

# *Turicibacter bilis* sp. nov., a novel bacterium isolated from the chicken eggshell and swine ileum

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## Abstract

Three novel, anaerobic, Gram-positive bacteria were isolated from the eggshell of two separate white leghorn chicken flocks and the ileum of a healthy pig, and designated MMM721<sup>T</sup>, ISU324 and PIG517 respectively. Cells were pleomorphic and capable of forming long chains of rods or coccoid clusters. Phylogenetic analysis of the 16S rRNA gene sequences identified these strains to be within the genus *Turicibacter*, of which only one species, *Turicibacter sanguinis*, has been formally described. However, whole genome sequencing of novel isolates returned a digital DNA–DNA hybridization value of 22.5% and average nucleotide identity (ANI) values of 76.4% (ANiB) and 86.0% (ANIm), indicating divergence between the type strain MMM721<sup>T</sup> and *T. sanguinis*, suggesting the strains represented a novel species. The major fatty acid methyl esters of strain MMM721<sup>T</sup> were C<sub>16:0</sub>, C<sub>18:1</sub> ω7c and C<sub>18:0</sub>. The strains mainly produced the volatile fatty acid lactate, along with smaller amounts of acetate and butyrate. Together, these data indicate that MMM721<sup>T</sup>, along with ISU324 and PIG517, represent a novel species within the genus *Turicibacter*. We propose the name *Turicibacter bilis* sp. nov. for the species. The type strain is MMM721<sup>T</sup> (=ATCC TSD-238<sup>T</sup>=CCUG 74757<sup>T</sup>).

## INTRODUCTION

The genus *Turicibacter* comprises obligately anaerobic, Gram-positive bacteria belonging to the phylum *Firmicutes*, class *Erysipelotrichia*, order *Erysipelotrichales* and family *Turicibacteraceae* (formerly considered a member of the family *Erysipelotrichaceae*) [1]. Currently, there is only one designated species within the genus *Turicibacter*, *Turicibacter sanguinis*, which was isolated from a blood culture of a febrile adult human [2]. Strains of *T. sanguinis* have been isolated from other animals, and *T. sanguinis* DNA sequences have been identified in culture-independent studies in many species, including turkeys, swine and even termites [3–7]. However, few strains within the genus have been isolated. Here, we describe novel *Turicibacter* strains MMM721<sup>T</sup>, ISU324 and PIG517, which were isolated from the eggshell surface of two distinct flocks of chickens and the ileum of a healthy pig, respectively. We propose that these strains represent a novel species within the genus and propose the name *Turicibacter bilis* sp. nov., with strain MMM721<sup>T</sup> as the type strain.

Chicken egg washes in 1×PBS and ileal contents were treated 1:1 with 70% ethanol and incubated overnight to enrich for spores by killing vegetative cells, as described previously [8]. Spore preparations were plated on brain–heart infusion (BHI) agar (Difco), supplemented with 0.1% (v/v) whole bile isolated from healthy white leghorn chickens, 0.05% (w/v) L-cysteine hydrochloride and 0.0001% resazurin, and incubated at 42 °C under anaerobic conditions (chamber inflated with 85% N<sub>2</sub>, 5% CO<sub>2</sub>, 10% H<sub>2</sub>) in a Coy anaerobic chamber to isolate spore-forming anaerobes. Bacterial colonies were randomly selected for 16S rRNA gene amplification PCR and Sanger sequencing after 72 h with the previously described bacterial primers 27F-YM and 1492R, using previously described methods [9, 10]. A total of 192 chicken isolates and 32 pig isolates were screened. The

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**Abbreviations:** ANI, average nucleotide identity; BHI, brain heart infusion; dDDH, digital DNA–DNA hybridization; SCFA, short chain fatty acid. The GenBank accession numbers for the 16S rRNA gene sequences of strains MMM721<sup>T</sup>, ISU324 and PIG517 are MT500785, MT500789 and MT500788, respectively. Accession numbers for the two sets of raw Illumina MiSeq reads for MMM721<sup>T</sup>, ISU324 and PIG517 are SRR11784102, SRR11784103, SRR11825064, SRR11825065, SRR11825066 and SRR11825067, respectively. Accession numbers for the *de novo* whole genome assemblies of MMM721<sup>T</sup>, ISU324 and PIG517 are JABTTG0000000000, JABTTH0000000000 and JABTTI0000000000, respectively. The BioProject accession number for these strains is PRJNA631282.

