

1 Data Release

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3 Genomic features of *Mycobacterium avium* subsp. *hominissuis* isolated from pigs in Japan.

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73 Abstract

74 *Mycobacterium avium* subsp. *hominissuis* (MAH) is one of the most important agents causing

75 non-tuberculosis mycobacterial infection in humans and pigs. Genome analysis on MAH of

76 human isolates has been proceeding, however, those of pigs are limited despite its potential

77 source of infection to human. In the current study, we obtained 30 draft genome sequences

of MAH of pigs reared in Japan. The 30 draft genomes consisted of 4,848,678 – 5,620,788 bp length, 4,652 – 5,388 coding genes and 46 – 75 (Med: 47) tRNAs. All isolates had restriction modification associated genes and 185 – 222 predicted virulence genes. Two isolates had tRNA arrays and one isolate had a clustered regularly interspaced short palindromic repeat (CRISPR) region. Our results will be useful for evaluation of the ecology of MAH by providing a foundation for genome-based epidemiological studies.

Research Areas: Microbiology

Classification: Molecular Genetics, Microbial Ecology

Data description

Context

To date, incidence of infection caused by non-tuberculous mycobacteria (NTM) has been increasing all over the world [1]. Among NTMs, *Mycobacterium avium* complex (MAC) is one of the most critical agents. MAC has 4 subspecies, namely *M. avium* subsp. *avium* (MAA), *M. avium* subsp. *paratuberculosis* (MAP), *M. avium* subsp. *silvaticum* (MAS) and *M. avium* subsp. *hominissuis* (MAH). MAH is known as a major pathogen for humans, causing lung disease and sometimes disseminated infection in immune suppressed patients [2, 3]. MAH is also a main causative agent of mycobacteriosis in pigs [4], showing mesenteric and mandibular lymphadenitis [5] and sometimes systemic infection [6]. Swine mycobacteriosis exerts severe economic impact in affected farms. MAH infected pigs are suspected as potential risk for human infection [7, 8, 9, 10].

Recently, genomic epidemiological study of MAH has extensively progressed. In our recent studies, MAH is divided into 6 major lineages (MahEastAsia1, MahEastAsia2, SC1 - SC4) and each lineage is predominant in specific regions on a global scale [11, 12]. For example, the

MahEastAsia1 and MahEastAsia2 are frequently isolated from human lung disease in Japan and Korea although SC1 – 4 are isolated from America and Europe [11, 12]. Japanese pig isolates are mainly classified into 2 lineages, SC2 and SC4 [11, 12]. However, from the one health point of view, to exactly clarify the ecology of MAH, the number of pig isolates used in these studies was insufficient.

As stated above, genome-based analysis of MAH has been proceeding and the most essential genes of MAH are thought to be mutual orthologues of genes in *Mycobacterium tuberculosis* (MTB) [13]. Although components of virulence systems have been investigated [14], reports about genome contents, even drug resistance genes are not available, despite the increasing the incidence of MAH disease [1]. To understand MAH evolution, distribution and to promote the identification of targets for antimicrobial drug discovery, the characterization of the defining genomic features of MAH is essential.

Here we obtained draft genome sequences of 30 MAH isolates derived from pigs reared in Japan, and identified genome features for bacterial defense systems, such as restriction modification (RM) system, clustered regularly interspaced short palindromic repeat (CRISPR), tRNA arrays, virulence factors and drug resistance genes. Our results in this study may provide a way to understand the epidemiological relationship of MAH in human and pigs.

Methods

a) Sampling

MAH isolates were collected from pigs reared at two areas, Tokai and Hokuriku in Japan, where about 10 % of pigs in Japan are reared. 48 mesenteric or mandibular lymph nodes of pigs reared in Tokai area were collected from Gifu meat inspection center from July – December, 2015. Samples (20: mesenteric lymph nodes, 1: mandibular lymph nodes, 1: liver) of Tokai and Hokuriku area were collected between August, 1998 – Mar, 2018 and archived in Toyama meat inspection center.

