

Linking carbohydrate structure with function in the human gut microbiome using hybrid metagenome assemblies

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18 Keywords: Nanopore Sequencing, CAZymes, colonic fermentation, metagenomics, starch

19

20 **Abstract** [350 words]

21 **Background**

22 Complex carbohydrates that escape digestion in the small intestine, are broken down in the
23 large intestine by enzymes encoded by the gut microbiome. This is a symbiotic relationship
24 between particular microbes and the host, resulting in metabolic products that influence
25 host gut health and are exploited by other microbes. However, the role of carbohydrate
26 structure in directing microbiota community composition and the succession of
27 carbohydrate-degrading microbes is not fully understood. Here we take the approach of
28 combining data from long and short read sequencing allowing recovery of large numbers of
29 high quality genomes, from which we can predict carbohydrate degrading functions, and
30 impact of carbohydrate on microbial communities.

31 **Results**

32 In this study we evaluate species-level compositional variation within a single microbiome in
33 response to six structurally distinct carbohydrates in a controlled model gut using hybrid
34 metagenome assemblies. We identified 509 high-quality metagenome-assembled genomes
35 (MAGs) belonging to ten bacterial classes and 28 bacterial families. We found dynamic
36 variations in the microbiome amongst carbohydrate treatments, and over time. Using these
37 data, the MAGs were characterised as primary (0h to 6h) and secondary degraders (12h to
38 24h). Annotating the MAG's with the Carbohydrate Active Enzyme (CAZyme) database we
39 are able to identify species which are enriched through time and have the potential to
40 actively degrade carbohydrate substrates.

41 **Conclusions**

42 Recent advances in sequencing technology allowed us to identify significant unexplored
43 diversity amongst starch degrading species in the human gut microbiota including CAZyme

44 profiles and complete MAGs. We have identified changes in microbial community
45 composition in response to structurally distinct carbohydrate substrates, which can be
46 directly related to the CAZyme complement of the enriched MAG's. Through this approach,
47 we have identified a number of species which have not previously been implicated in starch
48 degradation, but which have the potential to play an important role.

49

50 Microbial diversity within the microbiome and its interactions with host health and nutrition
51 are now widely studied[1]. An important role of the human gut microbiome is the metabolic
52 breakdown of complex carbohydrates derived from plants and animals (e.g. legumes, seeds,
53 tissue and cartilage)[2]. Short chain fatty acids (SCFA) are the main products of
54 carbohydrate fermentation by gut microbiota and provide a myriad of health benefits
55 through their systemic effects on host metabolism.[3, 4] However, we still do not have a
56 complete picture of the range of microbial species involved in fermentation of complex
57 carbohydrates to produce SCFA. Understanding the intricacies of complex carbohydrate
58 metabolism by the gut microbiota is a significant challenge. The function of many 'hard to
59 culture species' remains obscure and while advances in sequencing technology are
60 beginning to reveal the true diversity of the human gut microbiota, there is still much to be
61 learned.[5]

62 A key challenge is understanding the influence of structural complexity of
63 carbohydrates on microbiota composition. Carbohydrates possess immense structural
64 diversity, both at the chemical composition level (monomer and sugar linkage composition)
65 and at the mesoscale. Individual species, or groups of species, within the gut microbiota are
66 highly adapted to defined carbohydrate structures[6]. Starch is representative of the
67 structural diversity found amongst carbohydrates and serves as a good model system as
68 starches are readily fermented by several different species of colonic bacteria.[7] The gut
69 microbiota is repeatedly presented with starches of diverse structures from the diet.[8]
70 Consistent in starch is an α -1 \rightarrow 4 linked glucose back bone, interspersed with α -1 \rightarrow 6 linked
71 branch points. Despite this apparent structural simplicity, starches botanical origin and
72 subsequent processing (e.g. cooking) impacts its physicochemical properties, particularly
73 crystallinity and recalcitrance to digestion.[7] It has been shown *in vitro*[7], in animal

74 models[9] and in human interventions[8], that altering starch structure can have a profound
75 impact on gut microbiome composition.

