Taxonomic description and genome sequence of *Christensenella intestinhominis* sp. nov., a novel cholesterol-lowering bacterium isolated from human gut

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28

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31

32 Abstract

33 A Gram-staining-negative, non-spore-forming, short, straight rod, non-motile, obligate

34 anaerobic bacterial strain, AF73-05CM02^T, was isolated from faecal sample of thirty-years

35 old healthy male. Colonies were approximately 0.2mm in diameter, beige and circular after 4

36 days of incubation on PYG agar under anaerobic conditions at 37°C. Strain AF73-05CM02^T

37 grew in a temperature range between 30-42°C and pH range from 6.0 to 8.5, with optimum

38 growth at 37-42°C and pH 7.0. Base on the 16S rRNA gene sequence analysis, strain

39 AF73-05CM02^T belongs to the genus *Christensenella* and showed the highest level of

40 sequence similarity with *Christensenella minuta* YIT 12065^T. The predominant fatty acids of

41 strain AF73-05CM02^T were C_{10:0} (7.5%), iso-C_{11:0} (5.6%), C_{12:0} (7.2%), C_{14:0} (46.6%),

42 iso-C_{15:0} (7.4%), C_{16:0} (9.7%) and C_{18:1} ω_{9c} (6.9%). Acetic acid, formic acid, butyric acid and

43 lactic acid were end products of glucose fermentation. The isolate was negative for catalase,

44 indole production and hydrolysis of gelatin. Genomic relatedness analyses based on average

nucleotide identity indicated strain AF73-05CM02^T significantly differed from other species of the genus *Christensenella*, showing ANI values under 82.89% with the phylogenetically closest species. The G+C content of the genomic DNA was 52.07 mol% from genome sequence, which differs from that of *Christensenella minuta*. Several physiological biochemical and genotypic properties differentiated the novel bacterial strain from the related species, indicating that the isolate represent a new species of the genus *Christensenella* for which the name *Christensenella intestinhominis* sp. nov. is proposed, with strain AF73-05CM02^T (=CGMCC 1.5207^T =DSM 103477^T) being the type strain. The follow study approached the function of cholesterol-lowering for strains AF73-05CM02^T and DSM 22067^T revealed the two strains exhibit capacity for removing cholesterol with efficiency of 36.6% and 54.3%. Exopolysaccharide production of two strains were 234 and 271 mg/L, respectively.

Introduction

The human gut is colonised by a large and complex community of microorganisms ranging from 10¹³ to 10¹⁴ microbial cells (Ventura, 2009; Ghosh, 2013), which is equivalent to 10 times the number of human cells (Bäckhed F, 2005; Jeffery et al., 2016). The microbiome begin to resident the intestinal tract shortly after birth and develops over the first few years (Palmer et al., 2007). The composition of the microbiota is affected by many factors, including the genetic background of the host (Khachatryan et al., 2008; Benson et al., 2010; Fan et al., 2020), the host immune status (Hooper et al., 2012), living condition and daily diet (Turnbaugh et al., 2009; Fujimura et al., 2010). Approximately 90% of gut microbiota were affiliated with the two bacterial phyla,

67 including *Firmicutes* and *Bacteroidetes* (Turnbaugh et al., 2006; Turnbaugh et al., 2008;
68 Tremaroli and Backhed, 2012). *Christensenella minuta* YIT 12065^T, the type species of genus
69 *Christensenella* within the family *Christensenellaceae*, isolated from human faeces, was first
70 proposed by Morotomi, *et al* (Morotomi et al., 2012). Phylogenetically the isolate formed a novel
71 family-level lineage within the order *Clostridiales* with 86.9–86.1% 16S rRNA gene sequence
72 similarity with the closest relatives. *C. minuta* YIT 12065^T was identified as Gram-negative,
73 non-motile, non-spore-forming, short, straight rod with tapered ends and grows anaerobically. The
74 major fatty acids are iso-C_{15:0}, C_{14:0} and C_{16:0}. LL-diaminopimelic acid is present in the cell wall.
75 The draft genome of *C. minuta* YIT 12065^T has been reported previously (Rosa et al., 2017; Coil
76 et al., 2020). The recent research found that strain *C. minuta* as a beneficial bacteria has a
77 significantly effect in protecting against obesity (Goodrich et al., 2014), which might be a novel
78 approach in the treatment of obesity.

79 Cholesterol is an important basic substance for human body. However, a elevated level of blood
80 cholesterol increases the risk of cardiovascular diseases (CVDs) (Tok and Aslim, 2010; Tsai et al.,
81 2014), which remain the leading cause of deaths worldwide (Ishimwe et al., 2015). In recent years,
82 probiotics have been developed as a non-drug therapy to reduce blood lipids and cholesterol levels
83 and the risk of CVDs (Pan et al., 2010; Tsai et al., 2014). The mechanisms of cholesterol-lowering
84 was proposed for several hypotheses, including deconjugation of bile by bile salt hydrolase
85 activity (Lye et al., 2010a), assimilation and conversion of cholesterol by probiotics (Gilliland et
86 al., 1985; Lye et al., 2010b), and modulating the cholesterol absorption in the intestines of the host
87 (Huang and Zheng, 2010; Yoon et al., 2013).

88 In the present study, we focus on the polyphasic taxonomic approach for a novel strain, *C.*

89 *intestinihominis* sp. nov. AF73-05CM02^T, along with the whole genome sequencing and
90 annotation data, and investigating its cholesterol-lowering property.