Four supplementary tables and two supplementary figures are available with the online version of this article.

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**Table 1.** Comparison of strain MMM721<sup>T</sup> and *Turicibacter sanguinis* MOL361<sup>T</sup>

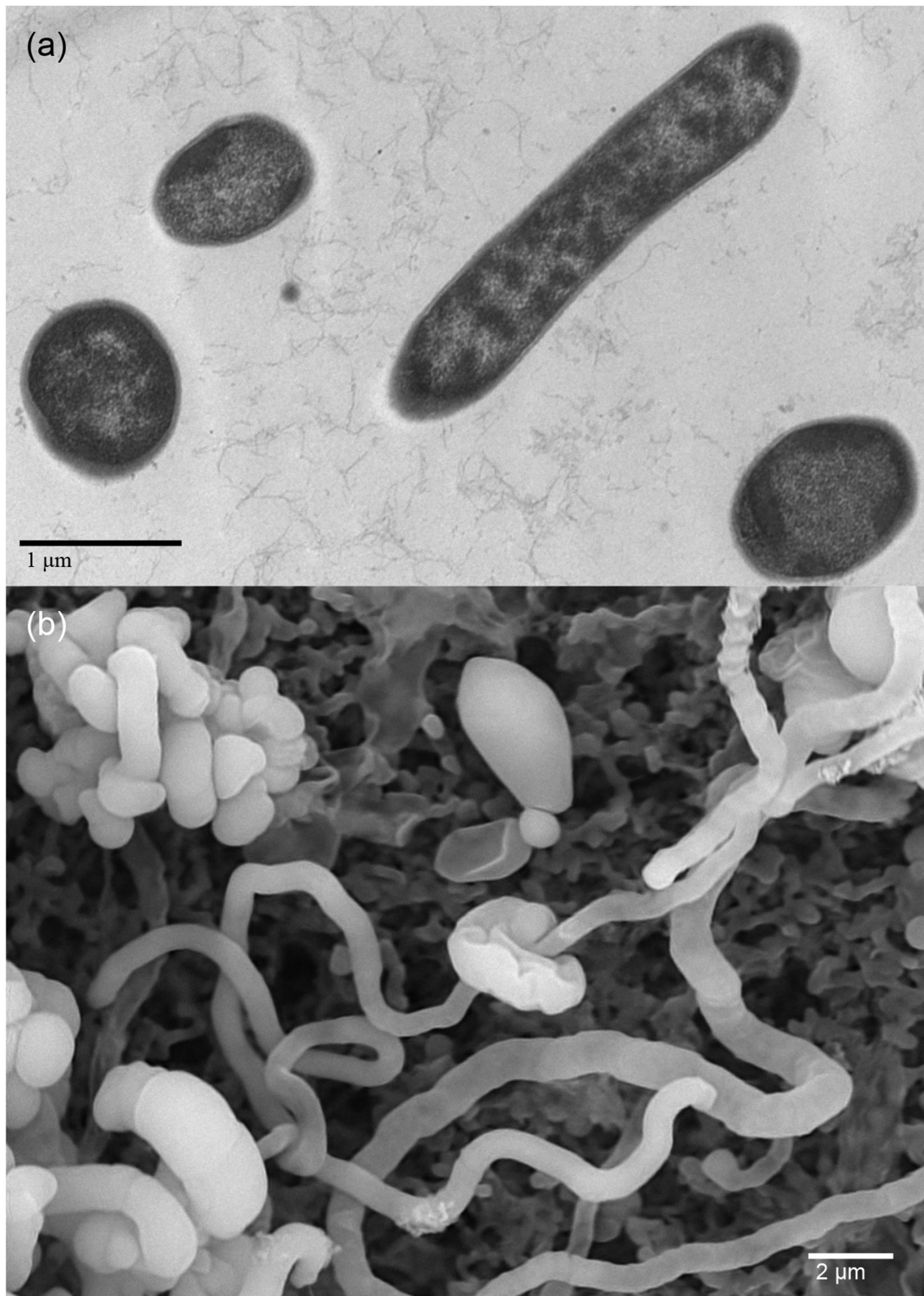
+, Positive; -, negative; w, weak; ±, variable; R, resistant; S, susceptible. Characteristics for *T. sanguinis* MOL361<sup>T</sup> were taken from Bosshard *et al.* [2]. SCFAs: Ac, acetic acid; But, butyric acid; Cap, caproic acid; Lac, lactic acid; Phen, phenylacetic acid; Val, valeric acid.

