caprae
Mycobacterium microti
Mycobacterium mungi
Mycobacterium oryssis
Mycobacterium pinnipedii
Mycobacterium africanum
Mycobacterium alsense
Mycobacterium avium MAV_120709
Mycobacterium avium MAV_120809
Mycobacterium avium subsp. paratuberculosis
Mycobacterium colombiense
Mycobacterium europaense
Mycobacterium gastri
Mycobacterium gastri ‘Wayne’
Mycobacterium heidelbergense
Mycobacterium interjectum
Mycobacterium kansasi
Mycobacterium kansasi 732
Mycobacterium lacus
Mycobacterium l.Diagnosticsdii
Mycobacterium liofilii 12FXT
Mycobacterium malmoense
Mycobacterium mantenii
Mycobacterium marinum
Mycobacterium marinum MB2
Mycobacterium montefioriense
Mycobacterium nebraskense
Mycobacterium palustris
Mycobacterium paraense
Mycobacterium paraffinicum
Mycobacterium parascrofulaceum
Mycobacterium paraseoulense
Mycobacterium pseudoshottsii J
Mycobacterium scrofulaceum
Mycobacterium shinjukuense
Mycobacterium ulcerans str. Harvey

Figure S41. Partial sequence alignment of an esterase protein showing a one amino acid insertion that is specific for members of the “Tuberculosis” clade.

"Tuberculosis" clade
(10/10)

Other Mycobacteriaceae
(0/30)
Figure S42. Partial sequence alignment of the hypothetical protein RN11_1864 showing an eight amino acid insertion that is specific for most members of the “Tuberculosis” clade and is absent from most other *Mycobacteriaceae*.
### “Tuberculosis” clade (11/11)

| Species                                      | Accession Number |
|----------------------------------------------|------------------|
| Mycobacterium tuberculosis                   | A1H39014         |
| Mycobacterium africana MAL010                | KP020086         |
| Mycobacterium bovis                          | WP_099217831     |
| Mycobacterium bovis AF2122/87                | YP_009359459     |
| Mycobacterium canetti                        | WP_014001127     |
| Mycobacterium caprae                         | APU2016          |
| Mycobacterium microti                         | APW97466         |
| Mycobacterium mungi                          | OAQ16566         |
| Mycobacterium orygis                         | EMT35678         |
| Mycobacterium pinnipedii                     | PRH89975         |
| Mycobacterium africana                       | WP_06184055      |
| Mycobacterium alsense                        | WP_083137868     |
| Mycobacterium angelicum                      | WP_083115490     |
| Mycobacterium arosienne                      | WP_083066950     |
| Mycobacterium asiaticum                      | WP_065034176     |
| Mycobacterium avium                         | WP_076221066     |
| Mycobacterium avium 09-5983                  | ETB25337         |
| Mycobacterium avium MAV_120709               | ETZ42095         |
| Mycobacterium avium XTB13-223                | KBR69579         |
| Mycobacterium avium subsp. avium             | EUA36540         |
| Mycobacterium avium subsp. hominissuis       | ETB41611         |
| Mycobacterium avium subsp. paratuberculosis  | QYO22606         |
| Mycobacterium bohemicum                      | WP_085180955     |
| Mycobacterium bohemicum DSM 44               | CPRO4576         |
| Mycobacterium branderi                       | WP_083131920     |
| Mycobacterium celatum                        | WP_082541814     |
| Mycobacterium chimaera                       | WP_054558322     |
| Mycobacterium colombiense                    | WP_086881452     |
| Mycobacterium conspicuum                     | WP_085232924     |
| Mycobacterium florentinum                    | WP_085220593     |
| Mycobacterium fragae                         | WP_085199881     |
| Mycobacterium gastrii                        | WP_086413019     |
| Mycobacterium genavense                      | WP_025735453     |
| Mycobacterium gordonae                       | WP_055579722     |
| Mycobacterium haemophilum                    | WP_047316462     |
| Mycobacterium hassiaca                       | WP_005628004     |
| Mycobacterium heckeshorrense                 | WP_048891217     |
| Mycobacterium heidelbergense                 | WP_083072431     |
| Mycobacterium insubricum                     | WP_083029933     |
| Mycobacterium intermedium                    | WP_069420335     |
| Mycobacterium intracellularere               | WP_064938952     |
| Mycobacterium iranicum                       | WP_064825721     |
| Mycobacterium kansasii                       | ORB88287         |
| Mycobacterium komaniense                     | WP_080273992     |
| Mycobacterium kubicae                        | WP_085075427     |
| Mycobacterium kyorinense                     | WP_065013412     |
| Mycobacterium lacus                         | WP_085159039     |
| Mycobacterium lentilavum                     | WP_090601413     |
| Mycobacterium leprae                         | WP_09082778      |
| Mycobacterium lepromatosis                   | WP_045843071     |
| Mycobacterium liflandii                      | WP_041300286     |
| Mycobacterium liflandii 12BFXT               | AGC62519         |
| Mycobacterium llatzerense                    | WP_082607985     |
| Mycobacterium malmeburyense                  | WP_090346536     |
| Mycobacterium malmoense                      | WP_065443144     |
| Mycobacterium marinum                        | WP_094360196     |
| Mycobacterium marinum str. Europe            | EP080767         |
| Mycobacterium marsellense                    | WP_083018822     |
| Mycobacterium microti OV254                  | PLV50158         |
| Mycobacterium montiferense                   | QBO29407         |
| Mycobacterium mucogenicum                    | WP_061001588     |
| Mycobacterium nebraskense                    | WP_085156685     |
| Mycobacterium neaurum                        | WP_030135490     |
| Mycobacterium neumannii                      | WP_094293434     |
| Mycobacterium noviomagenese                  | WP_085087419     |
| Mycobacterium novocastrense                  | WP_067389524     |
| Mycobacterium palustre                       | WP_085076295     |
| Mycobacterium paraense                       | WP_085101710     |
| Mycobacterium paraffinicum                   | WP_076873503     |
| Mycobacterium parafortuitum                  | WP_083144453     |
| Mycobacterium paraparvulense                 | WP_083172756     |
| Mycobacterium paratuberculosis               | WP_085266896     |
| Mycobacterium persicum                       | WP_083155888     |
Figure S43. Partial sequence alignment of a DEAD/DEAH box helicase protein showing one amino acid deletion that is specific for members of the “Tuberculosis” clade.
Figure S44. Partial sequence alignment of a phosphoglycerate mutase protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade.
"Tuberculosis" clade (10/10)

Other Mycobacteriaceae (0/78)

Figure S45. Partial sequence alignment of a hypothetical protein CAB90_02390 showing two amino acid insertion that is specific for members of the “Tuberculosis” clade.
Mycobacterium tuberculosis
Mycobacterium bovis
Mycobacterium bovis AF212/97
Mycobacterium bovis BCG
Mycobacterium canetti
Mycobacterium canetti CIPT 14
Mycobacterium caprae
Mycobacterium microti
Mycobacterium mungi
Mycobacterium orygis
Mycobacterium pinnipedii
Mycobacterium africanum

Mycobacterium alsenke WP_083137520
Mycobacterium saskatchewanense WP_085254603
Mycobacterium anglicum WP_083113552
Mycobacterium arosiense WP_083067335
Mycobacterium asiaticum WP_060536036
Mycobacterium avium WP_084041340
Mycobacterium branderi WP_083133306
Mycobacterium celatum WP_085168288
Mycobacterium chimaera WP_089151667
Mycobacterium chelonhe WP_085489056
Mycobacterium conceptionense CD021301
Mycobacterium conspicuum WP_085231261
Mycobacterium florentinum WP_085224170
Mycobacterium fragae WP_085199737
Mycobacterium gastri WP_056811467
Mycobacterium genavense WP_025735050
Mycobacterium gilvum WP_011893654
Mycobacterium gordonae WP_065043658
Mycobacterium haemophilum WP_047314644
Mycobacterium haffii WP_056283717
Mycobacterium heckeshornense WP_048895092
Mycobacterium heidelbergense WP_083074413
Mycobacterium holstastic WP_069404900
Mycobacterium houstonense WP_068697781
Mycobacterium interjectum WP_085202787
Mycobacterium intermedi WP_069421244
Mycobacterium intracellulare WP_064937169
Mycobacterium intracellulare s AF514106
Mycobacterium kansasi WP_063471996
Mycobacterium konicense WP_090277821
Mycobacterium lacus WP_085057457
Mycobacterium kyorinense WP_065014644
Mycobacterium lac WP_085161818
Mycobacterium lehmann WP_094288395
Mycobacterium lentiflavum WP_090603532
Mycobacterium leprae WP_080698112
Mycobacterium lepromat WP_085489056
Mycobacterium lepromat WP_085489056
Mycobacterium leprae WP_085489056
Mycobacterium lichen WP_085489056
Mycobacterium marin WP_012394991
Mycobacterium marseillense WP_083020436
Mycobacterium microti WP_101528625
Mycobacterium monteriense WP_100892140
Mycobacterium morlakense WP_085157569
Mycobacterium mucogin WP_064861032
Mycobacterium nebraskense WP_048184104
Mycobacterium neumanni WP_094291427
Mycobacterium neworleans WP_090509999
Mycobacterium noviogamense WP_083089022
Mycobacterium novocastrense WP_067390306
Mycobacterium obuense WP_046363408
Mycobacterium palust WP_085078183
Mycobacterium paraense WP_085101773
Mycobacterium paraffin WP_073974280
Mycobacterium paraffit WP_085346628
Mycobacterium paraintracel WP_014384524
Mycobacterium paraseoulen WP_083172793

"Tuberculosis" clade
(12/12)

