Detection of *Mycobacterium angelicum* in Human Urinary Tract, French Polynesia

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We definitively characterized *Mycobacterium angelicum*, an aquatic zoonotic opportunistic pathogen of the *M. szulgai* complex, using a polyphasic approach that included whole-genome sequencing. The sequence was obtained on the island of Tahiti, French Polynesia, from a urine specimen collected from a patient experiencing a urinary tract infection.

Mycobacterium angelicum, a slow-growing mycobacterium associated with animals living in freshwater environments, was delineated within the *M. szulgai* complex by 16S rRNA gene sequencing after its initial isolation from a freshwater angelfish (*Pterophyllum scalare*) in 2003 (*1*). In line with international recommendations (*2*), *M. angelicum* was formally described in 2015 as a new species including the seminal 2003 isolate, 2 additional isolates from freshwater fish, and a fourth isolate recovered from a freshwater tank containing tortoises (*1*). Meanwhile, another isolate was identified in Benin from the rodent *Crocidura olivieri* (*3*). A clinical isolate recovered from a respiratory tract sample taken from a patient in Northern Ireland was also tentatively identified as *M. angelicum* on the basis of 16S rRNA gene sequencing (*4*). We report another clinical isolate identified as *M. angelicum* on the basis of a polyphasic identification approach including whole-genome sequencing (WGS).

A middle-aged patient sought care for active struvite urolithiasis in the left kidney at the main hospital in Papeete, French Polynesia, 18 years after a right nephrectomy for obstructive pyonephrosis. We were unable to follow up with the patient beyond this medical episode. Of 3 successive urine samples collected over 3 consecutive days, which all lacked acid-fast bacilli after Ziehl-Neelsen staining, we successfully cultured 1 urine sample on MGIT (Becton, Dickinson and Company, https://www.bd.com) in 11 days. Culture on Löwenstein Jensen medium (Becton Dickinson) at 37°C under aerobic conditions remained negative after 3 months' incubation. This isolate positively stained by Ziehl-Neelsen staining; it exhibited rod-shaped, pink-stained bacteria measuring 3.225 ± 0.858 µm by 0.717 ± 0.048 µm under electron microscopy observation using a SU5000 SEM electron microscope (Hitachi, https://www.hitachi.com). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a Microflex spectrometer and software (Bruker Daltonics, https://www.bruker.com), as previously described (*5*), yielded a noninformative score of 1.31; a derived dendrogram clustered the isolate within the *M. szulgai* group. We conducted WGS concatenating Illumina (https://www.illumina.com) and Nanopore (https://nanoporetech.com) reads using Spades software version 3.15.4, as previously described (*6*); this process yielded 66.4% guanine-cytosine content, 0.23% gap ratio, and a 6,673,592-bp sequence distributed into 51 contigs encoding for 5,707 proteins, 56 tRNA, 3 rRNA, and 2 CRISPRs with a 90.5% total coding ratio. We deposited the sequence into GenBank (submission identification no. 2639860). As a first step, BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the 1,326,459-bp longest contig yielded 98% coverage and 99.3% similarity with an environmental *M. angelicum* isolate strain DSM 45057 WGS (GenBank accession no. NZ_MVHE01000100) (*1*) and a 98.6% DNA–DNA hybridization using the Type Strain Genome Server (Leibniz Institute DSMZ, https://www.dsmz.de). As a second step, we used the Orthologous Average Nucleotide Identity tool version 0.93.1 (*7*); the isolate clustered with the best BLAST hit *M. angelicum* isolate with 99.73% genome similarity, whereas further genome sequence similarity values were 93.25% with *M. szulgai*, 93.01% with *M. riyadhense*, and >85% with other mycobacteria (Figure). Those data identified our isolate as the *M. angelicum* Tahiti strain; we deposited it into the Collection de Souches de l'Unité des Rickettsies (CSUR Q5816). No antimicrobial resistance–encoding sequences were predicted in the *M. angelicum* Tahiti strain genome. In agreement with the qualification of *M. angelicum* as a human pathogen by the Center for Genomic Epidemiology online platform (http://www.genomicepidemiology.org), 24 pathogenicity-associated genes were identified in the *M. angelicum* Tahiti strain, all highly conserved in