76 The microbiome is known to harbour a huge repertoire of carbohydrate-active
77 enzymes (CAZymes) that can degrade diverse carbohydrate structures.[10, 11] However, it
78 is a formidable challenge to study this functionality in complex microbial communities due
79 to limitations in the depth of sequencing and coverage of all members in the community.

80 While metagenomic sequencing has become a key tool, identifying genomes and functional
81 pathways within the microbiome remains challenging in second generation sequencing due
82 to limitations associated with short (~300bp) reads. Third generation sequencing such as
83 nanopore sequencing (Oxford Nanopore Technologies (ONT)) promises to circumvent these
84 difficulties by providing longer reads (> 3 kilobase pairs [kbp]). This technology has become
85 popular in clinical metagenomics for rapid pathogen diagnosis[12] and in human genomics
86 research.[13] Long-read sequences can help bridge inter-genomic repeats and produce
87 better *de novo* assembled genomes.[14] While the MinION platform from ONT has been
88 used for metagenomic studies,[15] it cannot provide sufficient sequencing depth and
89 coverage to sequence the many hundreds of genomes present in the human gut

90 microbiome. PromethION (ONT) is capable of producing far greater numbers of sequences
91 compared to either MinION or GridION, averaging four-five times more data per flow cell
92 and the capacity to run up to 48 flow cells in parallel; this makes it suitable for
93 metagenomics and microbiome studies. For example, PromethION has been used for long-
94 read sequencing of environmental samples such as wastewater sludge, demonstrating its
95 potential to recover large numbers of metagenome-assembled genomes (MAGs) from
96 diverse mixtures of microbial species.[16] However, long error-prone reads aren't ideal for

97 species resolution metagenomics, therefore, a hybrid approach using short and long read
98 data has been found to be most effective for generating accurate MAGs.[17]

99 To achieve species-level resolution of the microbes present in the gut microbiome
100 during complex carbohydrate utilisation, we conducted a genome-resolved metagenomics
101 study in a controlled gut colon model. *In vitro* fermentation systems have been used
102 extensively to model changes in the gut microbial community as a result of external inputs,
103 e.g., changes in pH, protein and carbohydrate supply[7, 18, 19]. We measured the dynamic
104 changes in bacterial populations during fermentation of six structurally contrasting
105 substrates: two highly recalcitrant starches (native Hylon VII (“Hylon”) and native potato
106 starch (“potato”)); two accessible starches (native normal maize starch (“n.maize”) and
107 gelatinized then retrograded maize starch (“r.maize”); an insoluble fibre (cellulose) resistant
108 to fermentation (“Avicel”); and a highly fermentable soluble fibre (“inulin”). By generating
109 hybrid assemblies using PromethION and NovaSeq data, we obtained 509 MAGs. The
110 dereplicated set consisted of 151 genomes belonging to ten bacterial classes and 28
111 bacterial families. Using genome-level information and read proportions data, we identified
112 several species that have novel putative starch-degrading properties.

113

114 **Results**

115 **PromethION and NovaSeq sequencing of model gut samples enriched for carbohydrate**
116 **degrading species.** Fermentation of six contrasting carbohydrate substrates (inulin, Hylon,
117 n.maize, potato, r.maize and Avicel; see methods section) was initiated by inoculation of the
118 model colon with a carbohydrate and faecal material and the gut microbial community
119 composition was monitored over time (0h, 6h, 12h and 24h) by sequencing as shown in

120 Figure 1. In total, 23 samples and a negative control were sequenced (see Supplementary
121 Table 1 for the PromethION and NovaSeq summary sequencing statistics).

122 *PromethION sequencing:* The two sequencing runs generated 144 giga base pairs
123 (Gbp) of raw sequences. In the first run, all 23 samples were analysed while in the second
124 batch, 12 samples from hylon, inulin and r.maize were selected. The first run produced 7.87
125 million reads with an average read length of 3419 ± 57 bp and the second run generated
126 21.6 million reads with an average read length of 4707 ± 206 bp. Consolidating the runs,
127 trimming and quality filtering resulted in the removal of 33.3 ± 14.7 % of reads
128 (Supplementary Table 1). Median read lengths after trimming were 4972.5 ± 229 bp and the
129 median quality score was 9.7 ± 0.9 .

