Peptacetobacter felis sp. nov., a novel bile acid-converting bacterial species isolated from the feces of healthy cats in the United States

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Abbreviations:

MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

WGS: Whole genome sequencing

COG: Clusters of Orthologous Genes

MAG: metagenome assembled genomes

OMCL: Orthologous Markov Clustering

ABSTRACT

Six strains of a novel anaerobic bacterial taxa exhibiting bile acid-transforming activity were isolated from fecal samples of clinically healthy cats living in Oakland, California. 16S rRNA sequencing and phylogenetic analysis indicate that strain AB800^{T} belongs to the genus *Peptacetobacter*, with *Peptacetobacter hiranonis* strain JCM 10541 (98% coverage, 99.7% identity) as its closest relative. Whole genome sequencing shows an average nucleotide identity (ANI) of less than 96% (95.73% ANI, 90.53% AF) with the closest validly named species being *Peptacetobacter hiranonis DGF055142*. The size is about 2.58 Mb, containing 2,355 predicted coding sequences and 2,450 annotated genes. Phenotypic characterisation, and comprehensive genomic analysis, including ANI, digital DNA-DNA hybridization (dDDH) and core genome phylogenetic analysis, placing the strain AB800^{T} on a separate branch, supported the classification of a novel species, strain AB800^{T} (DSM 120482 = LMG 34035) with the proposed name *Peptacetobacter felis* sp. Nov., derived from "*felis*" the latin name of cat *felis catus*. These findings expand our understanding of host-associated bile acid converters and provide promising candidates for probiotic development.

INTRODUCTION

Comprehensive characterisation of the gut microbiome in companion animals and identification of live bacterial taxa are essential for developing next-generation microbial supplements, live biotherapeutic products, and advancing veterinary health [1, 2]. Yet, much of its diversity remains unexplored, especially at the species level. Among the critical microbial processes in the gut, bile acid metabolism plays a central role in regulating host physiology, microbial community structure and interactions, and intestinal homeostasis [3, 4]. In both humans and animals, gut microbes convert primary bile acids into secondary bile acids, modulating lipid absorption, intestinal signalling, and colonisation resistance effects mediated through host receptors such as FXR and TGR5 [5–7]. In dogs and cats, bile acid metabolism has been shown to have profound health implications, with alterations in bile acid profiles linked to gastrointestinal disorders and dysbiosis [5, 8].

To address this knowledge gap, culturomics, a high-throughput, culture-based microbial discovery method, has emerged as a powerful tool [9, 10]. By combining selective anaerobic cultivation, MALDI-TOF MS profiling, and whole genome sequencing, culturomics enables the isolation of novel gut microbes and the functional exploration of their metabolic capabilities [11, 12]. While metagenomics offers broad surveys of genomics content, only culture-based techniques provide direct insight into physiology, metabolic activity, and potential therapeutic applications. The gastrointestinal tracts of healthy cats and dogs provide a rich anaerobic environment that supports diverse microbial consortia involved in key transformations, such as protein fermentation and bile acid conversion [1, 13]. Protein-rich diets common to companion animals enhance microbial fermentation potential, but may also lead to the accumulation of ammonia and other intermediates that disrupt gut stability[14, 15]. In this context, bile acid-transforming bacteria may help stabilise

65 the microbial community and maintain gut homeostasis[5, 16]. One of the main bacteria reported
66 to modulate bile acid levels in companion animals with chronic enteropathy is *Pepetacetobacter*
67 *(Clostridium) hiranonis* [17].
68 The *Peptacetobacter* genus is classified in the *Peptostreptococcaceae* family and includes a group
69 of rod-shaped Gram-positive, obligatory anaerobic bacteria. There are two members of the genus
70 *Peptacetobacter*: *Peptacetobacter hiranonis* and *Peptacetobacter hominis*. The first species
71 isolated from this genus was initially classified as *Clostridium* based on the 16s rRNA similarity
72 and DNA-DNA hybridisation analysis [18]. Then, in 2020, the second species was isolated with a
73 ~95% 16S rRNA gene similarity, and exhibited ANI values of 66–76% (< 96%) and dDDH values
74 of 17–35% (< 70%), far below accepted genus and species thresholds. The whole-genome
75 phylogenomics further demonstrated that these organisms belonged to a distinct lineage, separated
76 from *Clostridium sensu stricto*, leading to the establishment of the genus *Peptacetobacter*, with *P.*
77 *hominis* as the type species and *P. hiranonis* reclassified accordingly ([19].
78 Here, we report the isolation and characterisation of a novel bile acid-transforming species from
79 the feces of healthy cats: *Peptacetobacter felis* sp. nov., expanding the genus *Peptacetobacter* to
80 three species. Using a culturomics framework, we identified and analysed these strains through
81 phenotypic, phylogenetic, chemo-taxonomic, and genomic methods. This discovery enhances our
82 ability to conduct additional studies to better understand the bile acid microbiome axis in
83 companion animals. It offers novel candidates for the development of precision probiotics to
84 improve gastrointestinal health and metabolic stability in dogs and cats.
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SAMPLE COLLECTION AND ISOLATION

Fecal samples were collected from five different healthy cats living in Oakland, California, USA, in 2022. Strain AB800^{T} was isolated from a fecal sample obtained from an 8-year-old, spayed domestic short-haired cat. AB403 was isolated from a fecal sample obtained from a 2-year-old, spayed female domestic short-haired cat. AB804 was isolated from a fecal sample obtained from a 7-year-old, spayed female domestic short-haired cat. AB807 and AB808 were isolated from the same 6-year-old, neutered male American short-haired cat. Strain AB811 was cultivated from a fecal sample obtained from a 1-year-old, spayed female domestic long-haired cat.

Freshly collected samples were transported in BIOME-Preserve AS-930 (Anaerobe Systems, Morgan Hill, CA) transport media in a one-to-one ratio and processed as part of the AnimalBiome culturomics process [20]. Culture and isolation were carried out between September 2022 and February 2023. In an AS500 Anaerobic chamber (Anaerobe Systems, Morgan Hill, CA), the sample was serially diluted, and the diluted samples were plated on Brain Heart Infusion Agar with Horse Blood and Taurocholate (BHIY-HT) (Anaerobe Systems, Morgan Hill, CA) and incubated anaerobically for 48 hours at 37°C. The grown colonies of strain AB800^{T} were repeatedly subcultured on homemade Columbia agar with 5% sheep blood until pure isolates were obtained. Colony identification of the strain was carried out using MALDI Biotyper smart System RUO microflex LT/SH smart with the BIDAL database v12 (Bruker Daltonics, Bremen, Germany) as previously described [20].