130

131 b) Bacterial isolation and DNA extraction

132 The method of bacterial isolation was available in protocols. io [15]. The mesenteric or
133 mandibular lymph nodes with mycobacterial granulomatous lesions were mixed with 400ul
134 of 2% NaOH and incubated at room temperature overnight. The samples were spread onto
135 2% Ogawa medium (Kyokuto Pharmaceutical, Tokyo, Japan) and incubated at 37 °C for 3 – 4
136 weeks. A single colony was inoculated onto 7H11 broth with 10% oleic acid-albumin-
137 dextrose-catalase as a supplement. The isolates were stored with Microbank (Pro Lab
138 Diagnostics Inc., Richmond Hill, ON, Canada) at -80°C. The method of extraction of genomic
139 DNA was also available in protocols. io [16]. In brief, cells were delipidated by treatment with
140 acetone, then lysed by lysozyme and Proteinase K. Genomic DNA was extracted by
141 phenol/chloroform treatment of the lysates.

142

143 c) Identification of MAH and insertion sequence profile

144 PCR amplification of *M. avium* 16S rRNA genes (MAV) was conducted for screening [17].
145 Isolates positive for MAV were identified by sequencing *hsp65* and *rpoB* genes [18, 19]. Basic
146 Local Alignment Search Tool (BLAST) analysis was conducted using partial sequences of *rpoB*
147 gene. Phylogenetic analysis of both genes was conducted by maximum likelihood method
148 using Molecular Evolutionary Genetics Analysis (MEGA) software ver. 7.0. Bootstrap values
149 were calculated from 1,000 replications. Insertion sequence patterns of IS900, IS901, IS902
150 and IS1245 were performed as described previously [20, 21, 22]. IS1311 and IS1613 were
151 searched for within draft genomes by using ISfinder (<https://isfinder.biotoul.fr>) with default
152 parameters [23].

153

154 d) Draft genome sequences and genome annotation

155 Extraction of genomic DNA was described above. An average 350-bp paired-end libraries
 156 were prepared from extracted genomic DNA by TruSeq DNA PCR-Free High Throughput
 157 Library Prep Kit (Illumina, San Diego, CA, USA). Pair-end sequencing (2× 150-bp) was
 158 conducted using the HiSeq X Ten sequencing platform (Illumina) at the Beijing Genomics
 159 Institute (Shenzhen, China). Output reads were trimmed by TrimGalore!
 160 (<https://github.com/FelixKrueger/TrimGalore>) and were corrected its mismatched reads by
 161 SPAdes ver 3.12.0. [24]. The reads were assembled and polished using Pilon [25] and
 162 Unicycler [26], and then genome completeness was estimated by CheckM [27]. Taxonomic
 163 classification of contigs was carried out using Kaiju [28] and Anvi'o [29]. Draft genome
 164 sequences were annotated via the National Center for Biotechnology Information (NCBI)
 165 Prokaryotic Genome Annotation Pipeline (PGAP) [30].

166

167 e) Detection of bacterial defence systems (RM system and CRISPR CAS system) in MAH
 168 genome

169 RM systems were determined by online tool, Restriction-ModificationFinder version 1.1
 170 (<https://cge.cbs.dtu.dk/services/Restriction-ModificationFinder/>) twice with the following
 171 settings (1: database: All incl. putative genes, threshold for %ID: 90%, minimum length: 80%
 172 to search the RM system of MAH and 2: database: All, threshold for %ID: 10%, minimum
 173 length: 20% to confirm the orthologue of MTB or the other Mycobacteria) [31]. CRISPR Cas
 174 systems were identified by the online tool CRISPRCasFinder program
 175 (<https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/Index>) with default setting [32, 33].

176

177 f) Detection of tRNA arrays in MAH genome

178 Total number of tRNAs in this study were retrieved from gb files annotated by PGAP. Draft
179 genomes of GM17 and OCU479 isolates, which had more tRNAs than the others (Table 1),
180 were inspected by tRNAscan-SE (<http://lowelab.ucsc.edu/tRNAscan-SE/>) to search tRNA
181 arrays [34]. tRNA gene isotype synteny (expressed by the single-letter amino acid code) of
182 both isolates and the reference strains were aligned and used for the maximum likelihood
183 method by MEGA 7.0. Classification of both isolates was conducted as previously described
184 [35].