130 *Illumina sequencing:* All 23 samples provided high quality sequences (Q value > 30)
131 generating a mean of 27 million reads per sample. Quality and read length (<60 bp) filtering
132 removed 2.96 % of reads (Supplementary Table 1).

133
134 **Dynamic shifts in taxonomic profiles among carbohydrate treatments.** Hierarchical
135 clustering for the taxonomic profiling using MetaPhlAn3 for each sample is shown in
136 Supplementary Table 2. At baseline (0h), profiles of the top 30 selected species by clustering
137 (using Bray-Curtis distances for samples and species, and a complete linkage) is similar for
138 all treatments, as expected (**Error! Reference source not found.**). This uniform profile was
139 distinct from the water control sample (a.k.a. 'the kitome'). The water blank also had less
140 than 3% (NovoSeq) and less than 0.2% (PromethION) of the reads of the samples.
141 Microbiome shifts were apparent from 6h in the n.maize treatment which showed a very
142 high abundance of *E. coli*, indicating contamination. After 12h, the profiles changed further

143 with a higher abundance of *E. coli* and *B. animalis* in the n.maize treatment while the
144 r.maize and inulin treatment profiles were similar, as were the potato and Hylon treatment
145 profiles. By the last sampling point (24h), potato and hylon had similar profiles which are
146 also similar to r.maize. The most abundant species in all the substrates was consistently
147 *Prevotella copri* which decreased in abundance over time but remained one of the most
148 abundant species throughout. After 6h and 12h, *Ruminococcus bromii* (a keystone starch
149 degrader) and *Bifidobacterium adolescentis* increased in abundance in the r.maize, potato
150 and Hylon treatments. *Faecalibacterium prauunitzii* decreased in abundance in inulin at 6h
151 and 12h and then increased in abundance for inulin and avicel at 24h.

152 Dynamic shifts in the microbiome were estimated using PCoA (Supplementary Figure
153 1), with 77% of total variance being explained by the first two components. As expected, the
154 0h profiles clustered closely together. The most distinct taxonomic change in microbial
155 community composition was apparent in the Avicel treatment after 24h. Inulin and r.maize
156 profiles clustered more closely together than potato and Hylon profiles. Inverse Simpson
157 index results followed a similar pattern for changes in diversity, which decreased after 0h
158 followed by a gradual increase (Supplementary Figure 2). However, in the Avicel treatment
159 there was a different pattern of taxonomic shifts with a large number of taxa increasing in
160 abundance after 12h.

161
162 **Hybrid metagenome assemblies vs short-read only assemblies.** Using Opera-MS, we
163 combined PromethION reads with Illumina assemblies to produce hybrid assemblies. The
164 assembly statistics for short-read-only and hybrid assemblies are shown in Supplementary
165 Table 3 and **Error! Reference source not found.** The longest N50 and the largest contig per

166 treatment were generated using hybrid assemblies as expected (figure 3b & 3c). The overall
167 length of assembled sequences was similar for both approaches (Figure 3d).

168 The reads from each treatment and collective T0 were co-assembled into hybrid
169 assemblies and binned into MAGs. In total we binned and refined 509 MAGs that met the
170 MIMAG quality score criteria[20] of which 65% (n=333) were high-quality (Figure 4;
171 Supplementary table 4). From the co-assemblies, thirty-five MAGs had an N50 of > 500,000
172 Mbp and 158 MAGs were assembled into < 30 scaffolds. The MAGs were dereplicated into
173 primary and secondary clusters according to Average Nucleotide identity (ANI) (primary
174 clusters <97%; secondary clusters <99%). In total, we identified 151 MAG secondary clusters
175 (Supplementary table 5). Each genome cluster consisted of between one and seven
176 genomes based on their genome similarity.