Characteristic	<i>T. sanguinis</i> MOL361 <sup>T</sup>	<i>T. bilis</i> MMM721 <sup>T</sup>
Morphology	Gram+ bacilli chains	Variable: Gram+ bacilli chains and Gram+ coccoid clusters
Cell size (µm)	0.5–2.0×0.7–7.0	Rods: 0.8–8.2×0.5–2.0 Cocci: 1.0–2.3
Temperature range for growth (°C)	25–46	30–45
pH range for growth (pH)	6.5–8.0	6.5–8.5
Motility	–	–
Catalase	–	–
Biochemical reactivity:		
α-D-Glucoside	+	–
α-D-Galactoside	+	–
Glycine	+	–
Arginine	–	+
Serine	+	–
Gelatin	+	+
Aesculin	±	±
maltose	+	–
Potassium 5-ketogluconate	w	–
Susceptibility to (50 µg ml <sup>-1</sup> ):		
Penicillin	S	S
Kanamycin	S	S
Vancomycin	S	S
Colistin	R	R
SCFAs	Ac, Lac	Ac, But, Lac, Val, Cap, Phen
DNA G+C content (mol%)	34.2	34.4

generated 16S rRNA gene fragments were 1392, 1397 and 1393 bp long for MMM721<sup>T</sup>, ISU324, and PIG517, respectively (accession numbers MT500785, MT500789 and MT500788). These sequences were nearly identical (>99%) to one another. When these sequences were assigned taxonomic identification using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST), they all grouped closely (99% 16S rRNA gene identity) to *T. sanguinis* MOL361<sup>T</sup>, the type strain and only described species within the genus *Turicibacter* [2, 11]. A 16S rRNA gene phylogeny was reconstructed with sequences from publicly available *Turicibacter* isolates downloaded from the Ribosomal Database Project (<http://rdp.cme.msu.edu/>). The resulting phylogenetic tree showed the three *T. bilis* isolates forming a clade separate from the *T. sanguinis* strains (Fig. S1, available in the online version of this article). All three strains were maintained on a modified BHI medium (BHIGL) supplemented with 1.0% (v/v) glycerol, 1.1% (w/v) sodium DL-lactate, 0.05% (w/v) L-cysteine hydrochloride and 0.0001% resazurin throughout the course of the study.

*Turicibacter sanguinis* MOL361<sup>T</sup> was obtained from DSMZ for use as a reference strain for comparison purposes. *Turicibacter sanguinis* MOL361<sup>T</sup> was capable of growth on BHIGL at 37°C, so this medium was used for strain maintenance throughout the course of this study.

Growth conditions were determined for MMM721<sup>T</sup> on BHIGL agar plates (Table 1). Determinations of growth rate and terminal OD<sub>600</sub> were determined in BHIGL broth inoculated with an agar plug of a single colony of MMM721<sup>T</sup> grown for 48 h on BHIGL. Cellular motility, catalase activity and sporulation activity were determined as described previously [8, 12–14]. Strain MMM721<sup>T</sup>



**Fig. 1.** (a) Transmission electron microscope image of MMM721<sup>T</sup> cells. Bar, 1 μm. (b) Scanning electron microscope image of MMM721<sup>T</sup> cells. Bar, 2 μm. Cells were cultured in BHIGL for 24 h at 42 °C prior to fixation and visualization.

**Table 2.** Fatty acid methyl esters of MMM721<sup>T</sup> and *Turicibacter sanguinis* MOL361<sup>T</sup>, grown on BHIGL media

Peak	<i>T. bilis</i> MMM721 <sup>T</sup>	<i>T. sanguinis</i> MOL361 <sup>T</sup>
10:0	0.29	0.12
11:0	–	0.07
12:0	1.08	0.56
13:0	–	0.11
14:0	2.84	1.88
15:0	0.86	2.65
C <sub>16:1</sub> ω9c	1.35	1.66
C <sub>16:1</sub> ω7c	1.41	1.33
C <sub>16:1</sub> ω5c	–	1.16
16:0	54.1	42.39
C <sub>17:1</sub> ω8c	–	0.75
C <sub>17:1</sub> ω6c	0.41	1.52
17:0	1.8	3.8
C <sub>16:0</sub> 3OH	–	0.15
C <sub>18:2</sub> ω6,9c	–	0.15
C <sub>18:1</sub> ω9c	5.78	5.78
C <sub>18:1</sub> ω7c	16.28	23.46
C <sub>18:1</sub> ω5c	1	1.65
18:0	11.76	8.92
19 cycloprop. 11,12	–	0.66
C <sub>20:1</sub> ω10c	1.04	0.75
20:0	–	0.47
Summed features:*		
C <sub>17:1</sub> ω8c and/or C <sub>17:2</sub> at 16.801	–	0.75
C <sub>18:1</sub> ω7c and/or unknown 17.834†	–	23.46

\*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total.