91

92 **Materials and Method**

93 **Strain isolation**

94 During study the composition of human gut microbiota and construct its taxonomic position by
95 using a polyphasic approach based on phenotypic characteristics and genotypic properties, we
96 isolated a novel *Christensenella*-like strain, designated AF73-05CM02^T. The fresh faecal sample
97 was collected from a healthy adult residing in Shenzhen, China, and brought back to the lab use
98 for bacteria isolation, The specific location of the studies (GPS coordinates) was 37°35'37"N
99 114°15'32"E. For cultivation, approximately 1 g fresh faecal was transferred into anaerobic box
100 (Bactron Anaerobic Chamber, Bactron□-2, shellab, USA) with a gas phase of N₂/H₂/CO₂ (90 : 5 :
101 5, v/v) and dispersed in 0.1 M PBS (pH 7.0). This suspension containing bacteria was mixed
102 thoroughly and serially diluted and spread onto peptone-yeast extract-glucose (PYG) plates as
103 described previously (Zou et al., 2019) . The plate was incubated at 37 °C for 1 week under
104 anaerobic condition. Single colonies were picked and purified by inoculation and subculturing on
105 the same medium. In this study, one of these strains, designated AF73-05CM02^T was maintained
106 as a glycerol suspension (20%, w/v) at -80°C. The type strain of genus *Christensenella*, *C. minuta*
107 DSM 22607^T, procured from the Deutsche Sammlung von Mikroorganismen und Zellkulturen
108 (DSMZ), Braunschweig, Germany, was used as reference strain for phenotypic characterization,
109 genomic comparison and analyses of cell fatty acids and maintained under the same conditions.

110 **16S rRNA gene sequencing and phylogenetic analysis**

111 The genomic DNA of strain AF73-05CM02^T was prepared from cells harvested from PYG broth
112 using the phenol:chloroform method (Cheng and Jiang, 2006). The 16S rRNA gene was amplified
113 using the universal bacterial primers 27F-1492R (5'-AGAGTTTGATCATGGCTCAG-3' and
114 5'-TAGGGTTACCTTGTACGACTT-3') and purified as described by Zou *et al.* (Zou et al.,
115 2013). Sequencing was performed by BGI-Shenzhen (Shenzhen, China). The resulting sequence
116 was compared with sequences of type strains retrieved from the EzBioCloud server (Yoon et al.,
117 2017) (<https://www.ezbiocloud.net/>) using BLAST. Phylogenetic analysis was performed using
118 software package MEGA 7.0 (Tamura et al., 2011) after multiple alignment of sequences data by
119 using CLUSTAL W program (Thompson et al., 1994). Evolutionary phylogenetic trees were
120 constructed using the neighbour-joining method (Saitou and Nei, 1987), maximum-likelihood
121 (Felsenstein, 1981) method and minimum-evolution method (Rzhetsky and Nei, 1993) and
122 bootstrap values were calculated based on 1000 replications.

123 **Genome sequencing, GC content and genome comparison**

124 For genome comparison of the novel isolate and the closely related species, we conducted genome
125 sequencing and assembly of strain AF73-05CM02^T. DNA extraction and purity were described
126 above. The draft genome sequence was carried out using a paired-end sequencing strategy with
127 Ion Proton Technology (Life Technologies) at BGI-Shenzhen (Shenzhen, China). The paired-end
128 library had an mean insert size of 500 bp. Reads were assembled using the SOAPdenovo 2
129 package (Luo et al., 2012). The genomic DNA base content (mol% G+C) was directly calculated
130 from the draft genome data. To determine the DNA relatedness between the isolate and most
131 closely related species, *C. minuta* DSM 22607^T and *C. hongkongensis* HKU16^T (Lau et al., 2007;
132 Lau et al., 2015), we calculated the average nucleotide identity values (Damodharan et al.), which

133 was thought to be able to corresponds to DNA–DNA hybridization (Goris et al., 2007; Tindall et
134 al., 2010), as described by Kim *et al.* (Kim et al., 2014), following the BLAST-based ANI
135 calculation using the EzGenome web service. ANI values of 95–96% corresponding to 70% DDH
136 has been proposed as a threshold value for species delineation in bacterial taxonomy. The obtained
137 draft genome sequences were annotated using the Rapid Annotation Subsystem Technology
138 (RAST) server (Kanehisa et al., 2016) and KEGG (Aziz et al., 2008) and COG databases
139 (Galperin et al., 2015). A visual genomic comparison across strain AF73-05CM02^T and most
140 closely related species was generated with CGView server (Grant and Stothard, 2008)
141 (http://stothard.afns.ualberta.ca/cgview_server/index.html).

142 **Morphological and growth characteristics**

143 Morphological and cultural characteristics were investigated with strain AF73-05CM02^T
144 incubated in PYG medium at 37°C. Morphological observations were examined using both phase
145 contrast microscopy (Olympus BX51, Japan) and transmission electron microscopy (TEM,
146 HITACHI-8100). The Gram reaction, spore formation and presence of flagella were performed by
147 staining using Gram stain kit (Solarbio), spore stain kit (Solarbio) and flagella stain kit (Solarbio)
148 according to the manufacturer's instructions. Cell motility was examined using semisolid PYG
149 (0.4% agar) (Tittsler RP, 1936). Colony morphology was observed for cultures grown on PYG
150 agar for 4 days at 37°C. Growth at 4, 10, 20, 25, 30, 35, 37, 45 and 50°C was tested on PYG
151 medium to determine the optimal temperature and temperature range for growth. The pH range for
152 growth was evaluated at pH 3.0–10.0 (at interval of 0.5 pH units) by adjusting the pH using the
153 appropriate buffers as described by Sorokin (Sorokin, 2005). Tolerance to NaCl was determined in
154 PYG broth containing different concentrations of NaCl (0-6%, in increments of 1.0%). Bilt

155 tolerance was also measured at different bile salt concentrations (0-5%, in increments of 1.0%) in
156 the PYG broth contained all of the ingredients. All the growth tests of incubation under anaerobic
157 condition for 2 weeks was determined by measuring the OD₆₀₀.