177

178 **Taxonomic annotation of MAGs.** Proposed bacterial taxonomy using GTDb was represented
179 in existing bacterial families: All MAG clusters had > 99% identity to existing genera
180 (Supplementary Table 6). Here, 49 of the 151 MAG clusters were named using alphanumeric
181 genus names and 19 MAG clusters were named using alphanumeric species names. In order
182 to provide a clear and stable genus and species names, the MAGs were directly compared in
183 NCBI to check whether these MAGs were already named or had any culture representatives.
184 We identified 12 MAGs that were previously named and/or cultured so the Latin binomial for
185 these MAGs was updated (Supplementary table 7). For the rest of the MAGs, we used the
186 approach described in Pallen *et al*[21] to provide novel Latin names to 56 MAG clusters. We
187 have provided 12 new genus names and 51 novel species names (Table 1). In addition, MAG
188 assembly statistics for the MAGs in the present study was compared to the representative
189 assemblies in GTDb (Supplementary Table 8). We found that while the average overall

190 assembly length was almost similar (an average of 2,250,870 bp in the present study vs.
191 2,541,312bp in GTDb), there were far fewer contigs in our assemblies (an average of 67
192 contigs in the present study vs. 160 in GTDb), and therefore our MAGs may be considered to
193 be of higher quality.

194

195 **Carbohydrate structure drives progression of bacterial diversity.** Relative abundance of
196 each MAG within treatments was calculated and log fold change of abundance between
197 treatments was used to estimate change in relative abundance (Supplementary table 9). In
198 total, 36 of 151 clusters exhibited ≥ 2 -log fold increase in relative abundance for all
199 treatments. Specifically, ≥ 2 -log fold change in abundance was seen in 6, 12, 11 and 18 MAGs
200 for Avicel, Hylon, potato and r.maize treatments, respectively (Figure 5). The genomes were
201 partitioned as early (0h up to 6h) and late degraders (12h to 24h) according to when they
202 first showed an increase in relative abundance (Supplementary Table 10).

203 Relative abundance of all MAGs from each treatment was aggregated and plotted for each
204 time period (Supplementary figure 3). Relative abundance was constant for Avicel
205 throughout indicating low activity of the MAGs in utilising crystalline cellulose, likely
206 reflecting the very limited fermentability of microcrystalline cellulose. As for other maize
207 starches (hylon, r.maize and potato), the read proportions showed an overall reduction in
208 abundance, with only starch degrading MAGs increasing in abundance.

209

210 **CAZyme family interplay with the carbohydrate treatments.** For identifying CAZymes in the
211 MAGs, genome-predicted proteins identified by Prodigal were compared with the CAZy
212 database using dbCAN2 (Supplementary table 11). CAZyme counts specifically for Glycoside
213 hydrolases (GH) and Carbohydrate binding modules (CBM) for all clusters showed a high

214 representation of the profiles with GH13, GH2 and GH3 accounting for 34.1% of all counts
215 (Supplementary Figure 4). CAZyme profiles for MAGs with > 2-log fold change are
216 highlighted in Supplementary table 12 and Figure 6. Although six genomes were identified
217 as associated with the degradation of cellulose, none contained any characteristic cellulose
218 active CAZy proteins indicating multiple cross feeders. *Collinsella aerofaciens*_J (cluster
219 29_1), *Candidatus Minthovivens enterohominis* (cluster 81_1) are novel genomes that
220 showed a 2x log -fold increase when in the presence of inulin and also harboured multiple
221 copies of inulinases (GH32). *Bacteroides uniformis*, a known inulin degrader also contained
222 multiple copies of GH32. We identified a large representation of the amylolytic (starch
223 degrading) gene family GH13 in Hylon (counts= 88), potato (counts=50) and r.maize
224 (counts=77) treatments. As expected, GH13 was weakly represented in Avicel (counts=19)
225 and inulin (counts=29) treatments (Figure 6). The presence of GH13 in MAGs was closely
226 associated with CBM48, which is commonly appended to starch degrading GH13
227 enzymes.[22] In total, we identified several novel degraders and previously discovered
228 degraders of the different carbohydrate treatments which are highlighted in Supplementary
229 table 10.

230

231 **Discussion**

232 Using a hybrid assembly approach (i.e., combining NovaSeq short-read and PromethION
233 long-read metagenomic data), we report species-level resolved taxonomic data identifying
234 distinct changes in microbiome composition in response to different substrates. The large
235 number of high-quality near-complete MAGs that we generated using this approach
236 enabled us to functionally annotate the CAZymes in the MAGs and identify potential

237 carbohydrate degrading species. Several of these species have not previously been
238 identified as playing a role in starch fermentation (Figure 6 and Supplementary Table 9).