†Unknown fatty acids are not listed in the MIDI system library; values are the equivalent chain lengths.

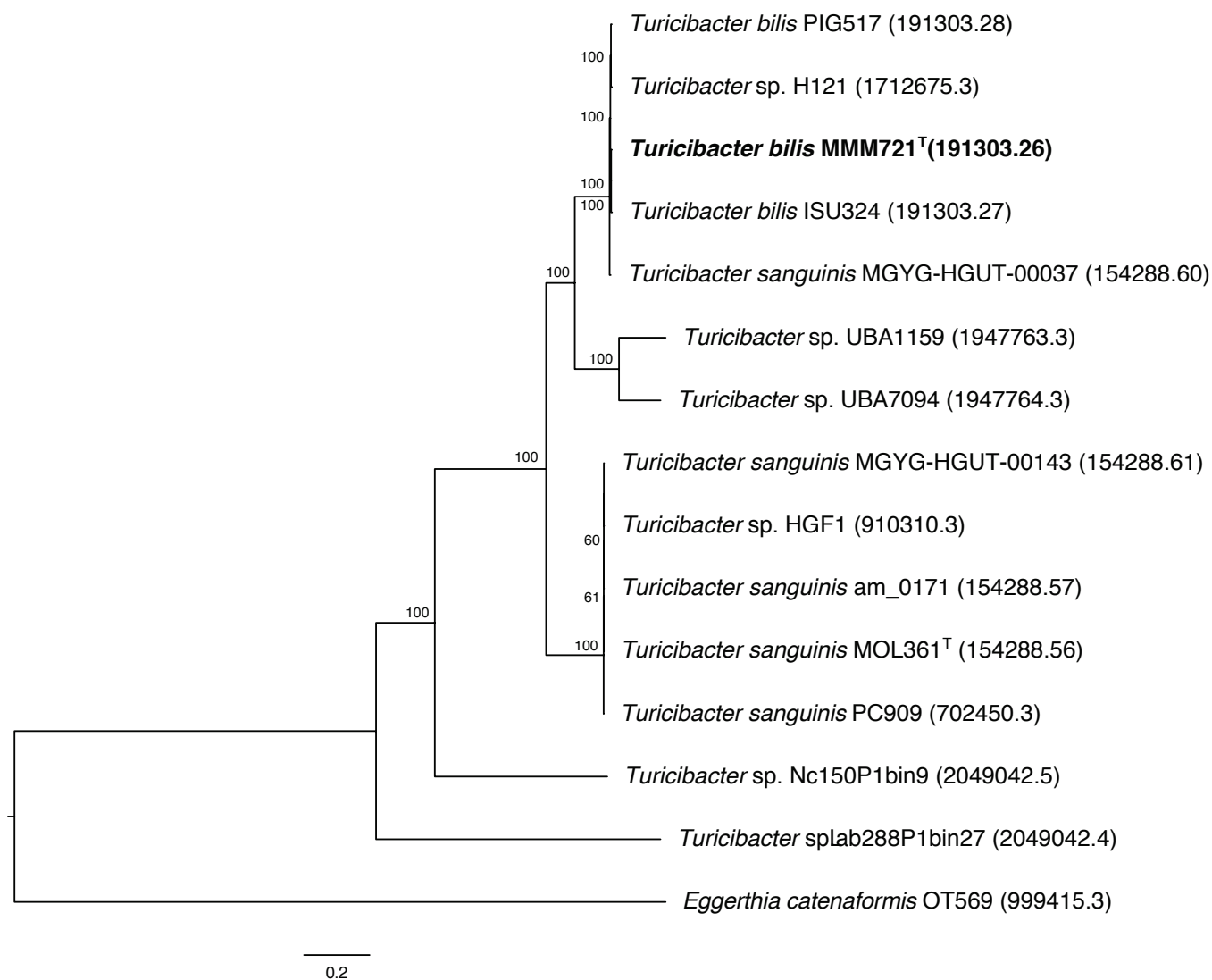
was strictly anaerobic, as exemplified by the absence of growth on aerobically incubated plates after 72 h. Strain MMM721<sup>T</sup> was capable of growth between 30 and 45 °C, with optimal growth achieved at 42 °C (Table 1). The pH range of MMM721<sup>T</sup> growth was between pH 6.5–8.5, with an optimal pH of 7.5 (Table 1). Resistance to 70% ethanol and survival under aerobic conditions suggested all strains were capable of forming spores. Strain MMM721<sup>T</sup> was negative for catalase activity (Table 1). The doubling time of MMM721<sup>T</sup> was estimated to be approximately 30 min. The terminal OD<sub>600</sub> was 0.600, corresponding to 3.75×10<sup>6</sup> c.f.u. ml<sup>-1</sup>.