185

186 g) Detection of virulence factors and drug resistance genes

187 Virulence genes were identified by using VFAnalyzer (<http://www.mgc.ac.cn/VFs/main.htm>)
188 [36]. We selected the following settings, genus: *Mycobacterium*, specify a representative
189 genome: *M. avium* 104 and choose genomes for comparison: blank and draft genome fasta
190 files were uploaded. Drug resistance genes were identified by Resistance Gene Identifier
191 (RGI) version 5.1.0 (<https://card.mcmaster.ca/analyze/rgi>) with the following settings, Select
192 Data Type: DNA sequence, Select Criteria: Perfect and Strict hit only, Nudge $\geq 95\%$ identity
193 Loose hits to Strict: Exclude nudge, Sequence Quality: high quality/coverage [37]. To confirm
194 the existence of mutations detected by RGI, we retrieved the respective drug resistance
195 associated genes from draft genome sequences, aligned by MEGA 7.0., and then manually
196 checked for mutations in the nucleotide sequences.

197

198 **Data Validation and quality control**

Identification of MAH

The experimental workflow from sampling to identification is shown in Fig. 1. We successfully obtained 13 MAH isolates derived from Tokai area and 8 out of 13 isolates (GM5 – GM44) with 22 isolates of Tokai and Hokuriku area (OCU467 – OCU486, Toy194, Toy195) were used for draft genome sequence analysis. We conducted multiple examinations to determine the isolates as MAH, IS possession patterns, sequence analysis of *hsp65* (Supplementary Table 1). Among MAH subspecies, the patterns of IS possession is different and is used for subspecies identification [38]. *IS900* and *IS901* are known as the indicator of MAP and MAA, respectively [21, 22]. MAH is usually positive for *IS1245* [39], and is negative for *IS900*, *IS901* and *IS902* [20], however, MAH strains without *IS1245* are frequently distributed in Japan [39, 40]. In our study, 10/30 isolates were negative for *IS1245* (33.3%) and none had *IS900*, *IS901* and *IS902* (Supplementary Table 1). In general, subspecies of *M. avium* is also identified by *hsp65* gene analysis, which had 17 variations of SNP among subspecies [19]. MAH has usually 1, 2, 3, 7, 8 or 9 *hsp* code [19], however, five isolates had unclassified *hsp* code (indicated by N) in this study (Supplementary Table 1). Therefore, we also conducted partial sequence analysis of the *rpoB* gene and the isolates were identified as MAH by BLAST analysis. In addition, we conducted phylogenetic analysis based on *hsp65* and *rpoB* genes retrieved from draft genome and all isolates in this study were also classified into MAH (Fig 2). All of these examinations confirmed that our isolates were MAH.

Draft genome data

All of our draft genome sequences had a total length between 4.85 – 5.62 Mb, similar to complete MAH genomes [41, 42]. All isolates had over 24kb N50 and over 40 fold genome coverage (average 233) (Table 1).