MORPHOLOGICAL, PHENOTYPICAL, AND BIOCHEMICAL CHARACTERISTICS

After isolation, a pure culture of strain AB800^{T} was morphologically characterised by phase-contrast microscopy and Gram staining. Motility was assessed by wet mount, and biochemical

111 profiling was conducted using API ZYM, API 50 CH, and API Rapid ID 32A strips (bioMérieux).
112 The ribosomal protein MALDI-TOF MS spectra profiling was obtained using MALDI Biotyper
113 smart System RUO microflex LT/SH smart with the software flexAnalysis 3.4 (Bruker Daltonics,
114 Bremen, Germany).
115 On Columbia blood agar, strain AB800^T exhibits white-grayish colonies in the form of a star
116 measuring approximately 1 to 2 mm in diameter. The wet mount reveals a non-motile bacterial
117 cell, while the Gram stain shows Gram-positive bacilli with no endospore formation detected. The
118 electron microscopy characterisation shows that strain AB800^T cells measure approximately 0.7–
119 1 µm in width and 3.2–8 µm in length. Growth of AB800^T is optimal on a Chocolate agar
120 (Anaerobe Systems, Morgan Hill, CA) plate at 37 °C in strict anaerobic conditions. The enzymatic
121 activity evaluation shows that strain AB800^T exhibits positive activity for N-acetyl-β-D-
122 glucosaminidase, acid phosphatase, alkaline phosphatase, naphthol-AS-BI-phosphohydrolase,
123 proline aminopeptidase, and esterase lipase (C8). Negative activity was observed for β-
124 glucosidases, β-galactosidase, valine, leucine arylamidases, lipase (C14), α-chymotrypsin, and
125 trypsin. Regarding carbohydrates, strain AB800^T can metabolise D-glucose and D-mannose but
126 shows no activity towards sucrose or sorbitol. Strain AB800^T shows no activity for urea utilisation,
127 nitrate reduction, indole production, and gelatin hydrolysis (Table 1)

128 **SEQUENCING AND GENOMIC ANALYSIS**

129 The total nucleic acid of strain AB800^{T} AB403, AB804, AB807, AB808, and AB811 was extracted
130 using the ZymoBIOMICS™ DNA Miniprep Kit for bacterial cells (Zymo Research, Irvine, CA,
131 USA) as previously described [20]. The concentration of the nucleic acid was measured using a
132 Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The strains AB800^{T},
133 AB403, AB804, AB807, AB808, and AB811 were sequenced using Illumina technology by
134 SeqCenter (Pittsburgh, PA, United States) as described in their protocol. Illumina sequencing
135 libraries were prepared using the tagmentation-based and PCR-based Illumina DNA Prep kit and
136 custom IDT 10 bp unique dual indices (UDI) with a target insert size of 280 bp. No additional
137 DNA fragmentation or size selection steps were performed. Illumina sequencing was performed
138 on an Illumina NovaSeq X Plus sequencer in one or more multiplexed shared-flow cell runs,
139 producing 2x151 bps paired-end reads. Demultiplexing, quality control, and adapter trimming
140 were performed with bcl-convert1 (v4.2.4).

141

142 **PHYLOGENY AND GENOME FEATURES**

143 Sequencing reads were screened for quality control using Fastqc v0.12.1 [21] and filtered with a
144 Phred score >30. We assembled them using SPAdes genome assembler v3.15.5 [22] implemented
145 in shovill (https://github.com/tseemann/shovill) with default parameters. The final scaffolds were
146 screened for quality using CheckM2 v1.0.2 [23] and taxonomically confirmed using GTDB-Tk
147 v2.4.0 (RefDB version r220) [24] and a Blastn search using BLASTN 2.17.0+ [25] against the 16S
148 rRNA database (NCBI). We annotated the genomes using Prokka v1.14.6 [26]. The genome was
149 searched for antimicrobial resistance and virulence genes using Abricate
150 (https://github.com/tseemann/abricate) (version 1.0.0) and the NCBI AMRFinderPlus [27],

151 ARGANNOT [28], ResFinder [28, 29], CARD [30], and MEGARes [31] databases downloaded
152 in November 2025. In addition, we conducted a screening for metabolism pathways associated
153 with bile acid metabolism using gapseq (version 1.3.1) [32] and searched for complete and
154 incomplete pathways.
155 The strain AB800^T was assembled into 149 contigs, totalling 2.58 Mb in genome size. Compared
156 with the other five strains, AB800^T is in the upper range of genome size, ranging from 2.43 to 2.86
157 Mb across all strains. The strain AB800^T encodes 2,355 coding sequences and 2,450 total genes,
158 values that fall centrally within the variation seen among other related strains (2,165–2,624 CDS;
159 2,261–2,719 genes). The strain carries 16 rRNA genes, 78 tRNA genes, and one tmRNA, with
160 three annotated repeat regions, reflecting the typical genomic architecture present across the set.
161 Regarding antimicrobial resistance determinants, strain AB800^T harbors both tetB(P) and tetA(P)
162 genes among the tetracycline group of genes, while no macrolide (ermQ) or lincosamide (lnuP)
163 genes are detected. This resistance gene profile is shared with the strains AB0403, AB804, and
164 AB811. However there were no genes associated to virulence. The genes associated with bile acid
165 metabolism are broadly conserved across all genomes examined, and strain AB800^T encodes the
166 complete set of core *bai* genes, including *baiN_1, baiN_2, baiB, baiE, baiCD_1, baiCD_2, baiF,*
167 *baiH* and *baiA2*. As in the other strains, *baiN_3, baiN_4* and *baiN_5* are absent (Table 2)
168
169