Strain MMM721<sup>T</sup> was grown for 3 days on BHIGL and BHIGL agar +0.1% whole chicken bile to determine colony and cellular morphology. On BHIGL, colonies appeared as small to medium-sized irregularly shaped, umbonate colonies with undulating edges. The raised centre of the colony was an opaque white that transitioned to a translucent tan or grey when moving toward the colony's outer edge. On BHIGL +0.1% whole chicken bile, colonies appeared larger, with an opaque white, almost-filamentous interior that was raised with translucent, mucoid margins that were undulating to lobate in nature. Cells stained Gram-positive (Table 1). Cellular morphology was assessed with both transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For TEM, the bacteriological samples were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. They were post-fixed with 2% osmium and placed in 2% agar. Then the samples were processed through graded alcohols, propylene oxide

**Table 3.** Pairwise comparison of ANIb values between MMM721<sup>T</sup>, ISU324, PIG517 and all publicly available genomes on the PATRIC genome database

Strains: 1, *T. sanguinis* MGYG-HGUT-00143; 2, *Turricibacter* sp. H121; 3, *T. sanguinis* PC909; 4, *Turricibacter* sp. UBA1159; 5, *T. sanguinis* am\_0171; 6, *Turricibacter* sp. HGF1; 7, *Turricibacter* sp. UBA7094; 8, *T. sanguinis* MGYG-HGUT-00037; 9, *T. sanguinis* MOL361<sup>T</sup>; 10, *Turricibacter* sp. Lab288P1bin27; 11, *Turricibacter* sp. Nc150P1bin9; 12, *T. bilis* ISU324; 13, *T. bilis* MMM721<sup>T</sup>. Bolded values exceed the 95–96% threshold for species assignment. The \* indicates 100% similarity when genomes were compared against themselves.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*	76.15	<b>99.43</b>	73.73	<b>99.38</b>	<b>99.4</b>	73.96	76.36	<b>99.38</b>	65.87	67.07	76.34	76.27	76.45
2	76.96	*	76.59	76.5	76.66	76.15	76.87	<b>98.34</b>	76.62	66.06	67.2	<b>98.11</b>	<b>98.66</b>	<b>98.1</b>
3	<b>99.55</b>	76.15	*	73.8	<b>99.58</b>	<b>99.36</b>	74.03	76.36	<b>99.73</b>	65.93	67.07	76.45	76.24	76.4
4	73.89	76.35	73.86	*	73.85	73.89	81.88	76.4	73.81	65.96	67.02	76.4	76.36	76.35
5	<b>99.44</b>	76.02	<b>99.58</b>	73.55	*	<b>99.4</b>	73.83	76.2	<b>99.51</b>	65.86	67.19	76.12	76.05	76.21
6	<b>99.31</b>	75.86	<b>99.25</b>	73.72	<b>99.26</b>	*	73.89	76.02	<b>99.2</b>	65.86	66.78	76.08	75.98	75.99
7	74.33	77.18	74.33	82.13	74.25	74.36	*	77.3	74.3	66.06	67.04	77.18	77.29	77.21
8	76.46	<b>98.04</b>	76.25	76.38	76.3	76.2	77.22	*	76.3	66.29	67.62	<b>98.14</b>	<b>98.04</b>	<b>98.04</b>
9	<b>99.46</b>	76.18	<b>99.68</b>	73.83	<b>99.47</b>	<b>99.33</b>	74.04	76.42	*	65.89	67.22	76.57	76.24	76.6
10	66	66.25	66.08	66.03	65.96	65.99	65.86	66.18	66.06	*	65.4	66.31	66.25	66.31
11	67.02	67.18	67.02	67.04	67.06	67.02	66.89	67.27	67.03	65.49	*	67.22	67.24	67.18
12	76.39	<b>97.79</b>	76.44	76.23	76.23	76.21	76.72	<b>98.04</b>	76.44	66.14	67.55	*	<b>97.97</b>	<b>98.76</b>
13	76.36	<b>98.5</b>	76.177	76.54	76.06	76.06	76.84	<b>98.25</b>	76.14	66.08	67.31	<b>98.17</b>	*	<b>97.93</b>
14	76.51	<b>97.77</b>	76.45	76.27	76.17	76.23	76.92	<b>97.98</b>	76.43	65.92	67.32	<b>98.76</b>	<b>97.71</b>	*