239

240 **High quality DNA for long-read sequencing was extracted using a bead beating protocol**

241 The N50 for the PromethION reads was 4,972 bp, which is comparable with another recent
242 study using bead-beating-based DNA extraction and provided adequate read lengths to be
243 useful for assembly of MAGs.[17] A recent publication by Moss *et al.*[23] and associated
244 protocol paper[24] suggested that bead beating DNA extraction protocols were unsuitable
245 for long-read sequencing as they led to excessive shearing of DNA and therefore enzymatic
246 cell lysis followed by phenol-chloroform purification were preferred to recover high
247 molecular weight (HMW) DNA. This was not reflected in our experience. The N50's obtained
248 by Moss *et al.* for sequencing DNA extracted from stool samples by phenol-chloroform on
249 the PromethION platform ranged from 1,432 bp to 5,205 bp, which on average was shorter
250 than the N50 we obtained using comparable samples extracted by a bead beating protocol.

251 This is in agreement with Bertrand *et al.*[14] who directly compared commercial bead
252 beating and phenol-chloroform extraction protocols for extracting HMW DNA from stool
253 samples for MinION sequencing and found that while phenol-chloroform gave higher
254 molecular weights of DNA, the DNA was of low integrity compromising sequencing quality.

255

256 **Hybrid assemblies allow generation of near complete MAGs.** We found larger N50s and
257 longest contigs when using hybrid assemblies compared with short-read assemblies; this is
258 in agreement with previous benchmarking data using a combined MinION and Illumina
259 hybrid approach to sequence mock communities, human gut samples,[14] and rumen gut
260 microbiota samples.[17] This allowed us to assemble 509 MAGs across all the major

261 phylogenetic groups (Supplementary file 5), with representatives from ten bacterial classes
262 and 28 families, including both Gram-positive and Gram-negative species. Bertand et al.[14]
263 found that phenol-chloroform extractions led to underrepresentation of 'hard to sequence'
264 gram-positive species such as those of the genus *Bifidobacterium*. In the present study near-
265 complete MAG's were recovered from 5 different species of *Bifidobacterium*, in contrast to
266 Moss et al.[23] who were unable to recover *Bifidobacterium* MAG's from the PromethION
267 data produced using their enzyme and phenol-chloroform based extraction method
268 (although they were able to recover *Bifidobacterium* MAG's from short-read data which was
269 obtained following a bead beating based DNA extraction of the same samples). This
270 indicates that bead beating is necessary to obtain accurate representations of the microbial
271 community in human stool samples. The bead beating DNA extraction protocol used in this
272 study was also recommended by the Human Microbiome Project to avoid biases in
273 microbiome samples.[25, 26]

274 We have provided *Candidatus* names to 70 bacterial species which do not currently have
275 representative Latin binomial names in the GTDB database (Table 1 and Supplementary
276 Table 7). Our decision to provide names for these species reflects the higher quality of
277 MAGs compared to those currently represented in the databases (Supplementary Table 8).

278

279 **Structural diversity in substrates drives changes in microbial communities.** Over the 24h
280 fermentation, microbial communities rapidly diverged depending on substrate. The smallest
281 change in community composition occurred in the Avicel treatment, as would be expected,
282 given that Avicel was the most recalcitrant substrate evaluated, with very limited
283 fermentability.[27] Each substrate resulted in distinct changes in microbial community

284 composition, supporting previous findings that chemically-identical but structurally-diverse
285 starches can result in distinct changes in microbial community composition.[7, 8]

286

287 **Changes in microbial composition are related to the ability to degrade structurally diverse**
288 **substrates.** To better understand potential mechanisms driving the changes in microbial
289 species composition in response to different substrates, we explored the CAZyme profiles of
290 our microbial community .[28] We found the greatest number and diversity of CAZyme
291 genes were in the genomes of *Bacteroidetes* (Figure 6 and Supplementary Figure 4), as has
292 previously been computationally estimated for the human gut microbiome.[10, 29] This is in
293 contrast to rumen microbiomes where *Fibrobacterales* are the primary fibre-degrading
294 bacterial group.[17]