170 The 16S rRNA gene sequence of strain AB800^{T}, when blasted against the 16S ribosomal RNA
171 database, showed 97% coverage and 99% identity to *P. hiranonis JCM 10541*. The maximum
172 likelihood phylogenetic inference with a General Time-Reversible (GTR) model shows strain
173 AB800^{T} to be a close parent of *P. hiranonis JCM10541(NR178263.1)* and *P. hiranonis strain TO-*
174 *931 (NR028611.1),* with a bootstrap of 98% and a midpoint rooting topology (Fig. 2: a). Genomic
175 taxonomic classification using Gtdbtk v2 showed that the closest species is *Peptacetobacter*
176 sp900539645 (GCA_900539645.1) with 98.67% ANI, *Peptacetobacter hiranonis*
177 *(GCF_008151785.1)*, ANI= 94.46%, AF=0.81, and *Peptacetobacter hominis*
178 *(GCF_006861675.1)*, ANI=80.74, AF=0.45. The DNA-DNA-Hybridization analysis with GGDC
179 showed that the closest from the database is *Peptacetobacter hiranonis DSM 13275* with dDDH =
180 67.7% (Suppl.Data: Table1). The Core-genome-based phylogeny showed strain AB800^{T} to be
181 close to or belong to the *Peptacetobacter* cluster but on a separate branch, as *P. hiranonis* and
182 close to the metagenome assembled genomes (MAG) of *Peptacetobacter* (*Pepetacetobacter* sp.
183 bin-44 and *Pepetacetobacter* sp. DOME MAG 11184) (Fig. 2: b). The closest species genomes
184 and other genomes were retrieved from NCBI, and ANI and AF were computed directly using
185 FASTANI (version 1.34) [33]. The results indicated that the closest are *Pepetacetobacter* sp.
186 DOME MAG11184 and *Pepetacetobacter* sp. bin-44 (ANI=98.72%, AF=81.95%, ANI=98.67%,
187 AF=74.17%). The closest known isolate is *Peptacetobacter hiranonis* DGF055145 (ANI=95.73%,
188 AF=81.95%) with ANI < 96%, making strain AB800^{T} a new species of the genus *Peptacetobacter*
189 (Fig. 3).

190 **COMPARATIVE GENOMIC**

191 A pangenome analysis using OMCL clustering showed that strain AB800^{T} shared only 1331 gene
192 clusters with *Peptacetobacter hiranonis* and *Peptacetobacter hominis* (core genome) while

193 conserving 500 genes as unique. It shares 405 with *Peptacetobacter hiranonis* and only 67 with *P.*
194 *hominis*, confirming that strain AB800^{T} is closer to *P. hiranonis* than *P. hominis* (Fig. 4: a). The
195 metabolic pathway analysis revealed that, while only *P. hiranonis* and strain AB800^{T} possess the
196 bile acid 7-alpha and 7-beta dehydroxylation pathways, only strain AB800^{T} lacks the
197 deconjugation pathways, as shown in Fig. 4: b.
198

199 **DESCRIPTION OF *PEPTACETOBACTER SP.* NOV**

200 *Peptacetobacter felis* (fe.lis. L. gen. n. felis, referring to the Latin name of cat, felis catus, the
201 species from which it was isolated).
202 A bile acid-converting, Gram-positive, non-motile, obligate anaerobic rod-shaped bacterium
203 isolated from the feces of a healthy cat living in Oakland, California. *Peptacetobacter felis AB800^{T}*
204 can be grown on Columbia blood agar, Chocolate agar, and Brain Heart infusion agar (with optimal
205 growth on Chocolate agar at 37 °C after 48h. On agar plates, it exhibits white-grayish colonies in
206 the form of a star measuring approximately 1 to 2 mm in diameter. Regarding enzymatic activity,
207 *Peptacetobacter felis* AB800^{T} shows the ability to hydrolyze specific peptide and amino sugar
208 substrates and active phosphatase and esterase systems, which translates to positive results for
209 naphthol-AS-BI-phosphohydrolase, leucine arylamidase, N-acetyl-β-D-glucosaminidase, alkaline
210 phosphatase, esterase (C4), esterase lipase (C8), and acid phosphatase. On the other hand, strain
211 AB800^{T} does not exhibit activity for cystine arylamidase, trypsin, lipase (C14), valine arylamidase,
212 α-mannosidase, α-fucosidase, α-chymotrypsin, β-glucosidase, or β-galactosidases, showing
213 limited glycosidic activity and proteolytic activity in comparison with other *Peptacetobacter* taxa.
214 Regarding carbohydrate, amino acid, and salt activity, *Peptacetobacter felis AB800^{T}* is urea- and
215 L-tryptophan-negative, suggesting the absence of urease and tryptophanase activities. For carbon

216 sources, the strain AB800^T can use D-saccharose (sucrose) and D-glucose, but does not use
217 amygdalin, D-melibiose, D-mannitol, D-sorbitol, L-rhamnose, L-arabitol, or inositol. In summary,
218 *Peptacetobacter felis AB800^T* is particularly characterized by its esterase and phosphatase
219 reactivity, but in general by its specific enzyme activities and limited substrate spectrum among
220 the tested compounds. Even though the 16S RNA similarity search and phylogeny did not show a
221 clear delineation between the strain *Peptacetobacter felis AB800^T* and other *P. hiranonis*, Genomic
222 taxonomic classification using Gtdbtk v2 showed that the closest species is Peptacetobacter
223 sp900539645 (GCA_900539645.1) with 98,67% ANI, which is a MAG. However, the nearest
224 known isolate is *Peptacetobacter hiranonis DGF055145* (ANI=95.73%, AF=81.95%), with ANI
225 < 96% and dDDH = 67.7% (< 70%), and Peptacetobacter hiranonis DSM 13275, making
226 *Peptacetobacter sp. AB800T,* a new species of the genus *Peptacetobacter*. The genome is
227 2,577,873 bp, comprises 149 contigs, has a GC content of 31%, and has been deposited in
228 GenBank under BioProject accession number PRJNA1200056. *Peptacetobacter felis* AB800^T was
229 deposited in two international collections with the following accession numbers: *Peptacetobacter*
230 *felis* AB800^T (=DSM 120482= LMG 34035

231 **Ethical statement**

232 The fecal sample used to isolate *Peptacetobacter felis* strain AB800^T, as well as the strains AB403,
233 AB804, AB808, AB807, and AB811, were all non-invasively obtained and donated by the cats'
234 owners with informed consent of the research that will be carried out.