Other Mycobacteria
(2/100)
Figure S46. Partial sequence alignment of the glycerol-3-phosphate dehydrogenase protein showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade and is absent from most other Mycobacteriaceae.
| Name                                      | Accession      | Insertion | Clade         |
|-------------------------------------------|----------------|-----------|---------------|
| Mycobacterium tuberculosis                | AIH9286        | 298-355   | “Tuberculosis” |
| Mycobacterium bovis                       | YP_090359775   | 134       | (9/9)         |
| Mycobacterium canetti                     | WP_015293669   | 115       |               |
| Mycobacterium caprae                      | APU26386       | 165       |               |
| Mycobacterium microti                     | AMC60094       | 195       |               |
| Mycobacterium mungi                       | WP_06431959    | L         |               |
| Mycobacterium orygis                      | WP_003412343   | 145       |               |
| Mycobacterium pinnipedi                   | PRH92206       | 145       |               |
| Mycobacterium africanum                   | WP_031666933   | 266       |               |
| Mycobacterium asiaticum                   | OBX18153       | 125       |               |
| Mycobacterium conceptionense              | CDD21896       | 135       |               |
| Mycobacterium europaeum                   | CDD11833       | 145       |               |
| Mycobacterium florentinum                 | WP_085221212   | 165       |               |
| Mycobacterium gastrí                       | WP_036419196   | 165       |               |
| Mycobacterium gordonae                    | WP_085133107   | 165       |               |
| Mycobacterium haemophilum                 | WP_054880917   | 165       |               |
| Mycobacterium hasiacum                    | WP_085977508   | 235       |               |
| Mycobacterium hassiacum DSM 44            | EKF23112       | 235       |               |
| Mycobacterium insubricum                  | WP_083028919   | 245       |               |
| Mycobacterium intermedium                 | WP_089417428   | 245       |               |
| Mycobacterium kansaii 732                 | KZS77658       | 245       |               |
| Mycobacterium kansaii 024                 | EAU13120       | 245       |               |
| Mycobacterium lacus                       | WP_085162908   | 255       |               |
| Mycobacterium litorale                    | WP_076021910   | 295       |               |
| Mycobacterium nebraskense                 | WP_05443420    | 295       |               |
| Mycobacterium palustre                    | ORW19959       | 295       |               |
| Mycobacterium paraense                    | WP_085244185   | 295       |               |
| Mycobacterium persicum                    | WP_089025285   | 295       |               |
| Mycobacterium phlei                       | WP_003890450   | 295       |               |
| Mycobacterium rhodesiae                   | WP_050950587   | 305       |               |
| Mycobacterium rhodesiae J880              | EH85367        | 305       |               |
| Mycobacterium saskatchewanense            | ORW65897       | 305       |               |
| Mycobacterium shinjukuense                | WP_083046349   | 305       |               |
| Mycobacterium sphagni                     | WP_09481366    | 305       |               |
| Mycobacterium szulgai                     | ORX13727       | 305       |               |
| Mycolicibacillus trivialis                | WP_085108973   | 305       |               |
| Mycolicibacter algericus                  | WP_083036319   | 305       |               |
| Mycolicibacter arupensis                  | KCC01354       | 305       |               |
| Mycolicibacter engbaeki                   | ORV42823       | 305       |               |
| Mycolicibacter heraktionensis             | KLO27872       | 305       |               |
| Mycolicibacter hiberniae                  | WP_085134854   | 305       |               |
| Mycolicibacter kumamotoensis              | OBY31474       | 305       |               |
| Mycolicibacter minnesotaense              | WP_083022314   | 305       |               |
| Mycolicibacter nonchromogenicus           | ORW16620       | 305       |               |
| Mycolicibacter senensis                   | WP_085035382   | 305       |               |
| Mycolicibacter sinensis                   | AEFS558        | 305       |               |
| Mycolicibacterium aromaticivorans         | WP_031660447   | 305       |               |
| Mycolicibacterium aurum                   | WP_04863168    | 305       |               |
| Mycolicibacterium brumae                  | WP_090593481   | 305       |               |
| Mycolicibacterium confluentis             | WP_109788561   | 305       |               |

Figure S47. Partial sequence alignment of the GTP-binding protein LepA showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade.
Figure S48. Partial sequence alignment of the type I restriction/modification system specificity determinant HsdS protein showing a four amino acid insertion that is specific for members of the "Tuberculosis" clade.
Figure S49. Partial sequence alignment of the hypothetical protein IQ38_12515 showing a two amino acid deletion that is specific for members of the “Tuberculosis” clade.

Mycobacterium tuberculosis
Mycobacterium bovis
Mycobacterium canetti
Mycobacterium microti
Mycobacterium mungi
Mycobacterium oryis
Mycobacterium pinnipedi
Mycobacterium africanum
Mycobacterium alsense
Mycobacterium angelicum
Mycobacterium arosiense
Mycobacterium asiaticum
Mycobacterium asiaticum DSM 44
Mycobacterium avium complex
Mycobacterium colombiense
Mycobacterium colombiense CECT
Mycobacterium conspicuum
Mycobacterium europaeum
Mycobacterium florentinum
Mycobacterium gastri
Mycobacterium genavense
Mycobacterium gordonae
Mycobacterium heidelbergense
Mycobacterium interjectum
Mycobacterium intracellulare
Mycobacterium intracellularare
Mycobacterium intracellularare M
Mycobacterium intracellularare s
Mycobacterium kansasi
Mycobacterium kansasi 662
Mycobacterium kansasi 732
Mycobacterium kubicai
Mycobacterium lacus
Mycobacterium lentilavum
Mycobacterium malmoense
Mycobacterium mantienii
Mycobacterium marcellense
Mycobacterium monteflorence
Mycobacterium nebraskense
Mycobacterium palustrum
Mycobacterium paraense
Mycobacterium paraense
Mycobacterium persicum
Mycobacterium riyadhense
Mycobacterium saskatchewanense
Mycobacterium scrofulaceum
Mycobacterium shigaense
Mycobacterium szulgai
Mycobacterium triplex
Mycobacterium ulcerans
Mycobacterium ulcerans str. Harvey
Mycobacterium vulneris
Mycobacteroides abscessus
Mycobacteroides abscessus subsp. abscessus
Mycobacteroides abscessus subsp. massiliense
Mycobacteroides chelonae
Mycobacteroides franki

"Tuberculosis" clade (8/8)