295 We identified genomes that increased in abundance during either early or late
296 stages of fermentation suggesting that their involvement in substrate degradation was
297 either as primary (early) or secondary (late) degraders (Figure 5). We also identified
298 differences in abundance of particular CAZyme-encoding genes amongst species which may
299 reflect their specialisation to specific substrates (Figure 6). *Bacteroides uniformis* has been
300 characterised as an inulin-degrading species,[30] and in our analysis it was identified during
301 inulin fermentation and had three copies of the GH32 (inulinase) gene and a gene encoding
302 the inulin binding domain, CBM38. *Candidatus Minthovivens enterohominis* also increased
303 in abundance early in inulin degradation, and its genome contained five copies of the GH32
304 gene. *Faecalibacterium prausnitzii* increased in abundance with inulin supplementation and
305 has been shown to have the ability to degrade inulin when co-cultured with primary
306 degrading species.[31, 32] *F. prausnitzii* was also found to increase in abundance for
307 cellulose, but not for the starch based substrates.

308 Avicel is a highly crystalline cellulose that is resistant to fermentation; the human gut
309 microbiota has a very limited capacity to degrade celluloses.[33] Interestingly, the largest
310 increase in abundance we observed was for *Blautia hydrogenotrophica*; which has been
311 reported in association with cellulose fermentation since it acts as an acetogen using
312 hydrogen produced by primary degraders of cellulose.[34]

313 In all starch treatments, there were large increases in the proportion of identified
314 genes that encoded GH13 (the major amylolytic gene family including α -amylase, α -
315 glucosidase and pullulanase) reflecting selection for starch-degrading species (Figure 6); this
316 was also the case for CBM48 which is also involved in starch degradation (Figure 6).[22] Our
317 analysis identified several well-known starch degrading species, most notably *R. bromii* and
318 *B. adoloscentis* (Figure 5). *R. bromii* is a well characterised specialist on highly recalcitrant
319 starch,[35] possessing specialised starch-degrading machinery termed the ‘amylosome’; it
320 was only identified in the most recalcitrant starch treatments (Hylon and potato). Previous
321 genome sequencing of an *R. bromii* isolate reported 15 GH13 genes;[35] 14 GH13 genes
322 were identified in the *R. bromii* MAG assembled in this study. In the potato treatment
323 another closely related but less well characterised *Rumminococcus* species with ten GH13
324 genes and one CBM48 gene was identified.

325 A previously uncultured *Blautia* species was identified possessing eight GH13 and
326 three CBM48 genes which increased in abundance in response to Hylon and potato. *Blautia*
327 species have previously been shown to increase in abundance in response to resistant
328 starch.[36, 37] We also identified four further previously-uncharacterised species that
329 increased in abundance and had more than five GH13 genes: *Candidatus Cholinenecus*
330 *caccae*, *Candidatus Eisenbergiella faecalis*, *Candidatus Enteromorpha quadrami* and
331 *Candidatus Aphodonaster merdae*.

332 Maize starch treatments (r.maize and Hylon) showed increases in abundance of
333 *Bifidobacterium* species. Previous studies have characterized *Bifidobacterium* as a starch-
334 degrading genus.[38] The only *Bifidobacterium* species to increase in abundance in response
335 to Hylon was *B. adolescentis*, which is known to utilise this hard-to-digest starch better
336 than other *Bifidobacterium* species,[39]; a broader range of *Bifidobacterium* species
337 increased in abundance in response to the more accessible r.maize.

338

339 **Conclusion**

340 We have demonstrated that deep long- and short-read metagenomic sequencing and hybrid
341 assembly has great potential for studying the human gut microbiota. We identified species-
342 level resolved changes in microbial community composition and diversity in response to
343 carbohydrates with different structures over time, identifying succession of species within
344 the fermenter. To provide functional information about these species we obtained over 500
345 MAGs from a single human stool sample. Annotating CAZyme genes in MAGs from species
346 enriched for by fermentation of different carbohydrates allowed us to identify species
347 specialised in degradation of defined carbohydrates, increasing our knowledge of the range
348 of species potentially involved in starch metabolism in the human gut.