235 **Author Contributions:**

236 ND: Conceived the idea, isolated the strains AB800^T, AB804, AB807, AB808, and AB811,
237 designed the experiments, supervised the characterization experiments, analyzed and validated the
238 data, created the figures, drafted the manuscript, and performed review and preparation. HHG:

Conceived the idea, validated the data, reviewed, and prepared the manuscript. KDM: Assembled the genomes, analyzed the data, conducted comparative genomic analyses, drafted the manuscript, and contributed to editing and review. GJ: Prepared the data and deposited the genomes to NCBI, reviewed the manuscript, and contributed to editing. ZM: Isolated strains AB0403, deposited the strains in the international collections (DSMZ and BCCM), and performed phenotypic characterization. SL: Performed the strain purification and cryobanking, DNA extraction, and prepared the DNA for sequencing

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Conflict of Interest

Niokhor Dione, Zara Marfori, Guillaume Jospin, and Holly Ganz are employees of and/or have equity in Animal Biome, a company that develops microbiome-based diagnostics and next-generation probiotic products for companion animals. These authors affirm that this affiliation did not influence the study design, data analysis, interpretation of results, or the conclusions presented in this manuscript. All other authors declare no competing financial or non-financial interests.

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260 **Supplementary Material**

261 Supplementary Table 1: DNA-DNA Hybridisation table from GGDC

262 **Data Availability Statement**

263 The genomes of strains AB800T, as well as the five strains reported in this study, were submitted
264 and referenced in the NCBI database with the accession numbers JBLOUI000000000 for
265 *Peptacetobacter felis* AB800^{T} and the temporary BioSample accession numbers for AB0403,
266 AB804, AB807, AB808, and AB811 are SAMN53260830, SAMN53260831, SAMN53260832,
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268

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List of Figures

Fig. 1: Phenotypic characterisation and structure of strain AB800^{T}. a1) and a2) show colony morphology on Chocolate agar and Brain Heart Infusion agar, respectively (Anaerobe Systems, Morgan Hill, CA); b1) shows transmission electron microscopy structure with a rod-shaped bacterium; b2) represents Gram staining under 100X light microscopy; c) displays the MALDI-TOF spectrum with the main proteic profile and associated molecular weight.

Fig. 2: Phylogenetic tree inference using the General Time-Reversible model and bootstrap 1000. **a**: 16S rRNA gene full-length phylogenetic tree with midpoint rooting topology showed strain AB800^{T} to be close to P. hiranonis. **b**: Core genome alignment phylogeny inference with midpoint rooting, strain AB800^{T} is shown on a separate branch and close to unknown metagenome assembly species.

Fig. 3: Whole genome taxonomic classification: **a:** Average Nucleotide Identity (ANI) of strain AB800^{T} compared to closed species retrieved from Gtdbtk classification output. **b:** Alignment Fraction (AF) of strain AB800^{T} compared to the closest species retrieved from Gtdbtk. The closest species based on the ANI (Average Nucleotide Identity) and Alignment Fraction (AF) are *Pepetacetobacter* sp. DOME MAG11184 and *Pepetacetobacter* sp. bin-44 (ANI=98.72%, AF=81.95%, ANI=98.67%, AF=74.17%). The closest known isolate is *Peptacetobacter hiranonis* DGF055145 (ANI=95.73%, AF=81.95%) with ANI < 96%

Fig. 4: Comparative genomic analysis of strain AB800^{T} compared to *P. hiranonis* and *P. hominis*. **a**: Venn diagram of the pangenome (ortholog genes) distribution. OMCL clustering showed that strain AB800^{T} shared only 1331 gene clusters with *P. hiranonis* and *P. hominis,* while conserving 500 genes as unique. It shares 405 with *P. hiranonis* and only 67 with *P. hominis*, confirming that strain AB800^{T} is closer to *P. hiranonis* than *P. hominis.* **b***:* Heatmap showing the distribution of the different components of bile acid metabolic pathways. While only *P. hiranonis* and strain AB800^{T} possess the bile acid 7-alpha and 7-beta-dehydroxylation pathways, only strain AB800^{T} lacks the deconjugation pathways.

393 **Tables:**

394

395 **Table 1:** Phenotypic characteristics of strain AB800^{T}, including morphology, enzymatic, amino acids, salts, utilization and activity,
396 carbon sources of strain AB800^{T} in comparison with existing members of the genus *Peptacetobacter*, TO-931^{T}, HD-17, and
397 ZHW00191^{T}

398

Substrates tested	*Peptacetobacter Felis* AB800^{T}	*Peptacetobacter hiranonis* TO-931^{T}	*Peptacetobacter hiranonis* HD-17	*Peptacetobacter hominis* ZHW00191^{T}
Basic characteristics				
Cell diameter (µm)		0.8 × 1.6–10	0.8 × 1.6–10	0.7–1 × 3.2–8
Oxygen requirement	Strict Anaerobe	Strict anaerobe	Obligate anaerobe	Obligate anaerobe
Gram stain	+	+	+	+
Motility	non-motile	non-motile	non-motile	non-motile
Endospore formation	-	+	+	-
Enzyme				
Alkaline phosphatase	+	NA	NA	+
Esterase (C 4)	+	NA	NA	w
Esterase Lipase (C 8)	+	NA	NA	+
Lipase (C 14)	-	NA	NA	NA
Leucine arylamidase	+	NA	NA	NA
Valine arylamidase	-	NA	NA	NA
Cystine arylamidase	-	NA	NA	NA
Trypsin	-	NA	NA	NA

α-chymotrypsin	-	NA	NA	NA
Acid phosphatase	+	NA	NA	+
Naphthol-AS-BI-phosphohydrolase	+	NA	NA	NA
α-galactosidase	-	NA	NA	-
ß-galactosidase	-	NA	NA	NA
ß-glucuronidase	-	NA	NA	NA
α-glucosidase	-	-	-	-
ß-glucosidase	-	NA	NA	NA
N-acetyl-ß-glucosaminidase	+	+	+	+
α-mannosidase	-	NA	NA	NA
α-fucosidase	-	NA	NA	NA
Amino acids / Salts / Carbon source	-	NA	NA	NA
Urea	-	NA	NA	NA
L-Tryptophan	-	NA	NA	NA
D-Glucose	+	+	+	+
D-Mannitol	-	-	-	w (acid)
Inositol	-	NA	NA	NA
D-Sorbitol	-	-	-	-
L-Rhamnose	-	-	-	-
D-Saccharose	+	+	+	-
D-Melibiose	-	NA	NA	NA