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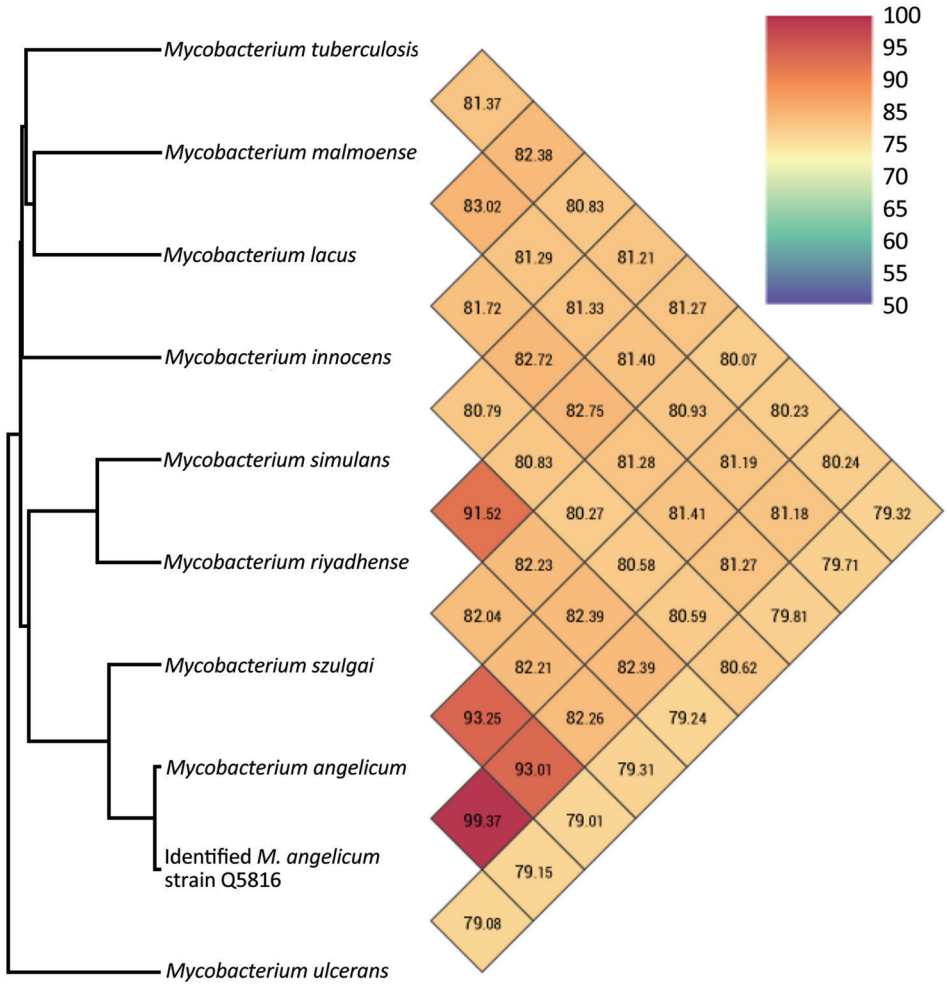

Figure. Whole-genome sequence–based clusterization of a *Mycobacterium angelicum* strain from a human urinary tract, French Polynesia. The strain clustered within the *M. szulgai* complex, based on orthoANI calculations (*7*).

Mycobacterium species (Appendix, https://wwwnc.cdc.gov/EID/article/29/7/22-1864-App1.pdf). The location of the *Clp*S gene in 74,165–74,377, its 97.14% sequence similarity with the homologous gene in *Mycobacterium*, and encoding an ATP-dependent Clp protease were predicted as nonpathogenicity factors affecting antimicrobial metabolism and rifampin resistance to protect the *Mycobacterium* cell wall against various stresses (*8*,*9*).