349

350 **Material and Methods**

351 A schematic overview of the workflow and experimental design is displayed in Figure 1.
352 **Substrates.** Native maize starch (catalogue no. S4126), native potato starch (catalogue no.
353 2004), Avicel PH-101 (catalogue no. 11365) and chicory inulin (catalogue no. I2255) were
354 purchased from Sigma-Aldrich, (Gillingham, UK). Hylon VII® was kindly provided as a gift by
355 Ingredion Incorporated (Manchester, UK).

356 Retrograded maize starch was prepared from 40g of native maize starch in 400 mL of
357 deionized water. The slurry was stirred continuously at 95°C in a water bath for 20 minutes.
358 The resulting gel was cooled to room temperature for 60 minutes, transferred to aluminium
359 pots (150 mL, Ampulla, Hyde UK), and stored at 4°C for 48 hours. The retrograded gel was
360 then frozen at -80°C for 12 hours and freeze-dried (Lyodry, MechaTech Systems Ltd, Bristol,
361 UK) for 72 hours.

362 Each substrate (0.500 ± 0.005 g, dry weight) was weighed in sterilized fermentation
363 bottles (100 mL) prior to start of the experiment.

364 **Inoculum collection and preparation.** A single human faecal sample was obtained from one
365 adult (≥ 18 years old), free-living, healthy donor who had not taken antibiotics in the 3
366 months prior to donation and was free from gastrointestinal disease. Ethical approval was
367 granted by Human Research Governance Committee at the Quadram Institute (IFR01/2015)
368 and London - Westminster Research Ethics Committee (15/LO/2169) and the trial was
369 registered on clinicaltrials.gov (NCT02653001). A signed informed consent was obtained
370 from the participant prior to donation. The stool sample was collected by the participant,
371 stored in a closed container under ambient conditions, transferred to the laboratory and
372 prepared for inoculation within 2 hours of excretion. The faecal sample was diluted 1:10
373 with pre-warmed, anaerobic, sterile phosphate buffer saline (0.1M, pH 7.4) in a double
374 meshed stomacher bag (500 mL, Seward, Worthing, UK) and homogenized using a
375 Stomacher 400 (Seward, Worthing, UK) at 200 rpm for two cycles, each of 60 seconds
376 length.

377 **Batch fermentation in the model colon.** Fermentation vessels were established with media
378 adapted from Williams *et al.*, [40] In brief, each vessel (100 mL) contained an aliquot (3.0 mL)

379 of filtered faecal slurry, 82 mL of sterilized growth medium, and one of the six substrates for
380 experimental evaluation: native Hylon VII or native potato starch (highly recalcitrant
381 starches); native maize starch or gelatinized, retrograded maize starch (accessible starches);
382 Avicel PH-101 (insoluble fibre; negative control); and chicory inulin (fermentable soluble
383 fibre; positive control). There was also a media only control with no inoculum (blank)
384 making a total of seven fermentation vessels.

385 For each fermentation vessel the growth medium contained 76 mL of basal solution,
386 5 mL vitamin phosphate and sodium carbonate solution, and 1 mL reducing agent. The
387 composition of the various solutions used in the preparation of the growth medium is
388 described in detail in **Supplementary Table 13**. A single stock (7 litres) of growth medium
389 was prepared for use in all vessels. Vessel fermentations were pH controlled and maintained
390 at pH 6.8 to 7.2 using 1N NaOH and 1N HCl regulated by a Fermac 260 (Electrolab Biotech,
391 Tewkesbury, UK). A circulating water jacket maintained the vessel temperature at 37°C.
392 Magnetic stirring was used to keep the mixture homogenous and the vessels were
393 continuously sparged with nitrogen (99% purity) to maintain anaerobic conditions. Samples
394 were collected from each vessel at 0 (5 min), 6, 12, and 24 hours after inoculation. The
395 biomass from two 1.8 mL aliquots from each sample were concentrated by refrigerated
396 centrifugation (4°C; 10,000 g for 10 min), the supernatant removed, and the pellets stored
397 at -80°C prior to bacterial enumeration and DNA extraction; one pellet was used for
398 enumeration and one for DNA extraction.