Amygdalin	-	NA	NA	NA
L-Arabitol	-	NA	NA	NA
Temperature range (°C)	37	30–50 (optimum ≈37)	30–50 (optimum ≈37)	25–45 (optimum 37–42)
pH range	NA	6.5–9.0 (optimum 7.5–8.0)	6.5–9.0 (optimum 7.5–8.0)	5.5–10.0 (optimum 6.5–7.0)
NaCl tolerance	NA	Up to ~1% (reported low)	Up to ~1% (reported low)	0–2% (optimum 0%)
Proline aminopeptidase	NA	+	+	+
Bile acid 7α-dehydroxylating activity	NA	High activity	High activity	NA
Origin	feline gut	Human gut	Human gut	Human gut

NA = Not available / not tested / data not reported ; **w** = Weak reaction ; **+** = Positive reaction / activity present ;
– = Negative reaction/activity absent

401 **Table 2:** Genomic features of all strains of strain AB403, strain AB800^T, strain AB804, strain AB807, strain AB808, strain AB811,
402 including antimicrobial resistance genes and genes involved in metabolism pathways.
403

	Description	**AB0403**	AB800^T	**AB804**	**AB807**	**AB808**	**AB811**
Genome features							
contigs		189	149	221	179	182	199
bases	Size	2518813	2583354	2529093	2692535	2857951	2430653
CDS	number of coding sequences	2206	2355	2299	2507	2624	2165
gene	Number of genes	2297	2450	2390	2599	2719	2261
rRNA	-	16	16	17	16	20	17
repeat_region	-	3	3	3	2	3	3
tRNA	-	74	78	73	75	74	78
tmRNA	-	1	1	1	1	1	1
Resistance Genes							
(Tet)tetA-P	Tetracycline resistance gene	-	-	-	-	-	-
(Tet)tetB-P	Tetracycline resistance gene	+	+	+	-	-	+
ERMQ	Macrolide resistance gene	-	-	-	-	-	-
LnuP	Lincosamide resistance gene	-	-	-	-	-	-
tetA(P)	Tetracycline resistance gene	+	+	+	-	-	+
Bile acid metabolism genes							
baiN_2	3-dehydro-bile acid delta(4,6)-reductase	+	+	+	+	+	+
baiN_1	3-dehydro-bile acid delta(4,6)-reductase	+	+	+	+	+	+
baiB	Bile acid-coenzyme A ligase	+	+	+	+	+	+
baiE	Bile acid-coenzyme A ligase	+	+	+	+	+	+
baiCD_1	3-oxocholoyl-CoA 4-desaturase	+	+	+	+	+	+
baiCD_2	3-oxocholoyl-CoA 4-desaturase	+	+	+	+	+	+
baiF	Bile acid-CoA transferase	+	+	+	+	+	+

baiH	7-beta-hydroxy-3-oxochol-24-oyl-CoA 4-desaturase	+	+	+	+	+	+
baiA2	Bile acid 7-alpha dehydratase	+	+	+	+	+	+
baiN_3	3-dehydro-bile acid delta(4,6)-reductase	-	-	-	-	-	-
baiN_4	3-dehydro-bile acid delta(4,6)-reductase	-	-	-	-	-	-
baiN_5	3-dehydro-bile acid delta(4,6)-reductase	-	-	-	-	-	-

+ = Presence of genes ; – = Absence of genes

406 **List of Supplementary data**

407 **Supplementary Table 1**: DNA-DNA Hybridization table from GGDC with strain AB800^{T} as the

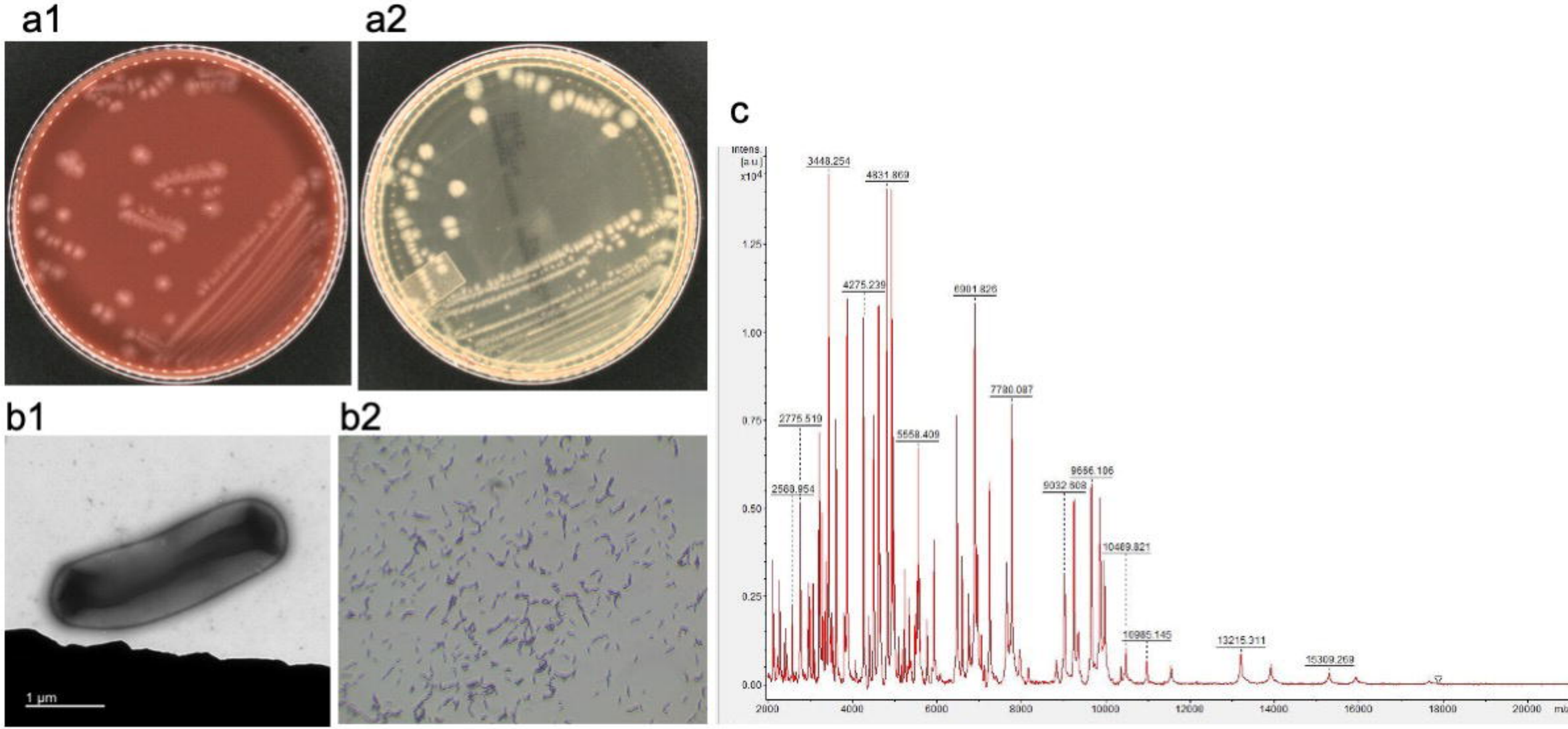
a1
a2
b1
b2
1 µm
c
Intens.
[a.u.]
x10^4
1.25
1.00
0.75
0.50
0.25
0.00
3448.254
4831.869
4275.239
6901.826
7780.087
2776.519
5558.409
2568.954
9686.106
9032.808
10469.821
10985.145
13215.311
15309.269
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000