We identified the Tahiti strain as *M. angelicum* by combining WGS with phenotypic data in the presence of controls. The identification pathway was an opportunity to make a clinical *M. angelicum* WGS available; 1 *M. angelicum* partial genome sequence (GenBank NZ_MVHE01000100.1) derived from a freshwater angelfish isolate had been described previously (*1*). We did not find reports of *M. angelicum* as a contaminant in that study, in a urine collection device, or as a laboratory contaminant. Furthermore, we did not find previous reports of *M. angelicum* analysis in either of the 2 laboratories that handled this patient's urine or the strain itself; we concluded that this *M. angelicum* isolate was not a contaminant. This isolate was the only microorganism we were able to isolate by culture from a urine sample; however, its role in the complex urinary tract pathology of this patient remained putative.

Previously, an isolate of *M. angelicum* was identified from a bronchoalveolar sample collected from a patient with chronic obstructive pulmonary disease in Northern Ireland; identification was based on 100% partial identity (817-bp) 16S rRNA gene sequencing with the reference (*3*). Our study underscores the need for WGS sequencing of bacterial pathogens not identified by first-line phenotypic schemes, including appropriate matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (*5*).

Acknowledgments

According to French law, anonymous case report does not require specific ethical approval when the patient does not oppose reporting.

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Authors' contributions: M.L.K. and M.M.: data collection, data cleaning, design of the study, data interpreting, validation and writing of the manuscript. M.L., G.B., and C.V.: clinical examination, data collection, validation, interpretation, and writing of the manuscript. M.D.: design of the study, data interpretation, validation, funding, critical review of the manuscript, coordination and direction of the work. All authors declare that they have read and approved the manuscript.

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Mr. Keita is a PhD student in the Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France. His primary research interest is in the clinical microbiology of mycobacteria of tropical sources, including *Mycobacterium ulcerans*. Dr. Morsli is an expert in the genomics and metagenomics of mycobacteria, working at the Centre Hospitalier Universitaire, Nîmes, France.

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Low Susceptibility of Pigs against Experimental Infection with HPAI Virus H5N1 Clade 2.3.4.4b

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We found that nasal and alimentary experimental exposure of pigs to highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b was associated with marginal viral replication, without inducing any clinical manifestation or pathological changes. Only 1 of 8 pigs seroconverted, pointing to high resistance of pigs to clade 2.3.4.4b infection.

Spread of highly pathogenic avian influenza (HPAI) virus H5N1 clade 2.3.4.4b of the goose/Guangdong (gs/GD) lineage, has exacerbated since early 2022 into a panzootic (*1*). Regional enzootic status in wild bird populations in Europe and North America, with lethal courses of HPAI virus infection in some species, produced large numbers of wild bird carcasses, easy prey for raptors and scavengers. Exposure of