158 **Physiological and biochemical characteristic**

159 For physiological and biochemical analyse, enzyme activities, hydrolytic activities, utilization of
160 various substrates as sole carbon sources and acid production from different carbohydrates, were
161 carried out for strain AF73-05CM02^T comparison with the closely related species, *C. minuta* DSM
162 22607^T, using API ZYM, API 20A and API 50CHL systems (bioMérieux, Marcy l'Etoile, France).
163 Sample preparation and test were performed following the directions of the manufacturer's
164 instructions with incubation at 37°C in an anaerobic condition. For API 50CHL test, CHL broth
165 was supplied with 0.05% cysteine hydrochloride for cells suspension and incubation. Catalase
166 activity was assessed by 3% H₂O₂ solution using cells collected from colonies incubated on PYG
167 agar at 37°C for 5 days (Smibert RM, 1994). The isolate and reference type strain were tested
168 under same laboratory conditions.

169 **Chemotaxonomical characteristic**

170 Chemotaxonomic characteristics of strain AF73-05CM02^T and the reference strain were
171 performed by detection of cellular fatty acids and cell wall composition. Strains were cultured on
172 PYG plates at 37°C for 5 days under anaerobic conditions and the fatty acid methyl esters
173 (FAMES) profile was prepared from lyophilized cells grown in PYG medium by extraction and
174 methylation as described previously (Chen and Dong, 2004). Determination of the fatty acid was
175 analysed by an Agilent HP6890 gas chromatograph and identified using MIDI microbial
176 identification system (M, 1990) and carried out by CGMCC (China General Microbiological

177 Culture Collection Center, Beijing, China). The cell-wall peptidoglycan of strain AF73-05CM02^T
178 was performed using wet cell biomass (incubated at 37°C for 5 days on PYG plates) and the
179 amino acid contents in peptidoglycan were determined by TLC as described by Zou, *et al.* (Zou et
180 al., 2013).

181 **Susceptibility tests and Hemolytic activity**

182 Susceptibility to antibiotics of strain AF73-05CM02^T was analysed by the disc diffusion method
183 according to Nizami *et al* (Duran et al., 2012). Antibiotic discs (HANG WEITM, China) were
184 placed on PYG agar plates inoculated with prepared suspensions of the test organisms. The
185 diameter of each zone was measured in millimeters after incubated at 37°C for 5 days. The
186 following antibiotic discs were tested: penicillin (10 ug), ampicillin (10 ug), carbenicillin (100 ug),
187 vancomycin (30), oxacillin (1 ug), piperacillin (100 ug), polymyxin B (300IU), compound
188 sulfamethoxazole (25), furazolidone (300), chloroamphenicol (30) and clindamycin (2).
189 Hemolytic activity was determined in sheep blood agar plates (Guangdong Huankai Microbial
190 Sci&Tech.Co., Ltd.). The plates were incubated under anaerobic conditions for 5 days at 37°C and
191 checked for hemolysis (Pineiro and Stanton, 2007).

192 **Metabolic end products analysis**

193 The metabolic end products of glucose fermentation, including short-chain fatty acids (SCFAs)
194 and organic acids, were performed using gas chromatograph (GC-7890B, Agilent) equipped with
195 capillary columns and detected using a flame-ionization detector (FID). The capillary column was
196 packed with Agilent 19091N-133HP-INNOWax porapak HP-INNOWax (30m × 0.25mm ×
197 0.25um) for SCFAs detection and Agilent 122-5532G DB-5ms (40m × 0.25mm × 0.25um) for
198 organic acids. The metabolic end products of strain AF73-05CM02^T were compared with the

199 closely related species of the genus *Christensenella*.

200 **The property of Exopolysaccharide (EPS) production**

201 The functional properties of strains AF73-05CM02^T and *C. minuta* DSM 22607^T were determined
202 by investigating the production of exopolysaccharide (EPS). The EPS were isolated from
203 fermentation solution of two strains using the method as described previously (Mercan et al.,
204 2015). Strains were inoculated in PYG broth at 37°C for 3 days and the cultures were boiled at
205 100°C for 15 min. The bacterial supernatant was collected after centrifugation at 10,000g for 30
206 min at 4°C and treated with 80% trichloroacetic acid solution and stirring overnight for
207 precipitating protein. Sample was centrifuged at 10,000g for 30 min at 4°C. The pH of the
208 supernatant was adjusted to 7.0 with 2 M NaOH. The supernatant was then precipitated by adding
209 double-volume chilled ethanol overnight and resuspended in distilled water with gentle heating.
210 EPS was dialyze by 3000 Da dialysis membrane for 24 h at 4°C and washed twice by distilled
211 water. The total EPS production levels was using phenol–sulfuric acid method with glucose as
212 standard (50-500 mg/L) (Dubois, 1956).

213 **Determination of cholesterol-lowering activity**

214 The capability of strain AF73-05CM02^T and the closely related reference strain *C. minuta* DSM
215 22607^T to lower cholesterol was determined according to a modified method of Damodharan *et al*
216 (Damodharan et al., 2015). PYG-CHO broth was prepared with addition of 0.1% (w/v) bile, 0.2%
217 (w/v) sodium thioglycollate and cholesterol dissolved in ethanol at a final concentration of
218 approximately 100µg/ml, w/v. The bacterial culture inoculated with log phase was incubated
219 anaerobically in PYG-CHO at 37°C for 4 days. After incubation, cells were harvested by
220 centrifugation at 10000 × g at 4°C for 10min. The concentration of cholesterol in the supernatant

was measured using the o-phthalaldehyde method as described by Rudel and Morris (Rudel, 1973).