Other Mycobacteriaceae (0/52)
**Mycobacterium tuberculosis**
AIH25735

**Mycobacterium africanum MAL010**
KB23920

**Mycobacterium bovis**
PR34488

**Mycobacterium bovis AF212/97**
YP_009360318

**Mycobacterium bovis BCG**
AMG52014

**Mycobacterium canettii**
WP_014001497

**Mycobacterium caprae**
WP_075744572

**Mycobacterium microti**
AMG54361

**Mycobacterium mungi**
OAQ17709

**Mycobacterium oryx**
WP_003414854

**Mycobacterium pinophilus**
WP_105826475

**Mycobacterium africanum**
WP_003910676

**Mycobacterium anglicum**
WP_083111679

**Mycobacterium asiaticum**
WP_065036022

**Mycobacterium bohemicum**
WP_085180269

**Mycobacterium branderi**
WP_083134348

**Mycobacterium caprae**
WP_105826136

**Mycobacterium cellatum**
WP_085168573

**Mycobacterium conceptionense**
CQD32689

**Mycobacterium fragae**
WP_085200231

**Mycobacterium gastri**
ORV65917

**Mycobacterium gordonae**
OBJ79446

**Mycobacterium gossiae**
WP_070355719

**Mycobacterium haemophilum**
WP_048793877

**Mycobacterium houstonense**
WP_066999183

**Mycobacterium interjectum**
WP_084454465

**Mycobacterium intermedium**
WP_069418873

**Mycobacterium kansasi**
KZ65402

**Mycobacterium koryonense**
WP_043373732

**Mycobacterium lacus**
WP_085157743

**Mycobacterium leprae**
WP_010908211

**Mycobacterium lepromatosis**
WP_082082362

**Mycobacterium liflandii**
WP_015355162

**Mycobacterium litorale**
WP_078019616

**Mycobacterium mageritense**
WP_085800454

**Mycobacterium malmense**
WP_090342510

**Mycobacterium malmoense**
WP_083012525

**Mycobacterium mantenii**
WP_083097797

**Mycobacterium marinum**
WP_084357877

**Mycobacterium monacense**
WP_084490096

**Mycobacterium neumannii**
WP_084294952

**Mycobacterium neworleansense**
WP_090513895

**Mycobacterium novomagensense**
WP_083089890

**Mycobacterium paracelense**
OB33916

**Mycobacterium parvum**
WP_085269581

**Mycobacterium peregrinum**
OBF40515

**Mycobacterium persicum**
WP_083153430

**Mycobacterium pseudohontalii J**
GAQ2151

**Mycobacterium rhodesiae**
WP_083120382

**Mycobacterium riyadhense**
WP_085252327

**Mycobacterium saskatchewanense**
WP_085253693

**Mycobacterium setense**
OB21522

**Mycobacterium szulgai**
WP_085670451

**Mycobacterium tamalense**
WP_071028710

**Mycobacterium thermoresistibile**
SWI6168

**Mycobacterium tusciae**
WP_083125368

**Mycobacterium ulcerans**
WP_017400048

**Mycobacterium xenopi**
WP_085197318

**Mycobacterium xenopi 3993**
EUA24313

**Mycobacterium xenopi 4042**
EAU68342

**Mycobacteroides abscessus**
WP_058624728

**Mycobacteroides chelonea**
WP_070918665

**Mycobacteroides franklinii**
WP_078334536

**Mycobacteroides immunogenum**
WP_064833126

**Mycobacteroides salmoniphilum**
WP_078326783

**Mycobacteroides saquaiense**
WP_08541140

**Mycolicibacterium agri**
WP_097944590

**Mycolicibacterium aromaticivorans**
WP_036337805

**Mycolicibacterium celeriflavum**
WP_083002234

**Mycolicibacterium conceptionense**
OM77444

**Mycolicibacterium confluens**
WP_105788489

**Mycolicibacterium farcinogenes**
WP_00365157

**Mycolicibacterium goodii**
WP_083453029

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**Figure S50. Partial sequence alignment of the polyketide synthase protein showing three amino acid insertion that is specific for most members of the “Tuberculosis” clade.**
**Figure S51.** Partial sequence alignment of a lipase protein showing one amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis       | CFE39138     | TRK5FSIAGTRVDAIKLSVFPQTL | GKSVDAGLAANVRDAVGNLAAE |
| Mycobacterium bovis BCG         | AM52153     |                          |                        |
| Mycobacterium canetti           | WP_015294138|                          |                        |
| Mycobacterium caprae            | APU26950    |                          |                        |
| Mycobacterium microti           | AMC0792     |                          |                        |
| Mycobacterium mogu              | OA10416     |                          |                        |
| Mycobacterium oryisis           | EMT34849    |                          |                        |
| Mycobacterium pinnipedii        | PRH90064    |                          |                        |
| Mycobacterium africanum         | CCC2813     |                          |                        |
| Mycobacterium alsense           | WP_083137013|                          |                        |
| Mycobacterium angelicu          | WP_083112114|                          |                        |
| Mycobacterium aroisiense        | WP_083064358|                          |                        |
| Mycobacterium asiaticum         | OBK28902    |                          |                        |
| Mycobacterium avium             | WP_003874932|                          |                        |
| Mycobacterium avium 05-4293     | ETA94798    |                          |                        |
| Mycobacterium avium 11-0986     | ETB50998    |                          |                        |
| Mycobacterium avium subsp. hominisiss | ETB52074           |                          |                        |
| Mycobacterium avium subsp. paratuberculosis | AA0565212 |                          |                        |
| Mycobacterium avium subsp. silvaticum | ETB13699     |                          |                        |
| Mycobacterium branderi          | WP_083130440|                          |                        |
| Mycobacterium celatum           | WP_062540237|                          |                        |
| Mycobacterium chimaera          | WP_083130726|                          |                        |
| Mycobacterium colombiense       | OB37709     |                          |                        |
| Mycobacterium colombiense CECT  | EJO87655    |                          |                        |
| Mycobacterium conspicuum        | WP_083523431|                          |                        |
| Mycobacterium europeae          | CQD20752    |                          |                        |
| Mycobacterium florentinum       | WP_085226078|                          |                        |
| Mycobacterium fragae            | WP_085193282|                          |                        |
| Mycobacterium gastrin           | ORV68882    |                          |                        |
| Mycobacterium gordonia          | WP_055577475|                          |                        |
| Mycobacterium heckeshornense    | WP_048890854|                          |                        |
| Mycobacterium heidelbergense    | WP_063074632|                          |                        |
| Mycobacterium intermedium       | WP_069421426|                          |                        |
| Mycobacterium intracellulare    | WP_01492702  |                          |                        |
| Mycobacterium intracellulare A  | ACF44977    |                          |                        |
| Mycobacterium intracellulare s  | AGP65349    |                          |                        |
| Mycobacterium kansassii         | KZ57495     |                          |                        |
| Mycobacterium kansassii 732     | EUA0055     |                          |                        |
| Mycobacterium kyorinense        | WP_04583517 |                          |                        |
| Mycobacterium lacus             | WP_083160569|                          |                        |
| Mycobacterium lentiflavum       | CD09980     |                          |                        |
| Mycobacterium liliandii         | WP_015355075|                          |                        |
| Mycobacterium malmoense         | WP_065442505|                          |                        |
| Mycobacterium mantellii         | WP_083029792|                          |                        |
| Mycobacterium marinum           | WP_020742952|                          |                        |
| Mycobacterium marseillense      | WP_083018890|                          |                        |
| Mycobacterium microti OV294     | PLV49948     |                          |                        |
| Mycobacterium montefiorensis    | OB36590     |                          |                        |
| Mycobacterium nebraskense       | WP_041845845|                          |                        |
| Mycobacterium noviomagens       | WP_083088360|                          |                        |
| Mycobacterium paraffinicum      | WP_073872329|                          |                        |
| Mycobacterium parascrofulaceum  | WP_007171235|                          |                        |
| Mycobacterium parvum           | WP_083267921|                          |                        |
| Mycobacterium saskatchewanense  | ORW72680    |                          |                        |
| Mycobacterium scrofulaceum      | WP_083177632|                          |                        |
| Mycobacterium sherrissi         | WP_069399772|                          |                        |
| Mycobacterium shigaense         | WP_096438370|                          |                        |
| Mycobacterium simiaeI           | WP_069395051|                          |                        |
| Mycobacterium simiae            | WP_083049586|                          |                        |
| Mycobacterium szulgai           | WP_085699650|                          |                        |
| Mycobacterium triplex           | CD087707    |                          |                        |
| Mycobacterium ulcerans          | WP_085602860|                          |                        |
| Mycobacterium vulgaris          | WP_08520116 |                          |                        |
| Mycobacterium xenopi            | WP_003922188|                          |                        |

**Figure S52.** Partial sequence alignment of a secreted protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade.
**Mycobacterium tuberculosis** WP_031727138
**Mycobacterium bovis** WP_04479225
**Mycobacterium canetti** WP_014001558
**Mycobacterium caprae** APU26952
**Mycobacterium microti** AMC60794
**Mycobacterium mungi** OAA16439
**Mycobacterium orygis** WP_003415985
**Mycobacterium pinnipedii** PRH00066
**Mycobacterium africanum** WP_031670343
**Mycobacterium angelicum** ORA01569
**Mycobacterium alesense** WP_083137012
**Mycobacterium arolesiense** WP_083064417
**Mycobacterium asiaticum** OB199292
**Mycobacterium avium** WP_003874930
**Mycobacterium avium 104** ABK67995
**Mycobacterium avium MAV_120709** ETZ56441
**Mycobacterium avium MAV_120809** ETZ47374
**Mycobacterium avium subsp. paratuberculosis** WPB07205
**Mycobacterium avium subsp. silvaticum** ETB13724
**Mycobacterium celatum** WP_062540236
**Mycobacterium colombiense** OBUJ24711
**Mycobacterium haemophilum** WP_058222266
**Mycobacterium intracellulare** WP_085241155
**Mycobacterium florentinum** WP_085220740
**Mycobacterium fragae** WP_085193285
**Mycobacterium genavense** WP_025735584
**Mycobacterium gordonae** WP_055577473
**Mycobacterium haemophilum** WP_058220740
**Mycobacterium heckeshorhense** WP_048990856
**Mycobacterium heidelbergense** WP_083074631
**Mycobacterium interjectum** WP_084453959
**Mycobacterium intermediense** WP_069420991
**Mycobacterium intracellularare** WP_036389998
**Mycobacterium intracellularare s** AGP65351
**Mycobacterium intracellularare s ARR79412
**Mycobacterium kubicae** WP_085057501
**Mycobacterium lacticum** WP_085160648
**Mycobacterium lentiflavum** WP_090608615
**Mycobacterium litorale** WP_078022147
**Mycobacterium malmoense** WP_062542523
**Mycobacterium montefioriense** WP_108920735
**Mycobacterium nebraskense** WP_046184852
**Mycobacterium noviomargense** WP_062808362
**Mycobacterium palustre** WP_085227373
**Mycobacterium paraense** WP_085093877
**Mycobacterium paraffinicum** WP_073871988
**Mycobacterium paraseoulense** WP_085715160
**Mycobacterium parvum** WP_085267919
**Mycobacterium rhodesiae** WP_005141565
**Mycobacterium riyadhense** WP_085251974
**Mycobacterium saskatchewanense** WP_085255005
**Mycobacterium scrofulaceum** WP_067274710
**Mycobacterium setense** WP_039318196
**Mycobacterium sherrisii** WP_069399724
**Mycobacterium simiae** WP_085295503
**Mycobacterium shinjukuense** WP_083049594
**Mycobacterium silesiae** WP_084952928
**Mycobacterium sphagni** WP_094476835
**Mycobacterium szulgai** WP_068025633
**Mycobacterium thermoresistibile** WP_085265521
**Mycobacterium triplex** CDD38705
**Mycobacterium aromaticivorans** WP_036345786