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Appendix

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INPUT SEQUENCE INFO:
Input Name ::    2_08_11_2022_090022_149104_input.fsa.translated
Sequences ::      5728
Total bpp ::      2042673
Longest seq ::    6302
Shortest seq ::   30
Avg seq lenght :: 356.0
RESULTS:
Min Identity Threshold ::    91.084
Z-THRESHOLD ::   3.0
Prediction Score ::    100.845
Probability of being human pathogen ::  0.83
Matches::  30
Genome Coverage (%) :: 0.52
Pathogenic Families Matched ::    26
Non-Pathogenic Families Matched :: 4
The organisms is predicted as human pathogenic ::   Yes
MATCHED SEQUENCES:
#input_seq: SEQ4_116 # 132658 # 136821 # 1 #
ID=4_116;partial=00;start_type=GTG;rbs_motif=3Base/5BMM;rbs_spacer=13-
15bp;gc_cont=0.651
#matched_seq:    15642 CP000717    Mycobacterium tuberculosis F11, complete
genome.    Actinobacteridae conserved hypothetical protein    ABR06151
      Yes   92.86
#input_seq: SEQ4_129 # 149255 # 151087 # 1 #
ID=4_129;partial=00;start_type=ATG;rbs_motif=None;rbs_spacer=None;gc_cont=
0.640
#matched_seq:    15642 CP000717    Mycobacterium tuberculosis F11, complete
genome.    Actinobacteridae conserved hypothetical protein    ABR06164
      Yes   93.61
#input_seq: SEQ9_126 # 138779 # 139858 # 1 #
ID=9_126;partial=00;start_type=GTG;rbs_motif=None;rbs_spacer=None;gc_cont=
0.686
#matched_seq:    15642 CP000717    Mycobacterium tuberculosis F11, complete
genome.    Actinobacteridae transcriptional regulatory protein, lacI-
family     ABR07933    Yes   92.2
#input_seq: SEQ21_20 # 15375 # 15761 # -1 #
ID=21_20;partial=00;start_type=ATG;rbs_motif=GGA/GAG/AGG;rbs_spacer=5-
10bp;gc_cont=0.654
```

#matched_seq: 16230 CP000325 Mycobacterium ulcerans Agy99, complete genome. Actinobacteridae conserved hypothetical secreted protein ABL03616 Yes 92.19
#input_seq: Seq1_332 # 390257 # 391426 # 1 # ID=1_332;partial=00;start_type=ATG;rbs_motif=AGxAGG/AGGxGG;rbs_spacer=5-10bp;gc_cont=0.639
#matched_seq: 16230 CP000325 Mycobacterium ulcerans Agy99, complete genome. Actinobacteridae prophage integrase ABL03228 Yes 91.26
#input_seq: SEQ4_125 # 143596 # 144498 # 1 # ID=4_125;partial=00;start_type=ATG;rbs_motif=GGAG/GAGG;rbs_spacer=5-10bp;gc_cont=0.662
#matched_seq: 16725 CP000854 Mycobacterium marinum M, complete genome. Actinobacteridae conserved proteinACC41119 Yes 97.33
#input_seq: Seq1_752 # 856607 # 857344 # -1 # ID=1_752;partial=00;start_type=ATG;rbs_motif=None;rbs_spacer=None;gc_cont=0.646
#matched_seq: 18883 CP000611 Mycobacterium tuberculosis H37Ra, complete genome. Actinobacteridae hexapeptide transferase family protein ABQ74848 Yes 91.84
#input_seq: SEQ5_205 # 256240 # 257085 # -1 # ID=5_205;partial=00;start_type=TTG;rbs_motif=GGAG/GAGG;rbs_spacer=5-10bp;gc_cont=0.656
#matched_seq: 16725 CP000854 Mycobacterium marinum M, complete genome. Actinobacteridae YrbE family protein, YrbE5A ACC42273 Yes 91.81