Fig.1

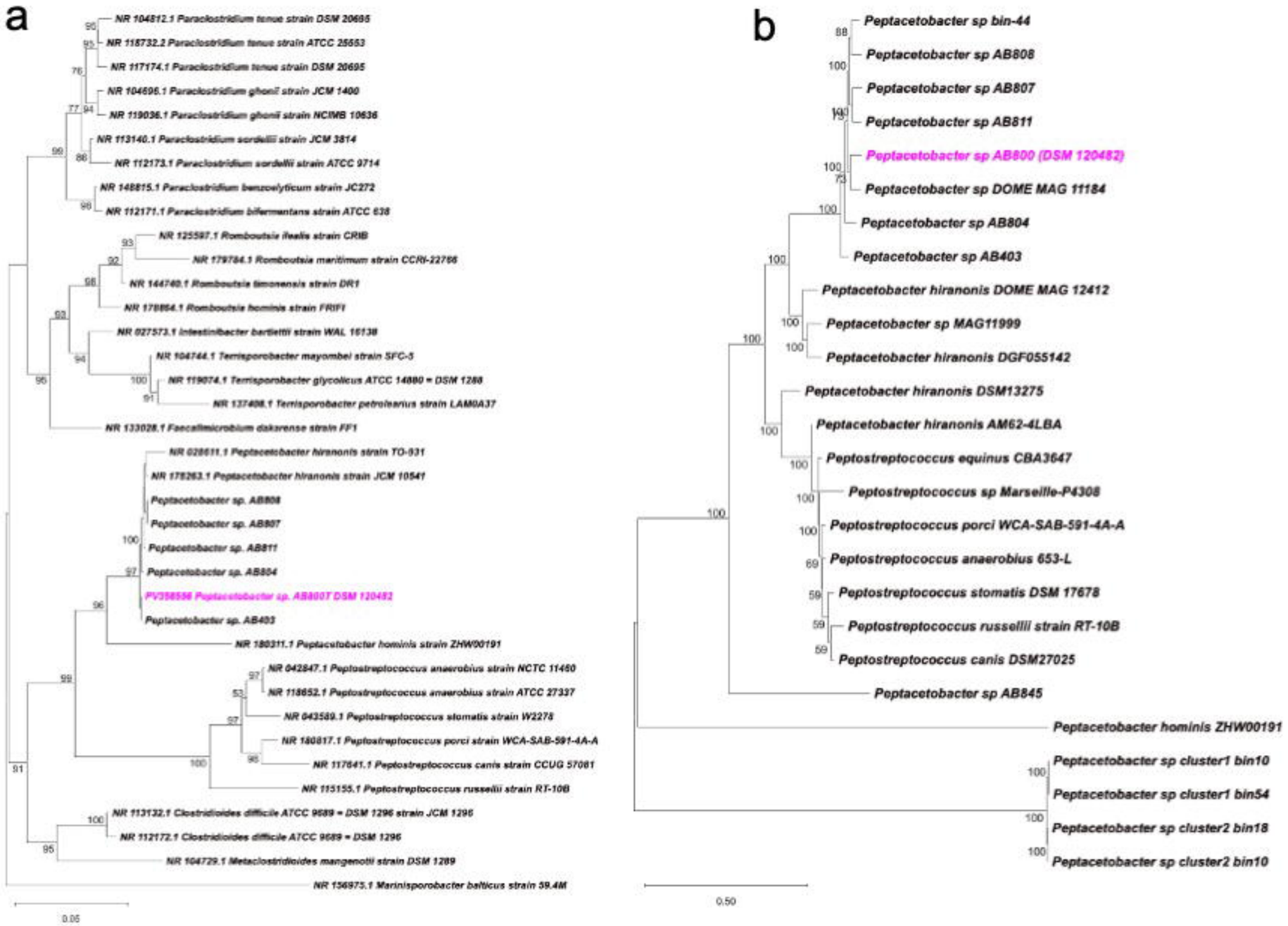
a
NR 104812.1 Paraclostridium tenue strain DSM 20695
NR 118732.2 Paraclostridium tenue strain ATCC 25553
NR 117174.1 Paraclostridium tenue strain DSM 20695
NR 104696.1 Paraclostridium ghonii strain JCM 1400
NR 119036.1 Paraclostridium ghonii strain NCIMB 10636
NR 113140.1 Paraclostridium sordellii strain JCM 3814
NR 112173.1 Paraclostridium sordellii strain ATCC 9714
NR 148815.1 Paraclostridium benzowlyticum strain JC272
NR 112171.1 Paraclostridium bifermentans strain ATCC 638
NR 125597.1 Romboutsia ilealis strain CRIB
NR 179784.1 Romboutsia maritimum strain CCRI-22766
NR 144740.1 Romboutsia timonensis strain DR1
NR 178864.1 Romboutsia hominis strain FRIFI
NR 027573.1 Intestinibacter bartlettii strain WAL 16138
NR 104744.1 Terrisporobacter mayombei strain SFC-5
NR 119074.1 Terrisporobacter glycolicus ATCC 14880 = DSM 1288
NR 137408.1 Terrisporobacter petroleanus strain LAM0A37
NR 133028.1 Faecalimicrobium dakarense strain FF1
NR 028611.1 Peptacetobacter hiranonis strain TO-931
NR 178263.1 Peptacetobacter hiranonis strain JCM 10541
Peptacetobacter sp. AB808
Peptacetobacter sp. AB807
Peptacetobacter sp. AB811
Peptacetobacter sp. AB804
PV358556 Peptacetobacter sp. AB800T DSM 120482
Peptacetobacter sp. AB403
NR 180311.1 Peptacetobacter hominis strain ZHW00191
NR 042847.1 Peptostreptococcus anaerobius strain NCTC 11460
NR 118652.1 Peptostreptococcus anaerobius strain ATCC 27337
NR 043589.1 Peptostreptococcus stomatis strain W2278
NR 180817.1 Peptostreptococcus porci strain WCA-SAB-591-4A-A
NR 117641.1 Peptostreptococcus canis strain CCUG 57081
NR 115155.1 Peptostreptococcus russellii strain RT-10B
NR 113132.1 Clostridioides difficile ATCC 9689 = DSM 1296 strain JCM 1296
NR 112172.1 Clostridioides difficile ATCC 9689 = DSM 1296
NR 104729.1 Metaclostridioides mangenotii strain DSM 1289
NR 156975.1 Marinisporobacter balticus strain 59.4M
0.05
b
Peptacetobacter sp bin-44
Peptacetobacter sp AB808
Peptacetobacter sp AB807
Peptacetobacter sp AB811
Peptacetobacter sp AB800 (DSM 120482)
Peptacetobacter sp DOME MAG 11184
Peptacetobacter sp AB804
Peptacetobacter sp AB403
Peptacetobacter hiranonis DOME MAG 12412
Peptacetobacter sp MAG11999
Peptacetobacter hiranonis DGF055142
Peptacetobacter hiranonis DSM13275
Peptacetobacter hiranonis AM62-4LBA
Peptostreptococcus equinus CBA3647
Peptostreptococcus sp Marseille-P4308
Peptostreptococcus porci WCA-SAB-591-4A-A
Peptostreptococcus anaerobius 653-L
Peptostreptococcus stomatis DSM 17678
Peptostreptococcus russellii strain RT-10B
Peptostreptococcus canis DSM27025
Peptacetobacter sp AB845
Peptacetobacter hominis ZHW00191
Peptacetobacter sp cluster1 bin10
Peptacetobacter sp cluster1 bin54
Peptacetobacter sp cluster2 bin18
Peptacetobacter sp cluster2 bin10
0.50