**Figure S5.** Partial sequence alignment of the DNA polymerase IV protein showing a one amino acid deletion that is specific for most members of the “Tuberculosis” clade and is absent from most other *Mycobacterium* species.
Mycobacterium tuberculosis A1H57612 378----
Mycobacterium bovis KF350291 426----
Mycobacterium bovis AF2122/97 YP_003965087 ----
Mycobacterium bovis BCG AHC52329 ----
Mycobacterium canetti WP_015288475 ----
Mycobacterium caprae APJ25707 ----
Mycobacterium microti WP_005799880 ----
Mycobacterium mungi OA016619 ----
Mycobacterium orygis WP_003416835 ----
Mycobacterium pinepiedi PRH96933 ----
Mycobacterium africanaum WP_031701650 ----
Mycobacterium literale AQT81202 ----
Mycobacterium sphagni WP_094483871 ----
Mycobacterium angelicum WP_083111080 ----
Mycobacterium aquaticum ORA31589 ----
Mycobacterium asiaticum WP_065035318 ----
Mycobacterium avium WP_062889609 ----
Mycobacterium avium WP_062531930 ----
Mycobacterium dioxanotrophicus ART71436 ----
Mycobacterium florentinum WP_085230888 ----
Mycobacterium fragae WP_085195852 ----
Mycobacterium gastri WP_085104986 ----
Mycobacterium gastri ‘Wayne’ ETW23146 ----
Mycobacterium gilvum WP_011895502 ----
Mycobacterium gordoniae WP_065133007 ----
Mycobacterium gregorie WP_070355596 ----
Mycobacterium haemophilum WP_047313270 ----
Mycobacterium holstii WP_069403967 ----
Mycobacterium hominis WP_052537384 ----
Mycobacterium insubricum WP_083030742 ----
Mycobacterium intermedium WP_069419047 ----
Mycobacterium iranicum WP_064281316 ----
Mycobacterium kansai WP_023571748 ----
Mycobacterium kansasii 824 EUA3109 ----
Mycobacterium kohomense WP_090281576 ----
Mycobacterium kyorinense WP_065016599 ----
Mycobacterium lacus WP_085161119 ----
Mycobacterium lehmannii WP_094286016 ----
Mycobacterium leprae WP_020993299 ----
Mycobacterium leprae WP_085177884 ----
Mycobacterium leprae DSW CD019903 ----
Mycobacterium melbourne WP_090338985 ----
Mycobacterium monoflorest WP_048637670 ----
Mycobacterium morio-kokka WP_083154172 ----
Mycobacterium morinificum WP_082440117 ----
Mycobacterium neonaurum WP_0440697 ----
Mycobacterium neumannii WP_084294317 ----
Mycobacterium neworleansense WP_090513897 ----
Mycobacterium novomexicanense WP_083086271 ----
Mycobacterium novocastrense WP_067395793 ----
Mycobacterium obuense WP_082133485 ----
Mycobacterium parasaense WP_085174271 ----
Mycobacterium paraffinicum WP_073967041 ----
Mycobacterium parafortuitum WP_083143065 ----
Mycobacterium paratuberculosis WP_065267695 ----
Mycobacterium perotense WP_080572052 ----
Mycobacterium persicum WP_089025042 ----
Mycobacterium phlei WP_061483543 ----
Mycobacterium porcinum WP_075921504 ----
Mycobacterium rhodesiae WP_014208804 ----
Mycobacterium rhodesiae JS5044887 ----
Mycobacterium riedelii WP_085252027 ----
Mycobacterium rumphi WP_006528979 ----
Mycobacterium ruminantium WP_084792472 ----
Mycobacterium shigae WP_069343805 ----
Mycobacterium shinmoidei WP_069396555 ----
Mycobacterium shinjukuense WP_083049868 ----

"Tuberculosis" clade (11/11)

Other Mycobacteriaceae (2/100)

- Mycobacterium tuberculosis
- Mycobacterium bovis
- Mycobacterium bovis AF2122/97
- Mycobacterium bovis BCG
- Mycobacterium canetti
- Mycobacterium caprae
- Mycobacterium microti
- Mycobacterium mungi
- Mycobacterium orygis
- Mycobacterium pinepiedi
- Mycobacterium africanaum
- Mycobacterium literale
- Mycobacterium sphagni
- Mycobacterium angelicum
- Mycobacterium aquaticum
- Mycobacterium asiaticum
- Mycobacterium avium
- Mycobacterium dioxanotrophicus
- Mycobacterium florentinum
- Mycobacterium fragae
- Mycobacterium gastri
- Mycobacterium gilvum
- Mycobacterium gordoniae
- Mycobacterium grossiae
- Mycobacterium haemophilum
- Mycobacterium holstii
- Mycobacterium hominis
- Mycobacterium insubricum
- Mycobacterium intermedium
- Mycobacterium iranicum
- Mycobacterium kansai
- Mycobacterium kansasii 824
- Mycobacterium kohomense
- Mycobacterium kyorinense
- Mycobacterium lacus
- Mycobacterium lehmannii
- Mycobacterium leprae
- Mycobacterium leprae DSW
- Mycobacterium melbourne
- Mycobacterium morio-kokka
- Mycobacterium morinificum
- Mycobacterium neonaurum
- Mycobacterium neumannii
- Mycobacterium neworleansense
- Mycobacterium novomexicanense
- Mycobacterium novocastrense
- Mycobacterium obuense
- Mycobacterium parasaense
- Mycobacterium paraffinicum
- Mycobacterium parafortuitum
- Mycobacterium paratuberculosis
- Mycobacterium paratuberculosis
- Mycobacterium perotense
- Mycobacterium persicum
- Mycobacterium phlei
- Mycobacterium porcinum
- Mycobacterium rhodesiae JS5044887
- Mycobacterium riedelii
- Mycobacterium ruminantium
- Mycobacterium rumphi
- Mycobacterium shigae
- Mycobacterium shinmoidei
- Mycobacterium shinjukuense
Mycobacteriaceae

- Mycobacterium smegmatis (WP_003893321)
- Mycobacterium szulgai (WP_068029251)
- Mycobacterium thermoresistibile (GAT1538)
- Mycobacterium timonense (WP_083187477)
- Mycobacterium tusciæ (WP_083125060)
- Mycobacterium vaccae (WP_003929197)
- Mycobacterium vulnéris (WP_065461062)
- Mycobacterium wolinskyi (WP_067854431)
- Mycobacterium xenopi 4042 (EU06820)
- Mycobacteroides abscessus (WP_062879200)
- Mycobacteroides abscessus 3A-0 (EIV49761)
- Mycobacteroides abscessus MAB (ESV56355)
- Mycobacteroides abscessus subspp. abscessus (SL26859)
- Mycobacteroides chelonae (WP_070919392)
- Mycobacteroides franklinii (WP_078336403)
- Mycobacteroides immunogenum (WP_064627996)
- Mycobacteroides marinphilum (WP_078323787)
- Mycobacteroides saopaulense (WP_083013600)
- Mycolicibacter koreensis (WP_085302500)
- Mycolicibacter koreensis trivialis (WP_069393355)
- Mycolicibacter algericus (WP_083058484)
- Mycolicibacter arupensis (WP_046911174)
- Mycolicibacter engbaekii (WP_085129321)
- Mycolicibacter heraklionensis (WP_064887988)
- Mycolicibacter hiberniae (WP_053139950)
- Mycolicibacter icosiamassiliensis (WP_067970803)
- Mycolicibacter kumamotoensis (WP_065287940)
- Mycolicibacter longobardus (WP_085243343)
- Mycolicibacter minnesotaensis (WP_083027542)
- Mycolicibacter nonchromogenicus (WP_085137937)
- Mycolicibacter seniorsis (WP_085081373)
- Mycolicibacter sinensis (WP_064855461)
- Mycolicibacter terrae (WP_085262250)
- Mycolicibacter agri (WP_097944507)
- Mycolicibacter aurum (WP_087020394)
- Mycolicibacter austroafricanum (WP_105887748)
- Mycolicibacter bacteremicum (WP_083035705)
- Mycolicibacter brisbanense (WP_109784468)
- Mycolicibacter canariensis (WP_062654522)
- Mycolicibacter chlorophenolicum (WP_048471357)
- Mycolicibacter chobuense (WP_014814699)
- Mycolicibacter confluens (WP_085146870)
- Mycolicibacter diernhoferi (WP_073587797)
- Mycolicibacter duvalii (WP_098004075)
- Mycolicibacter elephantis (WP_083042567)
- Mycolicibacter flavescens (WP_069415958)
- Mycolicibacter fortuitum (WP_061262700)
- Mycolicibacter fortuitum subsp. fortuitum (EI204493)
- Mycolicibacter goodii (WP_049747779)

Other Mycobacteriaceae (2/100)

- Mycolicibacter chelonae
- Mycolicibacter hiberniae
- Mycolicibacter icosiamassiliensis
- Mycolicibacter kumamotoensis
- Mycolicibacter longobardus
- Mycolicibacter minnesotaensis
- Mycolicibacter nonchromogenicus
- Mycolicibacter seniorsis
- Mycolicibacter sinensis
- Mycolicibacter terrae
- Mycolicibacter agri
- Mycolicibacter aurum
- Mycolicibacter austroafricanum
- Mycolicibacter bacteremicum
- Mycolicibacter brisbanense
- Mycolicibacter canariensis
- Mycolicibacter chlorophenolicum
- Mycolicibacter chobuense
- Mycolicibacter confluens
- Mycolicibacter diernhoferi
- Mycolicibacter duvalii
- Mycolicibacter elephantis
- Mycolicibacter flavescens
- Mycolicibacter fortuitum
- Mycolicibacter fortuitum subsp. fortuitum
- Mycolicibacter goodii