#input_seq: Seq1_1102 # 1250530 # 1251189 # 1 # ID=1_1102;partial=00;start_type=GTG;rbs_motif=None;rbs_spacer=None;gc_cont=0.626
#matched_seq: 15642 CP000717 Mycobacterium tuberculosis F11, complete genome. Actinobacteridae conserved transmembrane protein ABR07630 Yes 92.24
#input_seq: SEQ4_30 # 42264 # 43037 # 1 # ID=4_30;partial=00;start_type=ATG;rbs_motif=GGAGG;rbs_spacer=5-10bp;gc_cont=0.661
#matched_seq: 88 CP000479 Mycobacterium avium 104, complete genome. Actinobacteridae putative class II aldolase ABK65325 Yes 91.83
#input_seq: SEQ3_550 # 616803 # 617549 # 1 # ID=3_550;partial=00;start_type=ATG;rbs_motif=GGAG/GAGG;rbs_spacer=5-10bp;gc_cont=0.621
#matched_seq: 16725 CP000854 Mycobacterium marinum M, complete genome. Actinobacteridae succinate dehydrogenase (iron-sulfur subunit), Sdh ACC38974 Yes 94.76
#input_seq: SEQ9_127 # 139855 # 140481 # -1 # ID=9_127;partial=00;start_type=ATG;rbs_motif=AGGAG/GGAGG;rbs_spacer=11-12bp;gc_cont=0.652
#matched_seq: 91 AE016958 Mycobacterium avium subsp. paratuberculosis str. k10, complete genome. Actinobacteridae hypothetical protein AAS02808 Yes 94.71
#input_seq: SEQ3_46 # 40867 # 41241 # -1 # ID=3_46;partial=00;start_type=ATG;rbs_motif=GGA/GAG/AGG;rbs_spacer=11-12bp;gc_cont=0.635
#matched_seq: 15762 CP000384 Mycobacterium sp. MCS, complete genome. Actinobacteridae SSU ribosomal protein S12P ABG07098 No 98.39

#input_seq: SEQ4_157 # 180884 # 181501 # 1 # ID=4_157;partial=00;start_type=GTG;rbs_motif=GGAG/GAGG;rbs_spacer=5-10bp;gc_cont=0.670
#matched_seq: 88 CP000479 Mycobacterium avium 104, complete genome. Actinobacteridae transcriptional regulator, MerR family protein ABK68131 Yes 93.66
#input_seq: Seq1_142 # 175018 # 175335 # -1 # ID=1_142;partial=00;start_type=GTG;rbs_motif=GGAGG;rbs_spacer=3-4bp;gc_cont=0.651
#matched_seq: 15642 CP000717 Mycobacterium tuberculosis F11, complete genome. Actinobacteridae integration host factor mihF ABR05760 Yes 99.05
#input_seq: Seq1_1079 # 1225443 # 1225802 # 1 # ID=1_1079;partial=00;start_type=ATG;rbs_motif=GGA/GAG/AGG;rbs_spacer=5-10bp;gc_cont=0.653
#matched_seq: 15642 CP000717 Mycobacterium tuberculosis F11, complete genome. Actinobacteridae conserved hypothetical protein ABR07613 Yes 96.64
#input_seq: Seq1_373 # 429078 # 429446 # -1 # ID=1_373;partial=00;start_type=ATG;rbs_motif=AGGAG;rbs_spacer=5-10bp;gc_cont=0.653
#matched_seq: 21055 CP001658 Mycobacterium tuberculosis KZN 1435, complete genome. Actinobacteridae conserved hypothetical protein ACT24330 Yes 92.62
#input_seq: SEQ3_542 # 608730 # 609179 # 1 # ID=3_542;partial=00;start_type=ATG;rbs_motif=None;rbs_spacer=None;gc_cont=0.633
#matched_seq: 16230 CP000325 Mycobacterium ulcerans Agy99, complete genome. Actinobacteridae conserved hypothetical protein ABL03758 Yes 91.95
#input_seq: Seq1_92 # 117518 # 117751 # -1 # ID=1_92;partial=00;start_type=ATG;rbs_motif=GGA/GAG/AGG;rbs_spacer=5-10bp;gc_cont=0.594
#matched_seq: 18883 CP000611 Mycobacterium tuberculosis H37Ra, complete genome. Actinobacteridae protein-export membrane protein ABQ73192 Yes 96.1