**Fig. 2.** Whole genome sequence phylogeny of MMM721<sup>T</sup>, ISU324, PIG517 and all publicly available genomes on the PATRIC genome database as of November 2020. The tree is rooted by *Eggerthia catenaformis* OT569. Only bootstrap values >50% are noted for branch nodes. The PATRIC genome IDs are noted in parentheses after the strain name.

and Eponate 12 resin followed by a 48 h polymerization. An uranyl acetate and Reynold's lead stain were performed on the thin section before being examined with a ThermoFisher FEI Tecnai G2 BioTWIN electron microscope (FEI) and images were taken with Nanosprint12 camera (AMT) [15]. For SEM, bacterial cells were put through a 0.22  $\mu\text{m}$  Swinney filter and the filters were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. The samples were further processed through sequential exposure to osmium and thiocarbohydrazide [16]. Samples were dehydrated through graded alcohols and chemically dried with hexamethyldisilazane [17]. Samples were decorated with a thin coating of gold-palladium mixture and viewed on a TM3030Plus scanning electron microscope (Hitachi). Under magnification, strain MMM721<sup>T</sup> was pleomorphic in presentation, composed of long chains of rods (0.8–8.2 $\times$ 0.5–2.0  $\mu\text{m}$ ) as well as clusters of coccoid-like cells (1.0–2.3  $\mu\text{m}$ ) after 24 h of incubation (Fig. 1a, b, Table 1). Despite the appearance of multiple cellular morphologies, 16S rRNA gene sequencing of cells from the broth cultures pre- and post-microscopy sample collection confirmed the culture was free from contamination. Similar cellular pleomorphy was previously observed within the order *Erysipelotrichales*, most notably the animal pathogen *Erysipelothrix rhusiopathiae* [18].

The cellular fatty acid profile of strain MMM721<sup>T</sup> was compared to *T. sanguinis* MOL361<sup>T</sup> to assess the relatedness between the species. Both isolates were grown in BHIGL broth inoculated with an agar plug of a single colony and pelleted via centrifugation prior to submission to Microbial ID for cellular fatty acids analysis, using the MIDI Sherlock Microbial Identification System [19]. Abundant cellular fatty acids for MMM721<sup>T</sup> were similar to *T. sanguinis* MOL361<sup>T</sup>, though the proportions of those cellular fatty acids varied between the two species. The main cellular fatty acids were C<sub>16:0</sub>, C<sub>18:1</sub>  $\omega$ 7c and C<sub>18:0</sub> (Table 2).

The biochemical characteristic of the novel isolates MMM721<sup>T</sup>, ISU324 and PIG517 were assessed using the RapID Ana II (Remel), API 20A (bioMérieux) and API 50CHL (bioMérieux) systems according to the manufactures' instructions (Tables 1 and S1). All tests were conducted in duplicate. The RapID Ana II system and the API 20A systems are used for the identification of anaerobic bacteria while the API50 CHL system is used for the identification of *Lactobacillus* and other related genera. Strain MMM721<sup>T</sup> was only positive for arginine and gelatin biochemical reactivity, with variable aesculin hydrolysis activity (Table 1). Strain ISU324 was positive for gelatin and aesculin biochemical reactivity while strain PIG517 was capable of biochemical reactivity to  $\alpha$ -glucoside, arginine, gelatin and aesculin, with weak reactivity to serine, methyl  $\beta$ -D-xylopyranoside, D-glucose, arbutin, salicin, cellobiose and maltose (Table S1). Together, the novel strains presented biochemical reactivity profiles that differentiated them from *T. sanguinis* MOL361<sup>T</sup> (Tables 1 and S1).