Figure S54. Partial sequence alignment of an ATP-dependent DNA helicase protein showing a one amino acid deletion that is specific for members of the “Tuberculosis” clade and is absent from most other Mycobacteriaceae.
**“Tuberculosis” clade (10/10)**

| Mycobacterium tuberculosis | AIH73423 | 207 | 258 | partial sequence alignment of a membrane protein showing a one amino acid insertion that is specific for members of the “Tuberculosis” clade. | DROSIRTVQRGCGYEFRLTSCYNSFGD |
| Mycobacterium bovis | WP_080655140 | - | - | - | - |
| Mycobacterium bovis BCG str. K | AEG9244 | - | - | - | - |
| Mycobacterium cannetti | WP_015294267 | - | - | - | - |
| Mycobacterium caprae | PRH7739 | - | - | - | - |
| Mycobacterium microti | PR102565 | - | - | - | - |
| Mycobacterium mungi | WP_008901570 | - | - | - | - |
| Mycobacterium orygis | WP_003416858 | - | - | - | - |
| Mycobacterium pinnipedii | PRH91052 | - | - | - | - |
| Mycobacterium africanum | AMQ9892 | - | - | - | - |
| Mycobacterium alsense | WP_063140590 | - | - | - | - |
| Mycobacterium angelicum | WP_083111086 | - | - | - | - |
| Mycobacterium arosense | WP_083065571 | - | - | - | - |
| Mycobacterium asiaticum | WP_036353388 | - | - | - | - |
| Mycobacterium avium | WP_009978669 | - | - | - | - |
| Mycobacterium avium 10-5581 | ETB00793 | - | - | - | - |
| Mycobacterium avium subsp. paratuberculosis | GQ038462 | - | - | - | - |
| Mycobacterium bohemicum | ORU97556 | - | - | - | - |
| Mycobacterium chimaera | WP_095662930 | - | - | - | - |
| Mycobacterium colombiense | WP_064951701 | - | - | - | - |
| Mycobacterium colombiense CECT | EJ086266 | - | - | - | - |
| Mycobacterium canetti | ORW5132 | - | - | - | - |
| Mycobacterium europeum | WP_085241817 | - | - | - | - |
| Mycobacterium gastris | WP_051508106 | - | - | - | - |
| Mycobacterium gordoniae | WP_055577351 | - | - | - | - |
| Mycobacterium haemophilum | WP_047313276 | - | - | - | - |
| Mycobacterium heidelbergense | WP_053071338 | - | - | - | - |
| Mycobacterium interjectum | WP_085201869 | - | - | - | - |
| Mycobacterium intermedium | WP_069419037 | - | - | - | - |
| Mycobacterium intracellulare | WP_009955650 | - | - | - | - |
| Mycobacterium intracellulare M | ETZ1621 | - | - | - | - |
| Mycobacterium kansasi | ORB85931 | - | - | - | - |
| Mycobacterium kansasi 662 | A1R0398 | - | - | - | - |
| Mycobacterium kansasi 732 | EUA06382 | - | - | - | - |
| Mycobacterium kansasi 824 | EUA04801 | - | - | - | - |
| Mycobacterium lacus | WP_085161131 | - | - | - | - |
| Mycobacterium leprae | WP_010907946 | - | - | - | - |
| Mycobacterium lepromatosis | WP_045842708 | - | - | - | - |
| Mycobacterium liflandii | WP_051045779 | - | - | - | - |
| Mycobacterium liflandii 129FXT | ACG61472 | - | - | - | - |
| Mycobacterium malmoense | WP_050789988 | - | - | - | - |
| Mycobacterium marinum | WP_094358529 | - | - | - | - |
| Mycobacterium marinum MB2 | EPQ73299 | - | - | - | - |
| Mycobacterium microti 0V294 | PLV48167 | - | - | - | - |
| Mycobacterium nebraskense | WP_046185300 | - | - | - | - |
| Mycobacterium palustre | WP_085077983 | - | - | - | - |
| Mycobacterium paraense | WP_083108584 | - | - | - | - |
| Mycobacterium paraffinicum | WP_073876051 | - | - | - | - |
| Mycobacterium parascrofulaceum | EF676972 | - | - | - | - |
| Mycobacterium paravium | WP_083170104 | - | - | - | - |
| Mycobacterium parvum | WP_085267690 | - | - | - | - |
| Mycobacterium persicum | WP_083153873 | - | - | - | - |
| Mycobacterium pseudohontsii J | GAG37189 | - | - | - | - |
| Mycobacterium riyladhense | WP_085252023 | - | - | - | - |
| Mycobacterium saskatchewanense | ORW74006 | - | - | - | - |
| Mycobacterium scrofulaceum | WP_038169932 | - | - | - | - |
| Mycobacterium sherrisii | WP_069402505 | - | - | - | - |
| Mycobacterium shigaense | BAX91426 | - | - | - | - |
| Mycobacterium shinjukense | WP_083049890 | - | - | - | - |
| Mycobacterium smegi | WP_085670779 | - | - | - | - |
| Mycobacterium triple | ORX02884 | - | - | - | - |
| Mycobacterium ulcerans | WP_071497838 | - | - | - | - |
| Mycobacterium ulcerans Agly99 | ABL04871 | - | - | - | - |
| Mycobacterium ulcerans str. Harvey | EUB95555 | - | - | - | - |
Figure S56. Partial sequence alignment of an ATPase protein showing a one amino acid insertion that is specific for members of the “Tuberculosis” clade.
Mycobacterium tuberculosis

Mycobacterium bovis

Mycobacterium canetti

Mycobacterium caprae

Mycobacterium microti

Mycobacterium mungi

Mycobacterium orygis

Mycobacterium pinnipedii

Mycobacterium africanum

Mycobacterium taloniae

Mycobacterium doritum

Mycobacterium angeli

Mycobacterium arctisense

Mycobacterium asiaticum

Mycobacterium avium

Mycobacterium bohemicum

Mycobacterium branderi

Mycobacterium celatum

Mycobacterium colombiense

Mycobacterium conspicuum

Mycobacterium florentinum

Mycobacterium fragae

Mycobacterium genavense

Mycobacterium gordonae

Mycobacterium heckeshornense

Mycobacterium heidelbergense

Mycobacterium houstonense

Mycobacterium interjectum

Mycobacterium intracellulare

Mycobacterium kansasi

Mycobacterium kyorinense

Mycobacterium lacticum

Mycobacterium lentiflavum

Mycobacterium leprae

Mycobacterium malmoense

Mycobacterium marinum

Mycobacterium marseillense

Mycobacterium montefiorum

Mycobacterium nebraskense

Mycobacterium noviomagensis

Mycobacterium palustre

Mycobacterium paraseoulense

Mycobacterium parumense

Mycobacterium persicum

Mycobacterium pseudohotzii J

Mycobacterium riadyhense

Mycobacterium saskatchewanense

Mycobacterium sherrisii

Mycobacterium shigae

Mycobacterium shimodei

Mycobacterium shinjukuense

Mycobacterium simiae

Mycobacterium sphagni

Mycobacterium szulgai

Mycobacterium triflex

Mycobacterium ulcerans str. Harvey

Mycobacterium vulneris

Mycobacterium xenopi

Mycobacteriaceae clade
(9/9)

"Tuberculosis" clade

Other Mycobacteriaceae
(2/66)

Figure S57. Partial sequence alignment of a DNA glycosylase protein showing a four amino acid insertion that is specific for members of the “Tuberculosis” clade and is absent from most other Mycobacteriaceae.
### Mycobacterium tuberculosis
AIH36963

### Mycobacterium bovis
WP_069523478

### Mycobacterium bovis AF2122/97
WP_043368078

### Mycobacterium bovis BCG
ALAB0051

### Mycobacterium bovis BCG str. K
AGE69441

### Mycobacterium canettii
WP_044097375

### Mycobacterium caprae
CEJ51783

### Mycobacterium microti OV254
PPLQ5-A

### Mycobacterium mungi
OA18521

### Mycobacterium orgys
WP_003417948

### Mycobacterium pinnipedii
PHH93140

### Mycobacterium africanum
CCCC28476

### Mycobacterium angelicum
OJAJ09687

### Mycobacterium montefioreense
GBG36912

### Mycobacterium alsense
WP_083137392

### Mycobacterium aquaticum
ORA22348

### Mycobacterium ardisiense
WP_083065440

### Mycobacterium asiaticum
WP_065036120

### Mycobacterium avium
WP_062899852

### Mycobacterium boehmense
WP_085160177

### Mycobacterium bohemicum DSM 44
CP13539

### Mycobacterium branderi
WP_083100042

### Mycobacterium celatum
WP_085167998

### Mycobacterium chimera
WP_085087452

### Mycobacterium colombianum
WP_064883787

### Mycobacterium conceptionense
COD50989

### Mycobacterium concisum
WP_085236001

### Mycobacterium dixoanotrophicus
WP_087082773

### Mycobacterium europaeum
WP_048997824

### Mycobacterium flocculare
WP_085219672

### Mycobacterium fragae
WP_085198459

### Mycobacterium gastrin
WP_036411110

### Mycobacterium genavense
WP_025734741

### Mycobacterium gilvum
WP_013472639

### Mycobacterium gordonae
WP_065043273

### Mycobacterium gossiae
WP_083298132

### Mycobacterium hassiacum
WP_051007493

### Mycobacterium hassiacum DSM 44
EKF24782

### Mycobacterium heckeshornense
WP_048893275

### Mycobacterium heidelbergense
WP_083100072

### Mycobacterium holstaticum
WP_069407817

### Mycobacterium houstonense
WP_066903927

### Mycobacterium insubricum
WP_083031146

### Mycobacterium interjectum
WP_085202351

### Mycobacterium intermedium
WP_069417485

### Mycobacterium intracellularare
WP_064939839

### Mycobacterium iranicum
OA29688

### Mycobacterium kansasi
WP_063470070

### Mycobacterium kongiense
WP_090279652

### Mycobacterium kruegeriense
WP_085138580

### Mycobacterium lacus
WP_085160800

### Mycobacterium lentiflavum
WP_090600608

### Mycobacterium liflandii
WP_041300072

### Mycobacterium latzerense
WP_071289272

### Mycobacterium mageritense
WP_036428873

### Mycobacterium melmsburgense
WP_090345590

### Mycobacterium malmoense
WP_065441526

### Mycobacterium mantenii
WP_083100207

### Mycobacterium marinum
WP_094358828

### Mycobacterium marsellense
WP_095577754

### Mycobacterium morioakensense
WP_083154034

### Mycobacterium mucogenicum
WP_064980713

### Mycobacterium nebraskense
WP_085165031

### Mycobacterium neaourum
CDG6385

### Mycobacterium neaourum VKM Ac-
AH24455

### Mycobacterium neaourum
WP_053146416

### Mycobacterium neworleansense
WP_090514619

### Mycobacterium noviomagensense
WP_083087846

### Mycobacterium novocastrense
WP_067398338

### Mycobacterium obuense
WP_046676498

### Mycobacterium palustre
WP_085079337

### Mycobacterium paraense
WP_086936309

### Mycobacterium paraffinum
WP_073880252

### Mycobacterium parafortuitum
WP_083145848

### Mycobacterium parascrofulaceum
WP_007167994

### Mycobacterium paraseoulense
WP_083196973

### Mycobacterium parafortuitum
WP_083260966

### Mycobacterium peregrinum
OBRAV193

### Mycobacterium phlei
WP_003885609

### Mycobacterium phlei DSM 43070
KWI73927

---

**"Tuberculosis" clade**

(11/12)