Genome content analysis

In total, we identified 73 putative RM systems, including 24 type I RM systems, 48 type II RM systems, and 1 type III RM systems (Supplementary Table 2). All isolates had at least one Type II RM system and GM5, GM16, GM17, OCU468 – OCU470, OCU472, OCU473, OCU475, OCU476, OCU479, OCU483 and OCU484 had Type I, Type II RM systems, and GM44 had 3 types of RM systems. In these RM systems, 7 RM systems had homologues in MTB and 30 RM systems had homologues in *M. kansasii*. Orphan methyltransferase was detected in OCU473 and OCU479. CRISPR was detected only in GM44 (Supplementary Table 3). The sequences of the region were identical to MAH 104 (Query Cover: 100%, E value: 0.0, Per. Ident: 99.99%) which is the only MAH strain that had an intact CRISPR in the database (<https://crisprcas.i2bc.paris-saclay.fr/MainDb/StrainList>). The isolates had 185 – 222 virulence factors and 141 factors were common in all isolates (Supplementary Table 4). All isolates shared the same 2 drug resistance genes, *mtrA* which is associated with cell division and cell wall integrity [43] and resistance to macrolide antibiotics, and *RbpA* which regulates bacterial transcription and is associated with rifampicin resistance (Supplementary Table 5) [44]. In addition, single nucleotide polymorphisms (SNP) associated with drug resistance were found. All isolates had a C117D change in the *murA* gene conferring resistance to fosfomycin. A A2274G mutation in the *Mycobacterium avium* 23S rRNA which contributes to macrolide resistance was also detected by RGI, but when we examined the aligned nucleotide sequence, no point mutation was found in all isolates (Supplementary Table 5). CRISPR, virulence factor and drug resistance genes were selected from online tools. Original databases of each tool used in this study were updated in 2020, suggesting our data are based on the forefront of existing knowledge.

tRNA arrays

tRNA arrays were detected in isolates GM17 and OCU479 (Supplementary Table 6). tRNA array was discovered in some MAH isolates in the past study, and phylogenetic analysis

based on nucleotide sequences of tRNA array showed that tRNA array of MAH was classified into a specific group [35]. To confirm tRNA arrays in this study as authentic tRNA arrays, phylogenetic analysis was performed. Our tRNA arrays were classified into the group 3, as defined in a previous study (Fig. 3) [35].

Re-use potential

MAH is known as one of the most critical *M. avium* subspecies causing non-tuberculosis mycobacterial infection in human and pigs. Pigs are suspected to be the most dominant host of MAH in animal and a potential source of infection for human [7, 8, 9, 10]. However, the study about relationship with human and pig MAH isolates based on genome is limited [11, 12]. Our study provides 30 draft genome sequences of MAH isolated from pigs. We believe that these data will be useful for genome-based epidemiological studies to evaluate the importance of pigs as a source of infection. In addition, we provide molecular identification of defense systems, tRNA arrays, virulence factors and drug resistance genes. These data are expected to be used in future research of MAH classification, pathogenicity, and identification of antimicrobial drug targets. Principally, our draft genomes were derived from both cases of systemic and lymph node limited infection of MAH. Thus, the provided virulence factors can be included in the important candidate genes associated with systemic infection of pigs.

Data Availability

The summary information of draft genomes of the 30 MAH isolates are shown in Table 1. The genome sizes ranged to from approximately 4.8Mbps to 5.6Mbps. GC content was from 68.77% to 69.26%. All genome sequences have been deposited in GenBank under accession numbers VRUQ000000000, WEGO000000000 to WEGZ000000000 and WEHA000000000 to

WEHQ00000000, and SRA under accession numbers SRR13521605, SRR13556487 to SRR13556515.

Declarations

List of abbreviations

NTM: non-tuberculous mycobacteria; MAC: *Mycobacterium avium* complex; MAA: *M. avium* subsp. *avium*; MAP: *M. avium* subsp. *paratuberculosis*; MAS: *M. avium* subsp. *silvaticum*; MAH: *Mycobacterium avium* subsp. *hominissuis*; MTB: *Mycobacterium tuberculosis*; RM: restriction modification; CRISPR: clustered regularly interspaced short palindromic repeat; BLAST: Basic Local Alignment Search Tool; MEGA: Molecular Evolutionary Genetics Analysis; NCBI: National Center for Biotechnology Information; PGAP: Prokaryotic Genome Annotation Pipeline; SNP: single nucleotide polymorphism

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author's contributions

T.K, K.O and H.Y wrote the manuscript. K.M, A.H, S.S and K.S collected samples. K.O, J.O.O, S.S and K.S performed laboratory works. T.K, K.O, A.O, H.Y, J.O.O, T.Ito and M.K conducted computational analysis. Y.N, T.A, T.Y, H.F, T.W, S.Y, K.A designed methods. M.A, A.D.B, K.O, N.Y, T.Iwamoto and F.M designed whole research and advised on the interpretation of the study's findings. All authors reviewed the manuscript.