Next, short chain fatty acid (SCFA) production was determined for MMM721<sup>T</sup>, ISU324 and PIG517 in response to the substrates to which the isolates exhibited strong biochemical reactivity. Isolates were grown in BHIGL broth supplemented with 10 mM of substrate, or 0.1% (w/v) in the case of gelatin, and inoculated with an agar plug of a single colony. Broth cultures were incubated for 48 h at 42 °C, at which point cells were pelleted by centrifugation and 1.0 ml supernatant was collected for SCFA analysis via gas chromatography (GC) using previously described methods [20, 21]. Samples for GC analysis were prepared in duplicate. The major products of fermentation were acetate, butyrate, and lactate (Tables 1, S1 and S2).

Antibiotic susceptibility was measured using BHIGL agar plates supplemented with 50  $\mu$ g ml<sup>-1</sup> of either kanamycin, colistin, vancomycin or penicillin G, as these antibiotics were previously assessed for *T. sanguinis* MOL361<sup>T</sup> [2]. Strain MMM721<sup>T</sup> was streaked on plates containing each of the four antibiotics in duplicate and was incubated for 48 h at 42 °C, with lack of growth being interpreted as susceptibility. After 48 h, only plates supplemented with colistin displayed growth, suggesting MMM721<sup>T</sup> was resistant or insensitive to colistin but susceptible to kanamycin, vancomycin, and penicillin G (Table 1).

Genomic DNA was extracted from MMM721<sup>T</sup>, ISU324 and PIG517 using the Purelink genomic DNA extraction minikit (Invitrogen) according to the manufacturer's instructions, and DNA quality was determined using a NanoDrop (Thermo Fisher Scientific), Qubit fluorimeter (dsDNA Broad Range kit; Life Technologies) and gel electrophoresis. A Nextera Flex barcoding kit (Illumina) was used to prepare 2x250 bp paired-end read genomic libraries for sequencing on an Illumina MiSeq instrument according to the manufacturer's instructions. Each isolate was sequenced twice on separate MiSeq runs. The raw Illumina reads are available on NCBI's sequence read archive (accession numbers: SRR11784102, SRR11784103, SRR11825064, SRR11825065, SRR11825066 and SRR11825067). Raw Illumina reads were uploaded to the PathoSystems Resource Integration Centre (PATRIC) web resource [22, 23]. For each strain, the raw reads for the two MiSeq runs were pooled and assembled using PATRIC's online genome assembly tool, selecting the 'SPAdes' assembly strategy and using default settings [24]. Draft assemblies were annotated through PATRIC's RASTtk-enabled Genome Annotation Service [22, 25]. The MMM721<sup>T</sup> draft assembly was composed of 94 contigs, with an assembly length of 2717947 bp and a G+C content of 34.4mol%. The draft assembly for ISU324 had 118 contigs, with an assembly length of 2751587 bp and 34.2mol% G+C content. The PIG517 draft assembly had contigs and totalled 2585443 bp in size, with a G+C content of 34.3mol%. The three isolate assemblies had similar G+C content to the draft assembly of *T. sanguinis* MOL361<sup>T</sup> available through the PATRIC genome database, though the genome length for MOL361<sup>T</sup> was 2946097 bp, nearly 200 kb higher than ISU324, which possessed the longest genome length of the three isolates (Table S1).