**Other Mycobacteriaceae**

(2/>100)
Mycobacterium porcinum
Mycobacterium rhodesiae
Mycobacterium riyadhense
Mycobacterium rufum
Mycobacterium rutulum
Mycobacterium saskatchewanense
Mycobacterium scrofulaceum
Mycobacterium setense
Mycobacterium sher-Risili
Mycobacterium shigaense
Mycobacterium shimoidei
Mycobacterium shinjukuense
Mycobacterium simiae
Mycobacterium smegmatis
Mycobacterium smeagmatis MKDB
Mycobacterium sphagni
Mycobacterium sulgai
Mycobacterium thermoresistibile
Mycobacterium tineonense
Mycobacterium triplex
Mycobacterium tusciae
Mycobacterium ulcerans
Mycobacterium vaccae
Mycobacterium vaccae ATCC 2595
Mycobacterium vanBalenii
Mycobacterium vulneris
Mycobacterium wolinskyi
Mycobacterium xenopi
Mycobacterium abscessus subsp. abscessus
Mycobacterium algericus
Mycobacterium arupensis
Mycobacterium engbaeii
Mycobacterium heraklionensis
Mycobacterium hiberniae
Mycobacterium icosiumassiliensis
Mycobacterium kumamotoensis
Mycobacterium longobardus
Mycobacterium minnesotaensis
Mycobacterium nonchromogenici
Mycobacterium porcinum
Mycobacterium setense
Mycobacterium terrae
Mycobacterium virginensis
Mycobacterium agris
Mycobacterium aromaticivorans
Mycobacterium aurum
Mycobacterium austroafricans
Mycobacterium bacteremicum
Mycobacterium boenickei
Mycobacterium bradfordense
Mycobacterium canariense
Mycobacterium celeriaflavum
Mycobacterium cervinum
Mycobacterium chubbense
Mycobacterium conceptionense
Mycobacterium confluens
Mycobacterium cosmeticum
Mycobacterium dihnoferi
Mycobacterium doricum
Mycobacterium duvalii
Mycobacterium elephantis
Mycobacterium farcinnogenes
Mycobacterium flavescens
Mycobacterium fortuitum
Mycobacterium goodii

Other

Mycobacteria (2>100)

Figure S58. Partial sequence alignment of the hypothetical protein IQ47_16905 showing three amino acid insertion that is specific for most members of the "Tuberculosis" clade and is absent from most other Mycobacteriaceae.
"Tuberculosis" clade (9/9)

**Mycobacterium tuberculosis**  
CFB06816  
LSGVAAGRAGFAVGVGINRGT  
RAA  
GAALLRH0GADVYVTDLAE

**Mycobacterium bovis**  
WP_050899571  
---  
---

**Mycobacterium canetti**  
WP_000017299  
---  
---

**Mycobacterium caprae**  
APU27245  
---  
---

**Mycobacterium microti**  
AMC61178  
---  
---

**Mycobacterium mungi**  
AMC61178  
---  
---

**Mycobacterium orygis**  
EMT34339  
---  
---

**Mycobacterium pinnipedi**  
PRH49164  
---  
---

**Mycobacterium africanum**  
CCG28483  
---  
---

**Mycobacterium haemophilum**  
WP_047315611  
--- Q S GF  
--- V E K S

**Mycobacterium leprae**  
WP_010907705  
--- Q LS H GF  
--- V D EE I N

**Mycobacterium lepromatosis**  
WP_045842446  
--- Q S GF  
--- V TEE N

**Mycobacterium alsonse**  
WP_083137355  
--- Q GF VD  
--- E D N D

**Mycobacterium alsonse**  
WP_083137355  
--- Q GF VD  
--- E D N D

**Mycobacterium angelicum**  
ORA2140  
--- GF VD  
--- E D N

**Mycobacterium arosiense**  
WP_083065444  
--- GF VD  
--- E D N

**Mycobacterium avium**  
WP_003874438  
--- GF VD  
--- E D N S

**Mycobacterium bohemicum**  
WP_085180129  
--- GF VD  
--- E D KN

**Mycobacterium branderi**  
WP_083109045  
--- GF VD  
--- E D N D

**Mycobacterium celatum**  
WP_062540230  
--- GF VD  
--- E D N

**Mycobacterium colombiense**  
WP_040631117  
--- GF VD  
--- E D N

**Mycobacterium colombiense CECT**  
EJ088475  
--- GF VD  
--- E D N

**Mycobacterium fragae**  
WP_085198437  
--- GF VD  
--- E D N

**Mycobacterium gastris**  
WP_00141084  
--- GF VD  
--- E D N N

**Mycobacterium gordonae**  
KGM78797  
I Q GF VD  
--- EE DN

**Mycobacterium heckeshornense**  
KMV0750  
--- GF VD  
--- E D N I

**Mycobacterium interjectum**  
WP_066907845  
--- GF VD  
--- E D N D

**Mycobacterium intracellulare**  
WP_064940658  
--- GF VD  
--- E D N S

**Mycobacterium intracellulare s**  
WP_004110296  
--- GF VD  
--- E D N N

**Mycobacterium kansaii**  
WP_099223361  
--- GF VD  
--- E D N N

**Mycobacterium kansasii 824**  
EU405477  
--- GF VD  
--- E D N N

**Mycobacterium kyorinense**  
WP_057003436  
--- GF VD  
--- E D N D

**Mycobacterium lacus**  
WP_085160794  
--- GF VD  
--- E D N

**Mycobacterium malmense**  
WP_083009535  
--- GF VD  
--- E D N

**Mycobacterium manteii**  
WP_03093555  
--- GF VD  
--- E D N I

**Mycobacterium marinum**  
WP_020732019  
I Q GF VD  
--- QD N I

**Mycobacterium montefiorenses**  
GBQ36821  
--- GF VD  
--- E D KN

**Mycobacterium neworleansense**  
WP_090514655  
A K GF VD  
--- E D I M

**Mycobacterium noviomagenense**  
ORN14288  
--- GF VD  
--- E D N D

**Mycobacterium palustre**  
WP_085079333  
--- GF VD  
--- E D N D

**Mycobacterium paraense**  
WP_085096490  
--- GF VD  
--- E D N D

**Mycobacterium paraintracellulare**  
AFCC5855  
--- GF VD  
--- E D N S

**Mycobacterium peregrinum**  
WP_064878431  
--- GF VD  
--- E D I M

**Mycobacterium porcinum**  
WP_083671544  
--- GF VD  
--- E D M M

**Mycobacterium pseudohottowsii J**  
GAQ35438  
I Q GF VD  
--- E D N I

**Mycobacterium riyadhense**  
ORW75666  
--- GF VD  
--- E D S I G

**Mycobacterium saskatchewanense**  
WP_085255595  
--- GF VD  
--- E D D

**Mycobacterium scrofulaceum**  
WP_067280488  
I Q GF VD  
--- DD N I

**Mycobacterium septicum**  
WP_044516388  
A K GF VD  
--- E D I M

**Mycobacterium sherrisi**  
WP_00425831  
--- GF VD  
--- E D N D

**Mycobacterium shigaense**  
WP_096437636  
--- GF VD  
--- E SI S

**Mycobacterium shimoidei**  
WP_069395353  
--- GF VD  
--- DE S

**Mycobacterium shinjukuense**  
WP_083045845  
--- GF VD  
--- EA S

**Mycobacterium simiae**  
WP_061558416  
--- GF VD  
--- E D I

**Mycobacterium smegmatis**  
WP_058125639  
--- GF VD  
--- GE D Q

**Mycobacterium smegmatis HKD8**  
ELQ90785  
--- GF VD  
--- G D Q

**Mycobacterium smegmatis str. M**  
AFP38043  
--- GF VD  
--- G D Q

**Mycobacterium szulga**  
ORW92779  
--- GF VD  
--- EE D I

**Mycobacterium talmoniae**  
WP_071026537  
--- GF VD  
--- E A

**Mycobacterium triplex**  
WP_036469991  
--- GF VD  
--- E D NN

**Mycobacterium ulcerans**  
WP_065361604  
--- GF VD  
--- E D N I M

**Mycobacterium vulneris**  
WP_065438728  
A K GF VD  
--- E D M

**Mycobacterium wolinskyi**  
WP_085146714  
--- GF VD  
--- Q OE I

**Mycobacterium xenopi**  
WP_003912299  
--- GF VD  
--- H ED N

**Mycobacterium xenopi 4042**  
EUA06935  
--- GF VD  
--- H ED N

**Mycobacterium avium**  
WP_0343371  
--- GF VD  
--- DE N I A

**Mycobacterium aurum**  
WP_087019763  
--- GF VD  
--- E A T S

**Mycobacterium austroaficanum**  
WP_105387397  
--- GF VD  
--- SEA N I G

**Mycobacterium boenichelii**  
WP_077743977  
--- GF VD  
--- E D M

**Mycobacterium conceptionen**  
OBOD6710  
--- GF VD  
--- G D M

**Mycobacterium farcinogenes**  
CDP84081  
--- GF VD  
--- G D M

**Mycobacterium goodii**  
WP_049748048  
--- GF VD  
--- E D Q

Figure S59. Partial sequence alignment of a hydrolase protein showing a three amino acid insertion that is specific for members of the “Tuberculosis” clade and is absent from most other **Mycobacteriaceae**.
**Tuberculosis**