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Figure Legends

Figure 2. Phylogenetic analysis based on *rpoB* gene and *hsp65* gene. Phylogenetic tree was generated by maximum likelihood method using MEGA 7.0. All isolates in this study are indicated in bold font. **(a)** 30 MAH isolates in this study were classified as MAH and were differentiated from MAP and MAA/MAS node. **(b)** All the isolates in this study were classified into 5 *hsp* code, code 1, 2, 3, N1 and N4. These isolates were differentiated from MAP and MAA/MAS nodes. The bootstrap values were determined from 1,000 replications. The scale bar indicates genetic distances among strains.

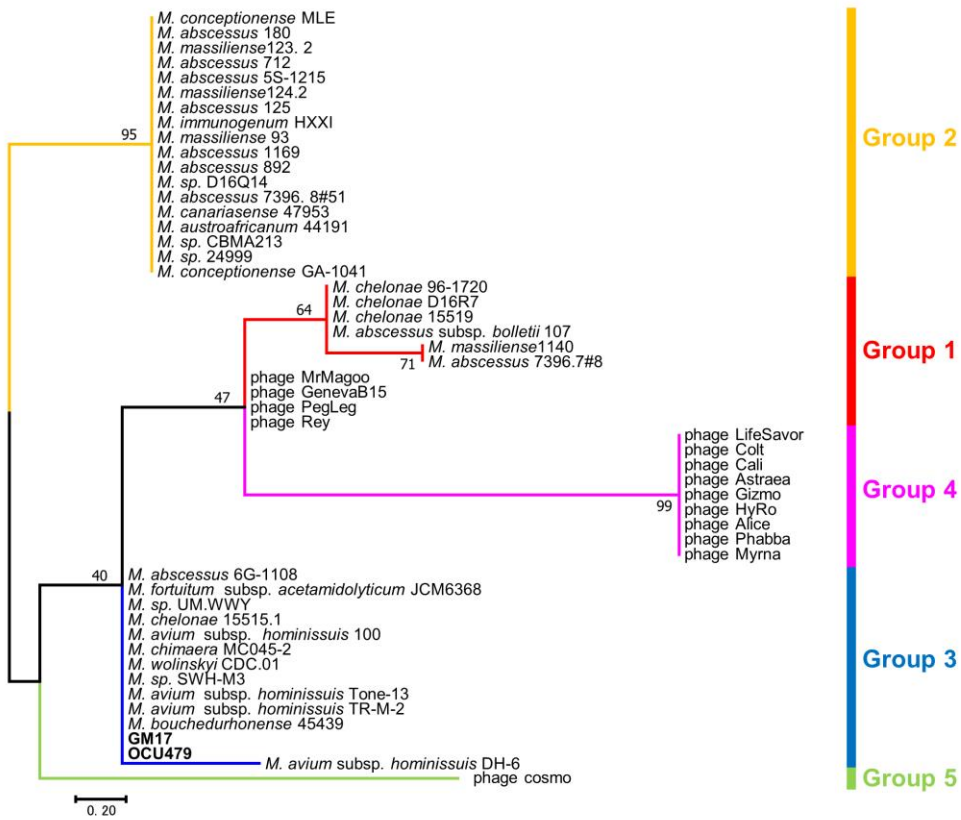


Figure 3. Phylogenetic tree based on the sequence of tRNA isotype located in tRNA array. Phylogenetic tree was generated by maximum likelihood method using MEGA 7.0. Two isolates (GM17 and OCU479 indicated in bold) were classified in Group 3. The bootstrap values were determined from 1,000 replications. The scale bar indicates genetic distances among strains.

Tables

336 **Table 1. Summary information for the draft genome sequences of 30 MAH isolates in this**
 337 **study.** * CDSs: coding sequences.