To determine divergence of the three *Turicibacter* isolates to other *Turicibacter* genomes, pairwise average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) calculations were conducted with MMM721<sup>T</sup>, ISU324, PIG517 and all publicly available *Turicibacter* species genomes on the PATRIC genome database (Tables 3, S3 and S4). Both ANI and dDDH provide an *in silico* method to delineate boundaries between species [26–28]. Pairwise ANI calculations were conducted using both BLASTN+ (ANIb) and MUMmer (ANIm) algorithms through the JSpecies Web Server (<http://jspecies.ribohost.com/jspeciesws/>) [29]. The dDDH calculations were made through the Genome-to-Genome Distance Calculator (version 2.1) using the recommended settings (<http://ggdc.dsmz.de/>) [28]. Typically, ANI values >95% and dDDH values >70% are considered to be the same species. Comparisons between MMM721<sup>T</sup>, ISU324 and PIG517 produced ANI values >97% (both ANIb/ANIm) and dDDH values >84%, suggesting all strains belonged to the same species. Compared to *T. sanguinis* MOL361<sup>T</sup>, MMM721<sup>T</sup>, ISU324 and PIG517 had ANIb values of 76.43, 76.44 and 76.14%, and ANIm values of 85.97, 86.04 and 85.79%, respectively, suggesting these isolates represent a distinct species from *T. sanguinis* (Tables 3 and S3). The ANI results were corroborated by the dDDH results, with values of 22.5, 22.5 and 22.1% obtained for MMM721<sup>T</sup>, ISU324 and PIG517 when compared to *T. sanguinis* MOL361<sup>T</sup> (Table S4). Interestingly, the ANI and dDDH results provided additional taxonomical information for several of the previously isolated and sequenced *Turicibacter* strains. Strains H121 and *T. sanguinis* MGYG-HGUT-00037 belong to the newly proposed species, along with MMM721<sup>T</sup>, ISU324 and PIG517 (Tables 3, S3 and S4). Strains UBA1159, UBA7094, Lab288P1bin27 and Nc150P1bin9 fell below the species threshold by ANIb, ANIm and dDDH metrics for both *T. sanguinis* MOL361<sup>T</sup> and MMM721<sup>T</sup>, suggesting that these strains could be divergent species within *Turicibacter* (Tables 3, S3 and S4).

To further compare the genomic features of the three *Turicibacter* isolates and *T. sanguinis* MOL361<sup>T</sup>, open reading frames (ORFs) from all four genomes were uploaded to eggNOG-mapper (<http://eggnog-mapper.embl.de>) for functional annotation and classification [30, 31]. A quadruple venn diagram of shared and unique ORFs was constructed using the 'venn()' function in the

statistical computing software R [32]. The four genomes shared a set of 1511 ‘core’ ORFs (Fig. S2). An additional 235 ORFs were shared between MMM721<sup>T</sup>, ISU324 and PIG517 while being absent from *T. sanguinis* MOL361<sup>T</sup>. *Turicibacter sanguinis* MOL361<sup>T</sup> possessed 748 unique ORFs. These large differences in conserved and unique ORFs between the three *Turicibacter* isolates and *T. sanguinis* MOL361<sup>T</sup> further suggest these strains represent a new species within *Turicibacter*.

A phylogenetic tree of publicly available *Turicibacter* genomes was reconstructed by using PATRIC’s Phylogenetic Tree Building Service using the ‘Codon Tree’ method on 100 single-copy genes allowing for up to five deletions/duplications (Fig. 2) [33]. This programme utilizes both DNA and amino acid sequences to build both gene and protein alignments, generating a phylogenetic tree from those alignments. *Eggerthia cateniformis* OT569 was used as an outgroup. Similar to the ANI and dDDH results, the codon tree had MMM721<sup>T</sup>, ISU324 and PIG517 together in a clade (including strains H121 and *T. sanguinis* MGYG-HGUT-00037), distinct from the clade containing *T. sanguinis* MOL361<sup>T</sup>, further evidencing these three strains constitute a novel species within the genus *Turicibacter* (Fig. 2).

## DESCRIPTION OF *TURICIBACTER BILIS* SP. NOV.

*Turicibacter bilis* (bi’lis. L. gen. n. *bilis* of bile).

Strictly anaerobic, Gram-positive, catalase-negative, non-motile, spore-forming, pleomorphic micro-organisms. Supplementation of media with 0.1% (v/v) chicken bile induces spore germination. Growth is poor in broth alone, but improves significantly in biphasic media or broth with an agar plug. Cells primarily exist as long chains of irregular rods (0.8–8.2×0.5–2.0 µm) or as individual or clusters of coccoid cells (1.0–2.3 µm). The organism is capable of growth between 30–45 °C and at pH 6.5–8.5, with optimums of 42 °C and pH 7.5, respectively. Colonies are visible on BHIGL agar after 1–2 days and appear as small to medium-sized irregularly shaped, umbonate colonies with undulating margins, with the raised centre an opaque white that transitions to a translucent tan or grey at the margins. Colonies are 2.5–5.0 mm after 3 days at 42 °C. The primary fermentation end products are acetate, butyrate and lactate. The organism is positive for arginine and gelatin biochemical reactivity.

The type strain is MMM721<sup>T</sup> and was isolated from the surface of a chicken eggshell (=ATCC TSD-238<sup>T</sup>=CCUG 74757<sup>T</sup>). The genome is 2.7 Mb with a G+C content of 34.4mol%.

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### Conflicts of interest

The authors declare that there are no conflicts of interest.

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