Cholesterol-lowering activity from PYG-CHO of each strain broth was calculated in terms of percent cholesterol-lowering as follows:

$$A=(B-C)/B*100\%$$

A=% of cholesterol-lowering, B= the concentration of cholesterol in the PYG-CHO, C= the concentration of cholesterol in the supernatant after inoculated with bacteria for 4 days.

Results and Discussion

Phylogeny based on 16S rRNA gene sequences

The nearly complete 16S rRNA gene sequence of strain AF73-05CM02^T (1,366 bp) was obtained.

The closest relatives of the isolate were *C. minuta* DSM 22607^T, *Catabacter hongkongensis*

HKU16^T (Lau et al., 2007), *Christensenella massiliensis* Marseille-P2438^T (Ndongo et al., 2016b)

and *Christensenella timonensis* Marseille-P2437^T (Ndongo et al., 2016a) with similarity value of

98.68%, 97.22%, 96.93% and 96.78%, respectively (**Table 1**). Phylogenetic analysis based on the

neighbour-joining, maximum-likelihood and minimum-evolution algorithm confirmed that strain

AF73-05CM02^T is most closely related to *C. minuta* DSM 22607^T and formed a tight phylogenetic

cluster with 99% bootstrap support (**Figure 1, Supplementary Figure S1 and S2**).

Table 1. Levels of 16S rRNA gene sequence similarity and ANI values (in percentages) based

on BLAST for strain AF73-05CM02^T and the phylogenetically related species.

Taxa:1, AF73-05CM02^T; 2, *C. minuta* DSM 22607^T; 3, *C. hongkongensis* HKU16^T; 4, *C.*

243 *massiliensis* Marseille-P2438^T; 5, *C. timonensis* Marseille-P2437^T.

Strain	Accession no.	1	2*	3*	4*	5*
16S rRNA gene sequence similarity (%)						
AF73-05CM02 ^T	KX078376	100				
<i>C. minuta</i> DSM 22607 ^T	AB490809	98.68	100			
<i>C. hongkongensis</i> HKU16 ^T	AB671763	97.22	96.69	100		
<i>C. massiliensis</i> Marseille-P2438 ^T	LT161898	96.93	97.51	95.99	100	
<i>C. timonensis</i> Marseille-P2437 ^T	LT223568	96.78	97.38	96.79	95.40	100
ANI values (%)						
AF73-05CM02 ^T	MAIQ00000000	100				
<i>C. minuta</i> DSM 22607 ^T	NZ_CP029256	83.31	100			
<i>C. hongkongensis</i> HKU16 ^T	LAYJ00000000	73.84	75.39	100		
<i>C. massiliensis</i> Marseille-P2438 ^T	LT700187	78.00	78.01	73.28	100	
<i>C. timonensis</i> Marseille-P2437 ^T	FLKP00000000	74.06	74.56	74.53	73.59	100

244 * Data from NCBI and EzBioCloud.

245

246 **Genome properties**

247 The chromosomes of strain AF73-05CM02^T was assembled from 3,145,728 reads resulting a total
 248 length of 3,026,655 bp in size and comprised 29 scaffolds including 36 contigs. The G+C content
 249 of DNA for strain AF73-05CM02^T is 52.07 mol% as calculated from the whole-genome sequence.

250 Circular maps of strain AF73-05CM02^T in comparison to related species is shown in **Figure 2**.

251 The general features of strain AF73-05CM02^T and related species are summarized in **Table 2**.

252

253 **Table 2. Genome features of *C. intestinihominis* AF73-05CM02^T and comparison with closely**
254 **related species**

Features	1	2	3	4	5
Approximate Genome Size (bp)	3,026,655	2,969,292	3,151,949	2,560,186	2,650,850
G+C content (mol%)	52.07	51.4	48.5	50.4	51.7
DNA scaffolds	29	1	38	1	2
N50 Length	294,532	2,969,292	166,940	2,560,186	2,314,156
Genes total number	2,642	2,875	2,986	2,515	2,483
rRNAs (5S, 16S, 23S)	4	6	3	8	9
tRNAs	47	49	47	51	51
ncRNA	4	8	4	4	4
Genes assigned to COGs	2,176	ND	ND	ND	ND

255 Taxa:1, AF73-05CM02^T; 2, *C. minuta* DSM 22607^T; 3, *C. hongkongensis* HKU16^T; 4, *C. massiliensis*
256 Marseille-P2438^T; 5, *C. timonensis* Marseille-P2437^T. ND, not data available

257

258 Among the 2,642 annotated genes in the *C. intestinihominis* AF73-05CM02^T genome, 2,176 genes
259 with specific functions were assigned to COGs. The distribution of genes into COGs functional
260 classification was presented in **Figure 3** and **Supplementary Table S1**, revealed that E (Amino
261 acid transport and metabolism), G (Carbohydrate transport and metabolism), M (Cell
262 wall/membrane/envelope biogenesis), C (Energy production and conversion), R (General function
263 prediction only), T (Signal transduction mechanisms), K (Transcription) and J (Translation,
264 ribosomal structure and biogenesis) were abundant categories. For compared the Individual