Fig.2

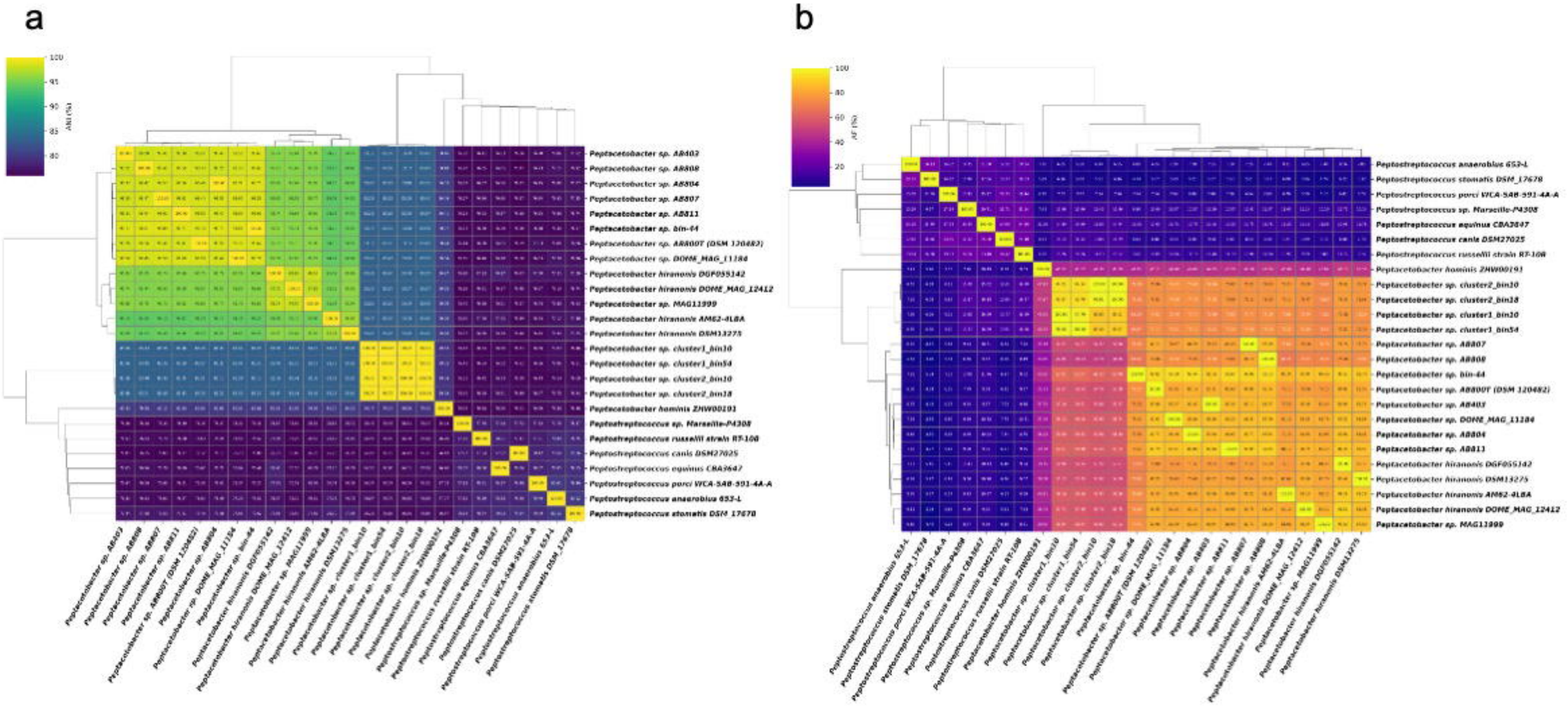
a
ANI (%)
100
95
90
85
80
Peptacetobacter sp. AB403
Peptacetobacter sp. AB808
Peptacetobacter sp. AB804
Peptacetobacter sp. AB807
Peptacetobacter sp. AB811
Peptacetobacter sp. bin-44
Peptacetobacter sp. AB800T (DSM 120482)
Peptacetobacter sp. DOME_MAG_11184
Peptacetobacter hiranonis DGF055142
Peptacetobacter hiranonis DOME_MAG_12412
Peptacetobacter sp. MAG11999
Peptacetobacter hiranonis AM62-4LBA
Peptacetobacter hiranonis DSM13275
Peptacetobacter sp. cluster1_bin10
Peptacetobacter sp. cluster1_bin54
Peptacetobacter sp. cluster2_bin10
Peptacetobacter sp. cluster2_bin18
Peptacetobacter hominis ZHW00191
Peptostreptococcus sp. Marseille-P4308
Peptostreptococcus russellii strain RT-108
Peptostreptococcus canis DSM27025
Peptostreptococcus equinus CBA9647
Peptostreptococcus porci VICA-SAB-591-4A-A
Peptostreptococcus anaerobius 653-L
Peptostreptococcus stomatis DSM 17678
b
AF (%)
100
80
60
40
20
Peptostreptococcus anaerobius 653-L
Peptostreptococcus stomatis DSM_17678
Peptostreptococcus porci VICA-SAB-591-4A-A
Peptostreptococcus sp. Marseille-P4308
Peptostreptococcus equinus CBA9647
Peptostreptococcus canis DSM27025
Peptostreptococcus russellii strain RT-108
Peptacetobacter hominis ZHW00191
Peptacetobacter sp. cluster1_bin10
Peptacetobacter sp. cluster1_bin54
Peptacetobacter sp. cluster2_bin10
Peptacetobacter sp. cluster2_bin18
Peptacetobacter sp. bin-44
Peptacetobacter sp. AB800T (DSM 120482)
Peptacetobacter sp. DOME_MAG_11184
Peptacetobacter sp. AB804
Peptacetobacter sp. AB403
Peptacetobacter sp. AB811
Peptacetobacter sp. AB807
Peptacetobacter sp. AB808
Peptacetobacter hiranonis AM62-4LBA
Peptacetobacter hiranonis DOME_MAG_12412
Peptacetobacter sp. MAG11999
Peptacetobacter hiranonis DGF055142
Peptacetobacter hiranonis DSM13275