Isolate	Genome size (bp)	N50 (bp)	Coverage	No. of contig	G+C content (%)	No. of CDSs*	No. of tRNAs
GM5	5,037,010	35,760	277	224	69.06	4,877	47
GM10	4,858,055	33,212	277	248	69.16	4,708	47
GM12	4,848,678	33,219	253	261	69.17	4,732	47
GM16	5,012,047	24,262	274	346	68.84	4,981	46
GM17	5,265,075	30,906	355	289	68.77	5,190	75
GM21	4,899,737	45,080	411	216	69.20	4,734	47
GM32	4,897,271	47,147	292	208	69.20	4,712	47
GM44	5,086,547	26,307	251	316	68.95	4,780	46
OCU467	5,110,693	243,182	207	75	69.16	4,803	46
OCU468	5,459,638	137,464	198	132	68.96	5,176	46
OCU469	5,167,480	190,329	191	57	69.19	4,886	47
OCU470	5,388,572	124,661	220	132	68.98	5,103	46
OCU471	4,990,913	193,095	237	70	69.24	4,713	47
OCU472	5,410,552	119,264	180	139	68.97	5,163	47
OCU473	5,237,229	105,027	232	118	69.11	4,981	47
OCU474	5,087,878	168,670	213	81	69.26	4,817	47
OCU475	5,376,580	113,114	243	130	68.99	5,121	46
OCU476	5,359,545	133,302	268	132	69.00	5,094	46
OCU477	5,087,664	218,065	221	85	69.22	4,779	47
OCU478	5,108,303	272,265	230	73	69.17	4,803	46
OCU479	5,620,788	112,152	167	143	68.78	5,388	75

OCU480	5,088,946	195,446	53	73	69.24	4,820	47
OCU481	5,100,722	163,519	247	101	69.19	4,802	47
OCU482	5,100,769	163,705	244	99	69.19	4,800	47
OCU483	4,943,024	200,611	228	68	69.24	4,652	47
OCU484	5,096,430	141,792	249	104	69.20	4,811	47
OCU485	5,109,020	243,182	258	80	69.16	4,805	46
OCU486	5,023,805	234,302	40	52	69.23	4,722	47
Toy194	5,347,524	216,164	273	93	68.97	5,018	47
Toy195	5,346,468	168,809	192	103	68.97	5,029	47

338

339

340 **Supplementary Table 1. Isolates information and molecular characteristics of 30 MAH in**
341 **this study.** a: Detected IS was 1213bp and shared 83% identity with IS900.

342

343 **Supplementary Table 2. Restriction modification system detected in 30 MAH isolates in**
344 **this study.** ^{*1}: These genes include the function of restriction enzyme/methyltransferase. ^{*2}:

345 These genes could be orphan methyltransferase. Yellow background: putative genes.

346

347 **Supplementary Table 3. Detected CRISPR-Cas systems in MAH GM44.**

348

349 **Supplementary Table 4. Virulence factors detected in 30 MAH isolates in this study.**

350

351 **Supplementary Table 5. Drug resistance genes detected in 30 MAH isolates in this study.**

352

353 **Supplementary Table 6. The information about tRNA array detected in MAH isolates GM17**
354 **and OCU479.**

References

1. Daley CL. *Mycobacterium avium* complex disease. Microbiol Spectr. 2017;5.
2. Uchiya K, Takahashi H, Nakagawa T, Yagi T, Moriyama M, Inagaki T, et al. Characterization of a novel plasmid, pMAH135, from *Mycobacterium avium* subsp. *hominissuis*. PLoS One. 2015;10:e0117797.
3. Uchiya KI, Asahi S, Futamura K, Hamaura H, Nakagawa T, Nikai T, et al. Antibiotic susceptibility and genotyping of *Mycobacterium avium* strains that cause pulmonary and disseminated infection. Antimicrob. Agents Chemother. 2018;62:e02035-17.
4. Agdestein A, Johansen TB, Polaček V, Lium B, Holstad G, Vidanović D, et al. Investigation of an outbreak of mycobacteriosis in pigs. BMC Vet Res. 2011;7;:63.
5. Agdestein A, Johansen TB, Kolbjørnsen Ø, Jørgensen A, Djønne B, Olsen I, et al. A comparative study of *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *hominissuis* in experimentally infected pigs. BMC Vet Res. 2012;8:11.
6. Hibiya K, Kasumi Y, Sugawara I, Fujita J. Histopathological classification of systemic *Mycobacterium avium* complex infections in slaughtered domestic pigs. Comp Immunol Microbiol Infect Dis. 2008;31:347-66 .
7. Agdestein A, Olsen I, Jørgensen A, Djønne B, Johansen TB. Novel insights into transmission routes of *Mycobacterium avium* in pigs and possible implications for human health. Vet Res. 2014;45:46.
8. Johansen TB, Olsen I, Jensen MR, Dahle UR, Holstad G, Djønne B. New probes used for IS1245 and IS1311 restriction fragment length polymorphism of *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *hominissuis* isolates of human and animal origin in Norway. BMC Microbiol. 2007;7:14..