265 predicted coding sequences of strain AF73-05CM02^T with *C. minuta* DSM 22607^T by RAST
 266 annotation, we found there were 10-11 RAST-annotated genes associated with diaminopimelic
 267 acid synthesis, 31-37 genes associated with metabolism of polar lipids, 14-16 genes associated
 268 with metabolism of polyamines, 4-5 genes associated with teichoic and lipoteichoic acids
 269 biosynthesis, and 3 genes associated with lipopolysaccharides biosynthesis present in the genomes
 270 (Table 3 and Supplementary Table S2). The number and kind of genes associated with
 271 diaminopimelic acid, polar lipids, polyamines and teichoic and lipoteichoic acids biosynthesis
 272 make strain AF73-05CM02^T distinguishable from the reference species, *C. minuta* DSM 22607^T.
 273
 274 **Table 3. Number of genes associated with biosynthetic pathway from whole genome**
 275 **sequences of strain AF73-05CM02^T and *C. minuta* DSM 22607^T identified by RAST.**
 276 Numbers of genes identified for mycolic acids and quinines (benzoquinones and naphthoquinones)
 277 were zero for all taxa studied.

Genes responsible for biosynthesis	AF73-05CM02 ^T	<i>C. minuta</i> DSM 22607 ^T
Diaminopimelic acid	10	11
Polar lipids	31	37
Polyamines	14	16
Teichoic and lipoteichoic acids	4	5
Lipopolysaccharides	3	3

278
 279 In order to further distinguish strain AF73-05CM02^T from the phylogenetically related species, the
 280 genome comparison was determined using BLAST average nucleotide identities (ANIb). The ANI
 281 values between strain AF73-05CM02^T and related reference species, *C. minuta* DSM 22607^T, *C.*

282 *hongkongensis* HKU16^T, *C. massiliensis* Marseille-P2438^T and *C. timonensis* Marseille-P2437^T
 283 were calculated as 83.51%, 78.92%, 79.66% and 78.76%, respectively (**Table 1**). The ANI values
 284 of strain AF73-05CM02^T with the related species were significantly below the cutoff of 95–96%,
 285 which is proposed as a threshold value for species delineation in bacterial taxonomy (Goris et al.,
 286 2007), indicating that strain AF73-05CM02^T is a distinct genomic species and should be classified
 287 as a representative of a novel species.

288

289 **Phenotypic features**

290 Strain AF73-05CM02^T was an obligate anaerobic and Gram-stain-negative bacterium. Cells were
 291 approximately 0.5µm in width and 1.0–2.0µm in length and occurring singly or in short chains.
 292 Under phase contrast microscope, cells were non-spore-forming, flagella were not observed. The
 293 bacteria formed punctiform colonies (approximately 0.2mm in diameter) with circular and beige
 294 after 4 days of growth at 37°C on PYG agar under anaerobic conditions. The growth temperature
 295 was from 30–42°C, with the optimum around 37–42°C, while no growth was observed below 30°C
 296 or at 45°C. Growth occurred at pH values from 6.0 to 8.5, with optimum growth between 6.5 and
 297 7.0. The strain tolerated salt concentrations up to 2% (w/v) NaCl and bile up to 0.3%. The cells
 298 were catalase-negative. The physiological and biochemical comparison of strain AF73-05CM02^T
 299 and related strain was carried out using API 20A, API 50CHL and API ZYM tests, the result were
 300 summarized in the species description and the differences of selected characteristics with the
 301 reference strain are given in **Table 4**. All the results of enzymatic characteristics and carbon source
 302 assimilation from API ZYM, API 20A and API 50CHL test are presented in **Supplementary**
 303 **Table S3** and **Supplementary Table S4**.

304

305 **Table 4. Comparison of phenotypic features between strain *C. intestinihominis***

306 **AF73-05CM02^T and the closest related reference strain, *C. minuta* DSM 22607^T.**

Phenotypic features	1*	2 [#]
Cell size (µm)	1.0×1.0–2.0	0.4×0.8–1.9
Growth:		
Temperature range (optimum) (°C)	30–42 (37–42)	25–45 (37)
pH range (optimum)	6.0–8.5 (7.0)	6.0–9.0 (7.5)
Salt tolerance (%)	2	3
Bile tolerance (%)	0.3	20
Aesculin hydrolysis	+	–
Acid from (API 20A and API 50CHL):		
Arbutin	+	w
D-Galactose	+	w
D-Maltose	+	w (– [#])
D-Sorbitol	+	w (– [#])
D-Sucrose	+	–
D-Turanose	+	–
Gentiobiose	+	–
L-Sorbose	+	w
Xylitol	+	w
D-Adonitol	–	w
L-Fucose	–	+
D-Melezitose	w	–
D-Raffinose	w	–
Enzyme activity (API ZYM):		
β-Glucosidase	–	+

307 Strains:1, *C. intestinihominis* sp. nov. AF73-05CM02^T, 2, *C. minuta* DSM 22607^T.

308 +, Positive; w, weakly positive reaction; –, negative; ND, no data available.

309 *Data from this study.

310 [#] Data from Morotomi, *et al* (Morotomi et al., 2012) and this study.

311

312 Chemotaxonomic characteristics of strain AF73-05CM02^T were consistent with the results of the

reference strain that were performed under identical conditions, confirming that the novel isolate belongs to the genus *Christensenella*. The cellular fatty acid composition of strain AF73-05CM02^T and DSM 22607^T are presented in **Table 5**, and the dominant fatty acids (representing > 5% of the total) for strain AF73-05CM02^T were C_{10:0} (7.5%), iso-C_{11:0} (5.6%), C_{12:0} (7.2%), C_{14:0} (46.6%), iso-C_{15:0} (7.4%), C_{16:0} (9.7%) and C_{18:1} *ω*9*c* (6.9%). The higher amount of C_{14:0} and less amount of iso-C_{15:0} and C_{16:0} significantly differentiated strain AF73-05CM02^T from the reference strains. The cell-wall diamino acid was LL-diaminopimelic acid.