Fig.3

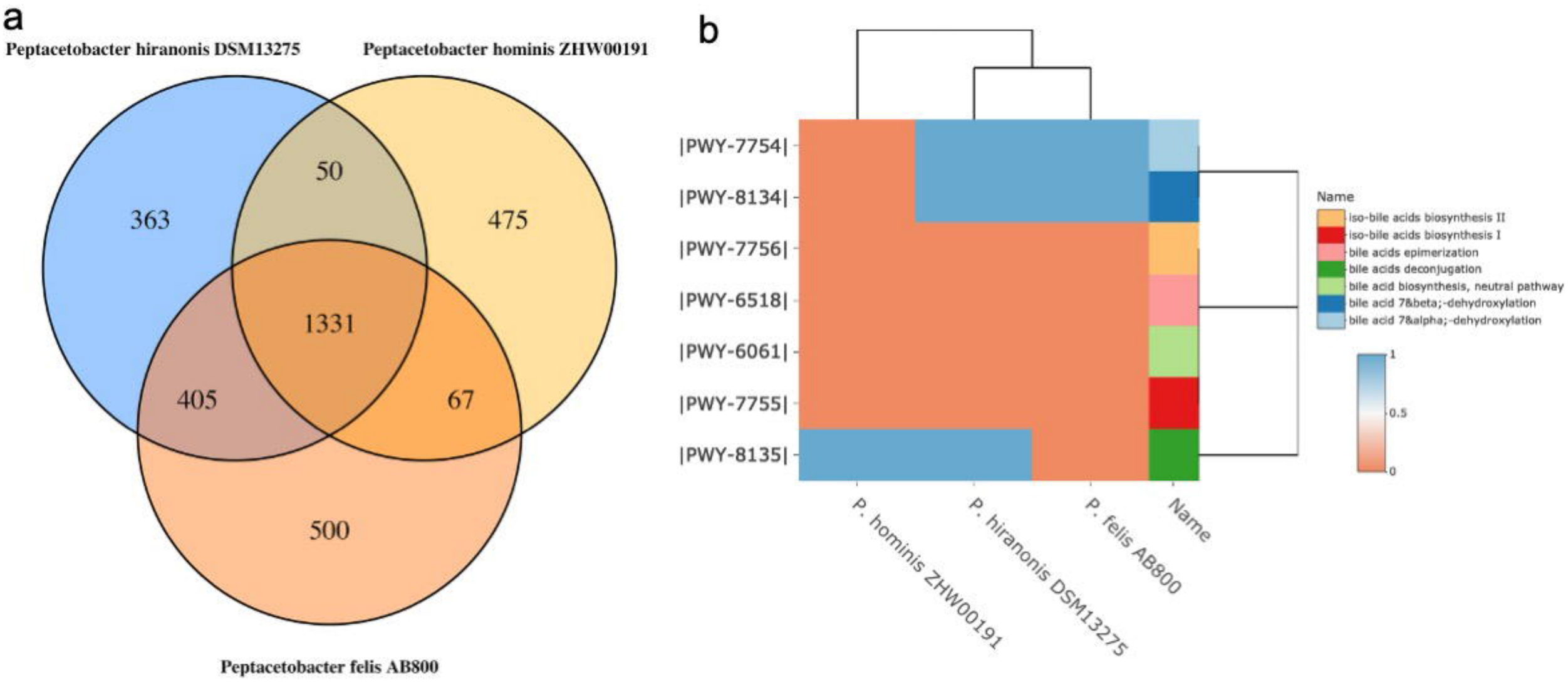
a
Peptacetobacter hiranonis DSM13275
Peptacetobacter hominis ZHW00191
363
50
475
1331
405
67
500
Peptacetobacter felis AB800
b
|PWY-7754|
|PWY-8134|
|PWY-7756|
|PWY-6518|
|PWY-6061|
|PWY-7755|
|PWY-8135|
P. hominis ZHW00191
P. hiranonis DSM13275
P. felis AB800
Name
Name
iso-bile acids biosynthesis II
iso-bile acids biosynthesis I
bile acids epimerization
bile acids deconjugation
bile acid biosynthesis, neutral pathway
bile acid 7β-dehydroxylation
bile acid 7α-dehydroxylation
1
0.5
0

Fig.4