- 379 9. Klanicova B, Slana I, Vondruskova H, Kaevska M, Pavlik I. Real-time quantitative PCR
380 detection of *Mycobacterium avium* subspecies in meat products. J Food Prot.
381 2011;74:636-40.
- 382 10. Slana I, Kaevska M, Kralik P, Horvathova A, Pavlik I. Distribution of *Mycobacterium*
383 *avium* subsp. *avium* and *M. a. hominissuis* in artificially infected pigs studied by
384 culture and IS901 and IS1245 quantitative real time PCR. Vet Microbiol.
385 2010;144:437-43.
- 386 11. Yano H, Iwamoto T, Nishiuchi Y, Nakajima C, Starkova DA, Mokrousov I, et al.
387 Population structure and local adaptation of MAC lung disease agent *Mycobacterium*
388 *avium* subsp. *hominissuis*. Genome Biol Evol. 2017;9:2403-17.
- 389 12. Yano H, Suzuki H, Maruyama F, Iwamoto T. The recombination-cold region as an
390 epidemiological marker of recombinogenic opportunistic pathogen *Mycobacterium*
391 *avium*. BMC Genomics. 2019;20:752.
- 392 13. Dragset MS, Iøerger TR, Loevenich M, Haug M, Sivakumar N, Marstad A, et al. Global
393 assessment of *Mycobacterium avium* subsp. *hominissuis* genetic requirement for
394 growth and virulence. mSystems. 2019;4:e00402-19.
- 395 14. Bruffaerts N, Vluggen C, Roupie V, Duytschaever L, Van den Poel C, Denoël J, et al.
396 Virulence and immunogenicity of genetically defined human and porcine isolates of
397 *M. avium* subsp. *hominissuis* in an experimental mouse infection. PLoS One.
398 2017;12:e0171895.
- 399 15. [dx.doi.org/10.17504/protocols.io.bujenuje](https://doi.org/10.17504/protocols.io.bujenuje)
- 400 16. [dx.doi.org/10.17504/protocols.io.bupvvnv6](https://doi.org/10.17504/protocols.io.bupvvnv6)
- 401 17. Chen ZH, Butler WR, Baumstark BR, Ahearn DG. Identification and differentiation of
402 *Mycobacterium avium* and *M. intracellulare* by PCR. J Clin Microbiol. 1996;34:1267-9.

18. Kim BJ, Lee SH, Lyu MA, Kim SJ, Bai GH, Chae GT, et al. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). J Clin Microbiol. 1999;37:1714-20.
19. Turenne CY, Semret M, Cousins DV, Collins DM, Behr MA. Sequencing of *hsp65* distinguishes among subsets of the *Mycobacterium avium* complex. J. Clin. Microbiol. 2006;44:433-40.
20. Ahrens P, Giese SB., Klausen J, Inglis NF. Two markers, IS901-IS902 and p40, identified by PCR and by using monoclonal antibodies in *Mycobacterium avium* strains. J Clin Microbiol.1995;33:1049-53.
21. Kunze ZM, Wall S, Appelberg R, Silva MT, Portaels F, McFadden JJ. IS901, a new member of a widespread class of atypical insertion sequences, is associated with pathogenicity in *Mycobacterium avium*. Mol Microbiol. 1991;5:2265-72.
22. Sanderson JD, Moss MT, Tizard ML, Hermon-Taylor J. *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. Gut. 1992;33:890-6.
23. Siguier P, Perochon J, Lestrade L, Mahillon J. Chandler M. ISfinder: the reference center for bacterial insertion sequences. Nucleic Acids Res. 2006;34:D32-6.
24. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol. 2013;20:714-37.
25. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One. 2014;9:e112963.
26. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13:e1005595.