Table 5. Cellular fatty acid composition of strain AF73-05CM02^T and closely related species, DSM 22607^T. Strains: 1, AF73-05CM02^T; 2, *C. minuta* DSM 22607^T; Data were obtained in this study. Numbers represent percentages of the total fatty acids. Only fatty acids amounting 1% or higher are shown. t, traces (<1%).

Fatty acids	1	2
C _{10:0}	7.5	8.6
C _{12:0}	7.2	1.1
C _{14:0}	46.6	13.0
C _{14:0} 2OH	t	1.3
C _{16:0}	9.7	21.1
C _{18:1} <i>ω</i> 9 <i>c</i>	6.9	6.8
C _{18:1} <i>ω</i> 7 <i>c</i>	t	3.9
C _{18:0}	1.8	3.7
Iso-C _{11:0}	5.6	2.9
Iso-C _{15:0}	7.4	27.4
Anteiso-C _{11:0}	t	1.3
Anteiso-C _{13:0}	t	2.4
Anteiso-C _{15:0}	1.3	3.2
Iso-C _{17:1} I/anteiso B	4.7	1.7
Antei-C _{18:0} /C _{18:2} <i>ω</i> 6,9 <i>c</i>	t	1.7

325

326 For Susceptibility tests, strain AF73-05CM02^T was resistant to oxacillin and compound
327 sulfamethoxazole, but sensitive to penicillin, ampicillin, carbenicillin, piperacillin, vancomycin,
328 polymyxin B, furazolidone, chloroamphenicol and clindamycin (**Supplementary Table S5**). The
329 hemolytic activity of the cells was not founded.

330 Metabolic end products from glucose for strain AF73-05CM02^T and DSM 22607^T are shown in
331 **Supplementary Table 6**. Acetic acid, formic acid, butyric acid and lactic acid were the major end
332 products (>1 mmol/L) for strain AF73-05CM02^T.

333

334 We found strain AF73-05CM02^T can be clearly differentiated from *C. minuta* DSM 22607^T based
335 on a lot of phenotypic and genotypic characteristics and ANI values obtained in this study can
336 separate this isolate from related species, which suggest that the strain AF73-05CM02^T represents
337 a novel species of the genus *Christensenella*. Therefore, we propose AF73-05CM02^T (=CGMCC
338 1.5207^T =DSM 103477^T) as the type strain of *Christensenella intestinhominis* sp. nov.

339

340 **EPS production**

341 EPS produced by probiotics have several biologically beneficial functions on the host, such as
342 improve the viscosity of the lactic acid bacteria fermented product (Li et al., 2014) and have
343 significant roles on colonization, stress resistance and adhesion (Delcour et al., 1999).
344 Furthermore, it has been suggested EPS may have probiotics properties on immune modulation
345 and antioxidative effects (Welman and Maddox, 2003; Fanning et al., 2012). In the present
346 research, both test strains, *C. intestinhominis* AF73-05CM02^T and *C. minuta* DSM 22607^T, were

347 capable of producing EPS with amount of 234 and 271 mg/L, respectively.

348

349 **Removal of Cholesterol**

350 The test the cholesterol-lowering activity was determined in PYG-CHO broth added with bile.

351 Both strain AF73-05CM02^T and *C. minuta* DSM 22607^T showed a capacity for removing

352 cholesterol from PYG-CHO broth. After incubated in PYG-CHO at 37°C for 4 days, the amount

353 of cholesterol in medium were reduced with efficiency of 36.6% and 54.3% by AF73-05CM02^T

354 and *C. minuta* DSM 22607^T, respectively. The control sample, containing no cultures,

355 demonstrated no cholesterol removal, as expected. The mechanisms of cholesterol-lowering by

356 probiotics from *in vitro* experiments have been reported including many hypotheses, such as

357 deconjugated bile acids via bile salt hydrolase activity, adsorption to cellular surface and

358 conversion by probiotics (Ishimwe et al., 2015). In the previous study, *in vivo* experiment of

359 cholesterol-lowering showed the probiotics have a useful and safe effect on modulating the

360 serum-lipid profile and reducing the host cholesterol level (Pan et al., 2010). A high cholesterol

361 level as a main production of obesity can increase the risk of CVDs. The genus *Christensenella* as

362 a especially common microorganism has been founded in lean people and showed a high

363 abundance population in the gut (Goodrich et al., 2014), suggesting *Christensenella* has a potential

364 function in protecting against obesity. Further studies will be required to focus on the cholesterol

365 reducing properties *in vitro* and *in vivo* and reveal the mechanism of lose weight for genus

366 *Christensenella*.

367

368 **Description of *Christensenella intestinhominis* sp. nov.**

369 *Christensenella intestinhominis* (in.tes.ti.ni.ho'mi.nis. L. gen. n. *intestini* of the intestine; L. gen. n.
370 *hominis* of a human being; N.L. gen. n. *intestinhominis* of the human intestine).