#input_seq: Seq1_378 # 432040 # 432342 # -1 # ID=1_378;partial=00;start_type=ATG;rbs_motif=GGAG/GAGG;rbs_spacer=5-10bp;gc_cont=0.620
#matched_seq: 15762 CP000384 Mycobacterium sp. MCS, complete genome. Actinobacteridae conserved hypothetical protein ABG08297 No 93.0
#input_seq: SEQ2_224 # 287984 # 288331 # 1 # ID=2_224;partial=00;start_type=GTG;rbs_motif=GGA/GAG/AGG;rbs_spacer=5-10bp;gc_cont=0.629
#matched_seq: 88 CP000479 Mycobacterium avium 104, complete genome. Actinobacteridae acyl carrier protein ABK65172 Yes 98.26
#input_seq: SEQ9_59 # 64828 # 65124 # -1 # ID=9_59;partial=00;start_type=ATG;rbs_motif=AGGAG;rbs_spacer=5-10bp;gc_cont=0.640
#matched_seq: 18883 CP000611 Mycobacterium tuberculosis H37Ra, complete genome. Actinobacteridae putative esat-6 like protein ABQ74144 Yes 93.88

#input_seq: SEQ4_122 # 142287 # 142586 # 1 #
ID=4_122;partial=00;start_type=ATG;rbs_motif=GGAG/GAGG;rbs_spacer=5-
10bp;gc_cont=0.667
#matched_seq: 16725 CP000854 Mycobacterium marinum M, complete
genome. Actinobacteridae PE family protein, PE19 ACC41116 Yes
94.95
#input_seq: SEQ4_123 # 142867 # 143163 # 1 #
ID=4_123;partial=00;start_type=GTG;rbs_motif=AGGAG;rbs_spacer=5-
10bp;gc_cont=0.657
#matched_seq: 16725 CP000854 Mycobacterium marinum M, complete
genome. Actinobacteridae EsaT-6 like protein Esx ACC43524 Yes
94.9
#input_seq: SEQ6_196 # 221559 # 221843 # -1 #
ID=6_196;partial=00;start_type=ATG;rbs_motif=AGGAG;rbs_spacer=5-
10bp;gc_cont=0.660
#matched_seq: 15642 CP000717 Mycobacterium tuberculosis F11, complete
genome. Actinobacteridae Esat-6 like protein esxN (Esat-6 like protein
5) ABR06159 Yes 96.81
#input_seq: SEQ6_311 # 348465 # 348749 # 1 #
ID=6_311;partial=00;start_type=ATG;rbs_motif=AGGA;rbs_spacer=5-
10bp;gc_cont=0.642
#matched_seq: 16230 CP000325 Mycobacterium ulcerans Agy99, complete
genome. Actinobacteridae EsaT-6 like protein EsxN ABL05326 Yes
92.55
#input_seq: SEQ5_52 # 74165 # 74377 # 1 #
ID=5_52;partial=00;start_type=GTG;rbs_motif=None;rbs_spacer=None;gc_cont=0
.587
#matched_seq: 15762 CP000384 Mycobacterium sp. MCS, complete genome.
Actinobacteridae ATP-dependent Clp protease adaptor protein ClpS
ABG09958 No 97.14
#input_seq: SEQ16_63 # 63548 # 63739 # -1 #
ID=16_63;partial=00;start_type=GTG;rbs_motif=AGGAG;rbs_spacer=5-
10bp;gc_cont=0.635
#matched_seq: 16230 CP000325 Mycobacterium ulcerans Agy99, complete
genome. Actinobacteridae ferredoxin FdxD ABL06117 Yes 93.65
#input_seq: SEQ9_8 # 9472 # 9633 # 1 #
ID=9_8;partial=00;start_type=ATG;rbs_motif=3Base/5BMM;rbs_spacer=13-
15bp;gc_cont=0.642
#matched_seq: 15762 CP000384 Mycobacterium sp. MCS, complete genome.
Actinobacteridae hypothetical protein ABG10925 No 96.23
#input_seq: SEQ10_21 # 33929 # 34123 # -1 #
ID=10_21;partial=00;start_type=ATG;rbs_motif=GGA/GAG/AGG;rbs_spacer=5-
10bp;gc_cont=0.677
#matched_seq: 88 CP000479 Mycobacterium avium 104, complete
genome. Actinobacteridae ribosomal protein L35 ABK68440 Yes 92.19