(20/22)

**Other**

*Mycobacterium tuberculosis*

*Mycobacterium africanaum G0411*

*Mycobacterium africanaum K95*

*Mycobacterium africanaum MAL010*

*Mycobacterium bovis*

*Mycobacterium bovis AF2122/97*

*Mycobacterium bovis A/N*

*Mycobacterium bovis BZ 7505*

*Mycobacterium bovis BCG*

*Mycobacterium bovis BCG str. A*

*Mycobacterium bovis BCG str. P*

*Mycobacterium bovis Bz 31105*

*Mycobacterium bovis MAL01093*

*Mycobacterium canetti*

*Mycobacterium canetti CIPT 14*

*Mycobacterium caprae*

*Mycobacterium microti*

*Mycobacterium mungi*

*Mycobacterium orygis 112400015*

*Mycobacterium pinnipedi*

*Mycobacterium africanum*

*Mycobacterium gordoniae*

*Mycobacterium lisseae*

*Mycobacterium angelicum*

*Mycobacterium arosiense*

*Mycobacterium arosiense ATCC B*

*Mycobacterium asiaticum*

*Mycobacterium avium*

*Mycobacterium boehmicum*

*Mycobacterium chimaera*

*Mycobacterium colombiensis*

*Mycobacterium conspicuum*

*Mycobacterium europeae*

*Mycobacterium fragae*

*Mycobacterium gastri*

*Mycobacterium heidelbergense*

*Mycobacterium interjectum*

*Mycobacterium intracellularare*

*Mycobacterium intracellularare 1*

*Mycobacterium intracellularare A*

*Mycobacterium intracellularare M*

*Mycobacterium intracellularare s*

*Mycobacterium kansasi 1*

*Mycobacterium kansasi 732*

*Mycobacterium kansasi 824*

*Mycobacterium kubicae*

*Mycobacterium lacus*

*Mycobacterium malmoense*

*Mycobacterium mantoni*

*Mycobacterium marseillense*

*Mycobacterium microti*

*Mycobacterium nebraskense*

*Mycobacterium orygis*

*Mycobacterium orygis 112400015*

*Mycobacterium palustrum*

*Mycobacterium paratuberculosis*

*Mycobacterium paratuberculosis parasprofulaceum*

*Mycobacterium paratuberculosis paraphenense*

*Mycobacterium paratuberculosis pinnipedi*

*Mycobacterium scrofulaceum*

*Mycobacterium shinmoedi*

*Mycobacterium shinjukense*

*Mycobacterium szulgai*

*Mycobacterium xenopi*

*Mycociliacillus trivalentis*

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**Figure S60.** Partial sequence alignment of the hypothetical protein RN11_1864 showing an eight amino acid insertion that is specific for most members of the “Tuberculosis” clade and is absent from most other *Mycobacteriaceae.*
### Mycobacteriaceae

#### “Tuberculosis” clade

| Genus                        | Accession         | Partial Sequence Alignment |
|------------------------------|-------------------|---------------------------|
| *Mycobacterium tuberculosis* | AM679382          | IDVKGLRLTLQAAHVAEDS ASRECPO PDADIVATAGFIAAEAGHRVA |
| *Mycobacterium bovis BCG*    | AM52727           |                           |
| *Mycobacterium canetti*      | WP_01400177       |                           |
| *Mycobacterium microti*      | AM61290           |                           |
| *Mycobacterium mungi*        | OAI18758          |                           |
| *Mycobacterium oryogis*      | EHT34247          |                           |
| *Mycobacterium pinnipedii*   | PRH53603          |                           |
| *Mycobacterium africanum*    | AM66029           |                           |

#### Other Mycobacteriaceae

| Genus                        | Accession         | Partial Sequence Alignment |
|------------------------------|-------------------|---------------------------|
| *Mycobacterium alsense*      | WP_08313782       | S-EI -E -S -D -I          |
| *Mycobacterium angelicum*    | WP_08311638       | K -EV -E -A -D           |
| *Mycobacterium avium subsp. paratuberculosis* | ETB3665          | S-L -E -A -D           |
| *Mycobacterium branderi*     | WP_08313104       | - - - - -D               |
| *Mycobacterium gordoniae*    | WP_06513298       | K -I -E -S-A -D         |
| *Mycobacterium grossiae*     | WP_07035421       | L -V -S-GL -QLE-DS-A    |
| *Mycobacterium haisiacum*    | WP_00563043       | A -V -IS-NL -ETE-N-A    |
| *Mycobacterium holstamicum*  | WP_06940443       | A -L -NI -EVE-N-A       |
| *Mycobacterium kansasi*      | WP_10846035       | K -L -EL -S-A -D        |
| *Mycobacterium kansasii 662* | EUA10305          | K -L -EL -S-A -D        |
| *Mycobacterium kansasii 732* | EUA00967          | K -L -EL -S-A -D        |
| *Mycobacterium komanimense*  | WP_09027987       | A -LS-L -V-N-A          |
| *Mycobacterium kubicae*      | WP_08507261       | S-V -S-A -D             |
| *Mycobacterium lacus*        | WP_08516216       | S-V -S-A -D             |
| *Mycobacterium litiandii*    | WP_01535735       | S-L -E -S             |
| *Mycobacterium latzerense*   | WP_04398460       | A -S -GL -E -N-A        |
| *Mycobacterium mageritense*  | WP_03643005       | LS-L -SS-A            |
| *Mycobacterium mallesburyense* | WP_09034260   | LS-GL -V-N-A         |
| *Mycobacterium marinum*      | WP_09436054       | S-L -E -S           |
| *Mycobacterium morioakense*  | WP_05315664       | A -V -LS-L -N-A        |
| *Mycobacterium mucogenicum*  | WP_05365393       | S -GL -E -N-A -D      |
| *Mycobacterium nebraskense*  | WP_04618478       | S-L -S-A -D           |
| *Mycobacterium nebraskense*  | WP_04732191       | S-L -S-A -D           |
| *Mycobacterium neumannii*    | WP_09429406       | S-V -S-A -D           |
| *Mycobacterium noviomagens*  | WP_060305418      | LS-L -GTE-SA-D        |
| *Mycobacterium novocastrense*| WP_06739849       | A -LS-L -L -G-A -D    |
| *Mycobacterium parvense*     | WP_08526828       | S-L -L -S-A -D        |
| *Mycobacterium phlei*        | WP_00390630       | LS-GL -EV-N-A         |
| *Mycobacterium pseudohotteii*| WP_08608532       | S-L -E -S             |
| *Mycobacterium rhodesiae*    | WP_014210733      | A -V -LS-L -VE-N-A     |
| *Mycobacterium riyadhense*   | WP_08552668       | S-V -S-A -D           |
| *Mycobacterium rutilum*      | WP_083407103      | A -LS-GL -V-N-A -D     |
| *Mycobacterium septicum*     | WP_044520595      | LS-L -V-S-SS          |
| *Mycobacterium shinjukuense* | WP_08304583       | K-SQ-I -A -D          |
| *Mycobacterium szulcasi*     | WP_060820595      | S-V -S-A -D           |
| *Mycobacterium thermoresistibile* | WP_003926320 | I -S-QM -V-M-A       |
| *Mycobacterium tuscae*       | WP_006246477      | A -V -LS-L -E-N-A     |
| *Mycobacterium ulcerans*     | WP_096369650      | S-L -E -S             |
| *Mycobacteroides abscessus*  | WP_100523804      | W -S-L -S-V -K -D    |
| *Mycobacteroides abscessus subsp. abscessus* | SHV1298    | A -S-GL -E -N-A -D  |
| *Mycobacteroides cheloneae*  | WP_070932666      | W -S-L -V -K -SD      |
| *Mycobacteroides franklinii* | WP_070932847      | W -S-L -V -K -SD      |
| *Mycobacteroides immunogenum* | WP_043076722    | W -S-L -V -K -D      |
| *Mycobacteroides salmoniphilum* | WP_078328081     | W -S-L -V -K -SD    |
| *Mycobacteroides saopaulense*| WP_070909104      | W -S-EL -V -K -D      |
| *Mycobacterium agri*         | WP_097944241      | A -V -LS-L -EV-N-A -D |
| *Mycobacterium celeriflavum* | WP_08307098       | A -LS-NL -V-S-A -D    |
| *Mycobacterium chobuense*    | WP_014817736      | I-A -LS-GL -EV-N-A    |
| *Mycobacterium confluens*    | WP_085153576      | I -LS-L -V-R-A        |
| *Mycobacterium flavescens*   | WP_089418315      | -LS-GL -V-N-A -D      |
| *Mycobacterium fortuitum*    | WP_064867998      | -LS-L -V-STA          |