371 Cells are Gram-stain-negative, obligately anaerobic, non-motile and short rods (1.0×1.0–2.0µm)
372 isolated from a faecal sample collected from a healthy adult. Colonies on PYG agar are 0.2mm in
373 diameter and punctiform with circular and beige after 4 days of growth at 37°C. Growth occurs at
374 temperatures from 30-42°C, with the optimum around 37-42°C. The pH range is from 6.0 to 8.5,
375 with optimum between 6.5 and 7.0. Able to grow in the presence of up to 2.0% (w/v) NaCl and
376 0.3% bile (w/v). Major end products of metabolism of glucose are Acetic acid, formic acid, butyric
377 acid and lactic acid. The cells exhibit resistance to oxacillin and compound sulfamethoxazole, but
378 are sensitive to penicillin, ampicillin, carbenicillin, piperacillin, vancomycin, polymyxin B,
379 furazolidone, chloroamphenicol, and clindamycin. The predominant cellular fatty acids are C_{10:0},
380 iso-C_{11:0}, C_{12:0}, C_{14:0}, iso-C_{15:0}, C_{16:0} and C_{18:1} ω9c. The diagnostic cell-wall diamino acid is
381 LL-diaminopimelic acid.

382 In API 20A and API 50CHL, the isolate was positive for utilization arbutin, D-arabinose,
383 D-fructose, D-fucose, D-galactose, D-glucose, D-lyxose, D-ribose, D-sorbitol, D-sucrose, D-tagatose,
384 D-turanose, D-xylose, gentiobiose, L-arabinose, L-rhamnose, L-sorbose,
385 methyl-β-D-xylopyranoside, salicin and xylitol, weakly reactions for D-maltose, D-mannose,
386 D-melezitose, D-raffinose, erythritol, L-xylose and salicin, and negative for amygdalin, cellobiose,
387 D-adonitol, D-arabitol, D-lactose, D-mannitol, D-melibiose, D-trehalose, dulcitol, gluconate,
388 glycerol, glycogen, inositol, inulin, L-arabitol, L-fucose, methyl-D-glucopyranoside,
389 methyl-α-D-mannopyranoside, N-acetyl-glucosamine, 2-ketogluconate and 5-ketogluconate.

390 Indole is not formed. Esculin can be degraded, but gelatin is not hydrolysed. Catalase is negative.

Results obtained from API ZYM showed positive enzymatic activity for naphthol-AS-BI-phosphohydrolase and negative for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and β -fucosidase.

In the result of RAST annotation, 11 genes/proteins are associated with biosynthesis of DAP, including 4-hydroxy-tetrahydronicotinate reductase (EC 1.17.1.8), 4-hydroxy-tetrahydronicotinate synthase (EC 4.3.3.7), aspartate-semialdehyde dehydrogenase (EC 1.2.1.11), aspartokinase (EC 2.7.2.4), diaminopimelate decarboxylase (EC 4.1.1.20), diaminopimelate epimerase (EC 5.1.1.7), L, L-diaminopimelate aminotransferase (EC 2.6.1.83), *N*-acetyl-L, L-diaminopimelate deacetylase (EC 3.5.1.47), *N*-succinyl-L, L-diaminopimelate desuccinylase (EC 3.5.1.18), UDP-*N*-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase (EC 6.3.2.13) and UDP-*N*-acetylmuramoylalanyl-D-glutamyl-2, 6-diaminopimelate-D-alanyl-D-alanine ligase (EC 6.3.2.10). 31 genes/proteins are associated with biosynthesis of polar lipids, including 1-acyl-sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51), acyl carrier protein (4 copies), acyl-phosphate:glycerol-3-phosphate O-acyltransferase PlsY, alcohol dehydrogenase (EC 1.1.1.1) (8 copies), acetaldehyde dehydrogenase (EC 1.2.1.10) (2 copies), aldehyde dehydrogenase (EC 1.2.1.3), aldehyde dehydrogenase B (EC 1.2.1.22), cardiolipin synthetase (EC 2.7.8.-), CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase (EC 2.7.8.5), diacylglycerol kinase (EC 2.7.1.107), dihydroxyacetone kinase family protein, glycerate kinase (EC 2.7.1.31), glycerol kinase (EC 2.7.1.30) (2 copies), glycerol-1-phosphate dehydrogenase [NAD(P)] (EC 1.1.1.261) (2 copies), glycerol-3-phosphate

413 dehydrogenase (EC 1.1.5.3), glycerol-3-phosphate dehydrogenase [NAD(P)⁺] (EC 1.1.1.94),
414 phosphate:acyl-ACP acyltransferase PlsX and phosphatidate cytidyltransferase (EC 2.7.7.41). 14
415 genes/proteins are associated with biosynthesis of polyamines, including agmatine deiminase (EC
416 3.5.3.12), agmatine/putrescine antiporter, associated with agmatine catabolism (2 copies), arginine
417 decarboxylase (EC 4.1.1.19) / Lysine decarboxylase (EC 4.1.1.18), carbamate kinase (EC 2.7.2.2),
418 carboxynorspermidine dehydrogenase, putative (EC 1.1.1.-), putrescine carbamoyltransferase (EC
419 2.1.3.6), putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1), S-adenosylmethionine
420 decarboxylase proenzyme (EC 4.1.1.50), prokaryotic class 1A and spermidine putrescine ABC
421 transporter permease component PotB (TC 3.A.1.11.1), spermidine putrescine ABC transporter
422 permease component potC (TC_3.A.1.11.1) (2 copies), spermidine synthase (EC 2.5.1.16) and
423 transcriptional regulator, MerR family, near polyamine transporter. 4 genes/proteins are associated
424 with biosynthesis of teichoic and lipoteichoic acids, including 2-C-methyl-D-erythritol
425 4-phosphate cytidyltransferase (EC 2.7.7.60), teichoic acid export ATP-binding protein TagH
426 (EC 3.6.3.40), teichoic acid translocation permease protein TagG and undecaprenyl-phosphate
427 *N*-acetylglucosaminyl 1-phosphate transferase (EC 2.7.8.-). 3 genes/proteins are associated with
428 biosynthesis of lipopolysaccharides, including lipopolysaccharide biosynthesis protein RffA (2
429 copies), lipopolysaccharide cholinephosphotransferase LicD1 (EC 2.7.8.-) and HtrA
430 protease/chaperone protein. There are no genes responsible for biosynthesis of respiratory
431 lipoquinones or mycolic acids.