27. Parks DH, Imelfort M, Skennerton C T, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* 2015;25:1043-55.
28. Menzel P, Ng KL, Krogh A. Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nat Commun.* 2016;7:11257.
29. Eren AM, Esen ÖC, Quince C, Vineis JH, Morrison HG, Sogin ML, Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ.* 2015;3:e1319.
30. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, et al. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids. Res.* 2016;44:6614-24.
31. Roer L, Hendriksen RS, Leekitcharoenphon P, Lukjancenko O, Kaas RS, Hasman H, et al. Is the Evolution of *Salmonella enterica* subsp. *enterica* Linked to Restriction-Modification Systems? *mSystems.* 2016;1:e00009-16.
32. Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, et al. CRISPRCasFinder, an update of CRISPRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. *Nucleic Acids Res.* 2018;46:W246-51.
33. Grissa I, Vergnaud G, Pourcel C. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res.* 2007;35:W52-7.
34. Lowe TM, Chan PP. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Res.* 2016;44:W54-7.
35. Morgado SM, Vicente ACP. Beyond the Limits: tRNA array units in *Mycobacterium* genomes. *Front Microbiol.* 2018;9:1042.
36. Liu B, Zheng D, Jin Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. *Nucleic Acids Res.* 2019;47:D687-92.

37. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: antibiotic resistance surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res.* 2020;48:D517-25.
38. Ichikawa K, Yagi T, Moriyama M, Inagaki T, Nakagawa T, Uchiya KI, et al. Characterization of *Mycobacterium avium* clinical isolates in Japan using subspecies-specific insertion sequences, and identification of a new insertion sequence, ISMav6. *J Med Microbiol.* 2009;58:945-50.
39. Mijs W, de Haas P, Rossau R, Van der Laan T, Rigouts L, Portaels F, et al. Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* for bird-type isolates and '*M. avium* subsp. *hominissuis*' for the human/porcine type of *M. avium*. *Int J Syst Evol Microbiol.* 2002;52:1505-18.
40. Hibiya K, Kazumi Y, Nishiuchi Y, Sugawara I, Miyagi K, Oda Y, et al. Descriptive analysis of the prevalence and the molecular epidemiology of *Mycobacterium avium* complex-infected pigs that were slaughtered on the main island of Okinawa. *Comp Immunol Microbiol Infect Dis.* 2010;33:401-21.
41. Matern WM, Bader JS, Karakousis PC. Genome analysis of *Mycobacterium avium* subspecies *hominissuis* strain 109. *Sci Data.* 2018;5:180277.
42. Uchiya K, Takahashi H, Yagi T, Moriyama M, Inagaki T, Ichikawa K, et al. Comparative genome analysis of *Mycobacterium avium* revealed genetic diversity in strains that cause pulmonary and disseminated disease. *PLoS One.* 2013;8:e71831.
43. Gorla P, Plocinska R, Sarva K, Satsangi AT, Pandeeti E, Donnelly R, et al. *MtrA* response regulator controls cell division and cell wall metabolism and affects susceptibility of *Mycobacteria* to the first line antituberculosis drugs. *Front Microbiol.* 2018;9:2839.

- 476 44. Newell KV, Thomas DP, Brekasis D, Paget MS. The RNA polymerase-binding protein
 477 *RbpA* confers basal levels of rifampicin resistance on *Streptomyces coelicolor*. Mol
 478 Microbiol. 2006;60:687-96.