Figure S61. Partial sequence alignment of the acyl-CoA dehydrogenase FadE27 protein showing an eight amino acid insertion that is specific for members of the “Tuberculosis” clade.
| Mycobacterium tuberculosis | A1H83748 | Q2AYLKTQPOAKTFAIEAR | PVAIRGSDLHMFSFYEKFPM |
| Mycobacterium bovis | WP_107193441 | | |
| Mycobacterium bovis AN5 | ESK70297 | | |
| Mycobacterium canetti CIPT 14 | CCC46093 | | |
| Mycobacterium caprae | APU28114 | | |
| Mycobacterium microti | AMC61535 | | |
| Mycobacterium mungi | OAO19131 | | |
| Mycobacterium oryis | WP_003399792 | S -VH -QDRC -T -SY -L -K | ESMGTWDLFR - G -MYTLOF-G |
| Mycobacterium pinnipedi | PRH1703 | | |
| Mycobacterium africanum | CC28820 | | |
| Mycobacterium angelicum | WP_083114354 | H -Q -S -R | DDIGTWDLFR - G -T -A |
| Mycobacterium aquaticum | WP_083163338 | Q -REH -GRN -L -S | GATGWTWDLFR - G -T -G |
| Mycobacterium asiaticum | WP_065144905 | Q -REH -GRS -L | GATGWTWDLFR - G -T -G |
| Mycobacterium boehemicum | WP_085181533 | Q -Q-R | GEIGTWDLFR - G -T |
| Mycobacterium conceptionense | COD19822 | H -QREH -GRYM -L | SAIGTWDLFR - G -T -G |
| Mycobacterium dioxanomorphicus | WP_087075042 | Q -QREH -GRN -L | GATGWTWDLFR - G -T -G |
| Mycobacterium gastri | WP_036410729 | H -YR -GT -L -G | ESIGTWSDLQ - G -MPS-G-G |
| Mycobacterium gastri ‘Wayne’ | ETW25669 | H -YR -GT -L -G | ESIGTWSDLQ - G -MPS-G-G |
| Mycobacterium gordonae | OBS03650 | Q -Q -G | ADIGTWDLFR - G -T -A |
| Mycobacterium houstonense | WP_066897409 | QREH -RSY | GATGWTWDLFR - G -T -G |
| Mycobacterium intermedium | WP_069418835 | QKOH -GR -Y -L | GATGWTWDLFR - G -T -G |
| Mycobacterium kanssii | ORBB4657 | QA -H | DDIGTWDLFR - G -T -A |
| Mycobacterium kansasii ATCC 12 | AGBS2121 | | |
| Mycobacterium lacus | WP_085161105 | | |
| Mycobacterium liflandii | WP_015357597 | QEH | NDIGTWDLFR - G -E -NT -A |
| Mycobacterium litorale | WP_078020230 | LGH -ATL -G | DAIGTWDLFR - G -T -G |
| Mycobacterium latzerense | WP_043984748 | Q -QREH -GRS -L -S | GATGWTWDLFR - G -T -G |
| Mycobacterium mageritense | WP_036432246 | H -FR -T -V -L -G | DSIGTWNLQD - G -MPT-G-G |
| Mycobacterium marinus | WP_012396787 | QEH | NDIGTWDLFR - G -T -G |
| Mycobacterium mucogenicum | WP_053564206 | Q -QREH -GRS -L -S | GATGWTWDLFR - G -T -G |
| Mycobacterium neaurum | WP_030136205 | QREH -GRD -L -S | QSTGTWDLFR - G -T -G |
| Mycobacterium rhoesiae | WP_005141504 | MOH -V-G | -DS | EAIGTWDLFR - G -T -G |
| Mycobacterium riyadhense | WP_064874682 | H -YR -GT -L -G | DSIGTWSDLQ - G -MPT-G-G |
| Mycobacterium setense | WP_030136205 | QREH -GRD -L -S | QSTGTWDLFR - G -T -G |
| Mycobacterium shinjukuense | WP_083046741 | H -QK -A | ADIGTWDLFR - G -T -A |
| Mycobacterium sphagni | WP_094476643 | LGH -V-Q -G | ADGWTWDLFR - G -T -G |
| Mycobacterium szulgai | WP_086676537 | H -Q | DDIGTWDLFR - G -T -G |
| Mycobacterium tusciae | WP_083127306 | QKOH -GRS -L -S | GASGTWDLFR - G -T -G |
| Mycobacterium ulcerans | WP_0117411956 | QEH | NDIGTWDLFR - G -T -A |
| Mycobacterium ulcerans str. Harvey | EUB06658 | QEH | NDIGTWDLFR - G -T -A |
| Mycobacteroides abscessus subsp. abscessus | SHM22920 | Q -QREH -GRS -L -S | GATGWTWDLFR - G -T -G |
| Mycolicibacterium aromaticivorans | WP_051659881 | MOH -A-G | -G | DAIGTWDLFR - G -T -G |
| Mycolicibacterium aurum | WP_03026162 | QOHR -GRD -L -S | GATGWTWDLFR - G -T -G |
| Mycolicibacterium brisbane | WP_02630665 | Q -QREH -GRS -L -S | GATGWTWDLFR - G -T -G |
| Mycolicibacterium canariense | GQ89011 | QREH -GRS -L -S | GATGWTWDLFR - G -T -G |
| Mycolicibacterium conceptionense | WP_064894335 | QREH -GRYM -L -S | SAIGTWDLFR - G -T -G |
| Mycolicibacterium cosmeticum | WP_036396092 | QREH -GRS -L -S | GATGWTWDLFR - G -T -G |
| Mycolicibacterium diernhoferi | WP_073859792 | QRDG -GRS -L -S | GATGWTWDLFR - G -T -G |
| Mycolicibacterium farcinogenes | WP_03026162 | QOHR -GRD -L -S | GATGWTWDLFR - G -T -G |
| Mycolicibacterium fortuitum | ALI28406 | QREH -GRS -L -S | GATGWTWDLFR - G -T -G |

**Other Mycobacteriaceae (0/40)**

*Figure S62. Partial sequence alignment of an oxidoreductase protein showing an eleven amino acid deletion that is specific for most members of the “Tuberculosis” clade.*
| Mycobacterium tuberculosis | AIH40693 | MIAAGACAVATAIGVG | VDVAPPAPASPTAGH1TVSKPAPV | |
| Mycobacterium bovis | WP_044797969 | -------- | VA | |
| Mycobacterium bovis BCG | AMC3222 | | |
| Mycobacterium canettii | WP_014002029 | -------- | VA | |
| Mycobacterium caprae | APU24247 | | |
| Mycobacterium microti | WP_105799879 | | |
| Mycobacterium mungi | OAG18867 | | |
| Mycobacterium oryinis | ETM37754 | | |
| Mycobacterium pinnipedi | RHR91305 | | |
| Mycobacterium africanum | AM66536 | | |
| Mycobacterium amselense | WP_083139489 | AV - V - VG - LA - | T AA | |
| Mycobacterium aroriense | WP_053064680 | T - A - | T AA | |
| Mycobacterium asiaticum | WP_065034065 | LA - | T AA | |
| Mycobacterium avium | WP_033730589 | A - | VA | |
| Mycobacterium avium | WP_038430586 | A - | T AA | |
| Mycobacterium avium 09-5983 | ETB18351 | - | VA | |
| Mycobacterium bochumica | WP_085179808 | VV - | VA - | |
| Mycobacterium cellatum | WP_085167563 | VV - | VA - | |
| Mycobacterium chimaera | WP_072501544 | L - | VA - | |
| Mycobacterium colombiense | OBJ58540 | T - | TAA | |
| Mycobacterium europeaum | WP_085239316 | V - | TAA | |
| Mycobacterium florentinum | WP_085219527 | VA - | TA | |
| Mycobacterium gastritidis | WP_036418423 | IL - | AA | |
| Mycobacterium genavense | WP_025737867 | LA - | TAA | |
| Mycobacterium gordonae | WP_055579943 | VV - | TAA | |
| Mycobacterium haemophilum | WP_047315650 | V - | TAA | |
| Mycobacterium heidelbergense | WP_083075756 | LAV - VG - LA - | TAA | |
| Mycobacterium intermedium | WP_066917037 | LA - | TAA | |
| Mycobacterium intermedium | WP_069421178 | V - | TAA | |
| Mycobacterium intracellulare | WP_014383556 | L - | TAA | |
| Mycobacterium kansasi | OOK75776 | IL - | TAA | |
| Mycobacterium lacus | WP_085163139 | VL - | TAA | |
| Mycobacterium lindii ATCC 128FXT | AGC65128 | IL - | TAA | |
| Mycobacterium malnose | WP_065441537 | VL - | TAA | |
| Mycobacterium mantenii | WP_083099529 | T - | TAA | |
| Mycobacterium marinum | WP_012399767 | IL - | TAA | |
| Mycobacterium microti OV254 | PLV49966 | IL - | TAA | |
| Mycobacterium nebraskense | WP_047322614 | VV - | TAA | |
| Mycobacterium paraense | WP_085104579 | LA - | TAA | |
| Mycobacterium paraffinicum | WP_073880701 | V - | TAA | |
| Mycobacterium paraintracellulare | WP_014386141 | L - | TAA | |
| Mycobacterium paraseouline | WP_083171234 | VV - | TAA | |
| Mycobacterium parvum | WP_085268192 | LV - | TAA | |
| Mycobacterium persicum | WP_083154545 | IL - | TAA | |
| Mycobacterium riyadhense | WP_085252970 | V - | TAA | |
| Mycobacterium sinoferlicum | WP_067297825 | VV - | TAA | |
| Mycobacterium shinjukuense | WP_085163139 | VL - | TAA | |
| Mycobacterium ulcerans | WP_011745218 | IL - | TAA | |
| Mycobacterium vulneris | WP_085290981 | T - | TAA | |

**Figure S63.** Partial sequence alignment of the hypothetical protein IQ42_20035 showing a two amino acid deletion that is specific for most members of the “Tuberculosis” clade.