432 The type strain AF73-05CM02^T (=CGMCC 1.5207^T =DSM 103477^T) was isolated from the faecal
433 samples of a healthy adult residing in Shenzhen, China (37°35'37"N, 114°15'32"E). The DNA
434 G+C content of strain AF73-05CM02^T is 52.07 mol% calculated from the genome sequence. The

435 genome size is 3.02Mbp.

436

437 **Data Availability Statement**

438 The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of
439 *Christensenella intestinihominis* AF73-05CM02^T are KX078376. The draft genome of *C.*
440 *intestinihominis* AF73-05CM02^T have been deposited at DDBJ/EMBL/GenBank under the
441 accession numbers MAIQ00000000. The data that support the findings of this study have also
442 been deposited into CNGB Sequence Archive (CNSA) (Guo et al., 2020) of China National
443 GeneBank DataBase (CNGBdb) (Chen et al., 2020) with accession number CNPhis0003415.

444

445 **Author Contributions**

446 Conceived and designed the experiments: Y.Z. and L.X. Performed the experiments: Y.Z., W.X.,
447 M.L. and Y.D. Analyzed the data: Y.Z., L.X., G.L., and X.L. Contributed
448 reagents/materials/analysis tools: Y.Z., W.X., M.L. and Y.D. Wrote the paper: YZ.

449

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454

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458

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641 **Figure legends**

642 **Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing**
643 **the phylogenetic relationships of strains AF73-05CM02^T and the representatives of related**
644 **taxa.** *Bacillus subtilis* subsp. *subtilis* NCIB 3610^T (ABQL01000001) was used as an out-group.
645 Bootstrap values based on 1000 replications higher than 70% are shown at the branching points.
646 Bar, substitutions per nucleotide position.

647

648 **Figure 2. Graphical circular map of the genome from strain *Christensenella intestinhominis***
649 **sp. nov. AF73-05CM02^T, *Christensenella minuta* DSM 22607^T, *Catabacter hongkongensis***
650 **HKU16^T, *Christensenella massiliensis* Marseille-P2438^T and *Christensenella timonensis***
651 **Marseille-P2437^T using CGView server using default parameters. From inner to outer: Ring 1**

652 and Ring 2, G+C positive skew (green) and G+C negative skew (purple); Ring 3, GC% content;
653 Ring 4-Ring 8, Contig, rRNA, tmRNA, rRNA and CDS from AF73-05CM02^T; Ring 9,
654 *Catabacter hongkongensis* HKU16^T; Ring 10, *Christensenella timonensis* Marseille-P2437^T; Ring
655 11, *Christensenella massiliensis* Marseille-P2438^T; Ring 12, *Christensenella minuta* DSM 22607^T.

656

657 **Figure 3. The distribution of the genes associated with the COG functional categories in**
658 **strain AF73-05CM02^T.** The number of genes is shown in parentheses.

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663 **Figure 1**

0.020

Figure 2

Figure 3

Supplementary Material

684 **Supplementary Table S1. Number of genes associated with general COG functional**
 685 **categories in the genome of *C. intestinihominis* AF73-05CM02^T and *C. minuta* DSM 22607^T.**

686 **Supplementary Table S2. The specific genes/protein related to biosynthesis of DAP, polar**
 687 **lipids, polyamines and lipoteichoic and teichoic acids and their positions in the genome in**
 688 **comparasion of strain AF73-05CM02^T and *C. minuta* DSM 22607^T identified by Rapid**
 689 **Annotation Subsystem Technology (RAST).**

690 **Supplementary Table S3. Enzymatic characteristics of strain AF73-05CM02^T from API**
 691 **ZYM test.**

692 **Supplementary Table S4. Carbon source assimilation of strain AF73-05CM02^T from API**
 693 **20A and API 50CHL test.**

694 **Supplementary Table S5. Antibiotic sensitivity of strain AF73-05CM02^T.**

695 **Supplementary Table S6. Metabolic end products from glucose for strain AF73-05CM02^T**
 696 **and *C. minuta* DSM 22607^T.**

697

698 **Supplementary Figure S1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene**
 699 **sequences showing the phylogenetic relationships of strains AF73-05CM02^T and the**
 700 **representatives of related taxa. *Bacillus subtilis* subsp. *subtilis* NCIB 3610^T (ABQL01000001)**
 701 **was used as an out-group. Bootstrap values based on 1000 replications higher than 70% are shown**
 702 **at the branching points. Bar, substitutions per nucleotide position.**

703 **Supplementary Figure S2. Minimum-evolution phylogenetic tree based on 16S rRNA gene**

704 sequences showing the phylogenetic relationships of strains AF73-05CM02^T and the
 705 representatives of related taxa. *Bacillus subtilis subsp. subtilis* NCIB 3610^T (ABQL01000001)
 706 was used as an out-group. Bootstrap values based on 1000 replications higher than 70% are shown
 707 at the branching points. Bar, substitutions per nucleotide position.

708 **Supplementary Figure S3. Certification. Deposit certification of CGMCC.**

709 **Supplementary Figure S4. Certification. Deposit certification of DSMZ.**