399 **Bacterial cell enumeration.** All materials used for bacterial cell enumeration were
400 purchased from Sigma-Aldrich (Gillingham, UK), unless specified otherwise. To each frozen
401 pellet, 400 µL of PBS and 1100 µL of 4% paraformaldehyde (PFA) were added and gently

402 thawed at 20°C for 10 minutes with gentle mixing. Once thawed, each resuspension was
403 thoroughly mixed and incubated overnight at 4°C for fixation to occur. The resuspensions
404 were then centrifuged for 10 minutes at 8000 x g, the supernatant removed, and the
405 residual pellet washed with 1 mL 0.1% Tween-20. This pellet then underwent two further
406 washes in PBS to remove any residual PFA and was then resuspended in 600 µL PBS: ethanol
407 (1:1).

408 The fixed resuspensions were centrifuged for 3 minutes at 16000 x g, the
409 supernatant removed, and the pellet resuspended in 500 µL 1 mg/mL lysozyme (100 µL 1M
410 Tris HCl at pH 8, 100 µL 0.5 M EDTA at pH 8, 800 µL water, and 1 mg lysozyme, catalogue no.
411 L6876) and incubated at room temperature for 10 minutes. After thorough mixing and
412 centrifugation for 3 minutes at 16000 x g, the supernatant was removed, and the pellet
413 washed with PBS. The resulting pellet was then resuspended in 150 µL of hybridisation
414 buffer (HB, per mL: 180 µL 5 M NaCl, 20 µL 1M Tris HCl at pH 8, 300 µL Formamide, 499 µL
415 water, 1 µL 10% SDS), centrifuged, the supernatant removed and the remaining pellet
416 resuspended again in 1500 µL of HB and stored at 4°C prior to enumeration. For bacterial
417 enumeration, 1 µL of Invitrogen SYTO 9 (catalogue no. S34854, Thermo Fisher Scientific,
418 Loughborough, UK) was added to 1 mL of each fixed and washed resuspension. Within 96-
419 well plate resuspensions were diluted to 1:1000 and the bacterial populations within them
420 enumerated using flow cytometry (Luminex Guava easyCyte 5) at wavelength of 488nm and
421 Guava suite software, version 3.3.

422 **DNA extraction.** Each pellet was resuspended in 500 µL (samples collected at 0 and 6 hr) or
423 650 µL (samples collected at 12 and 24 hr) with chilled (4°C) nuclease-free water (Sigma-
424 Aldrich, Gillingham, UK). The resuspensions were frozen overnight at -80°C, thawed on ice
425 and an aliquot (400 µL) used for bacterial genomic DNA extraction. FastDNA® Spin Kit for

426 Soil (MP Biomedical, Solon, US) was used according to the manufacturer's instructions
427 which included two bead-beating steps of 60s at a speed of 6.0m/s (FastPrep24, MP
428 Biomedical, Solon, USA). DNA concentration was determined using the Quant-iT™ dsDNA
429 Assay Kit, high sensitivity kit (Invitrogen, Loughborough, UK) and quantified using a
430 FLUOstar Optima plate reader (BMG Labtech, Aylesbury, UK).

431 **Illumina NovaSeq Library preparation and sequencing.** Genomic DNA was normalised to 5
432 ng/µL with elution buffer (10mM Tris-HCl). A miniaturised reaction was set up using the
433 Nextera DNA Flex Library Prep Kit (Illumina, Cambridge, UK). 0.5 µL Tagmentation Buffer 1
434 (TB1) was mixed with 0.5 µL Bead-Linked Transposomes (BLT) and 4.0 µL PCR-grade water in
435 a master mix and 5 µL added to each well of a chilled 96-well plate. 2 µL of normalised DNA
436 (10 ng total) was pipette-mixed with each well of tagmentation master mix and the plate
437 heated to 55°C for 15 minutes in a PCR block. A PCR master mix was made up using 4 µL
438 kapa2G buffer, 0.4 µL dNTP's, 0.08 µL Polymerase and 4.52 µL PCR grade water, from the
439 Kap2G Robust PCR kit (Sigma-Aldrich, Gillingham, UK) and 9 µL added to each well in a 96-
440 well plate. 2 µL each of P7 and P5 of Nextera XT Index Kit v2 index primers (catalogue No.
441 FC-131-2001 to 2004; Illumina, Cambridge, UK) were also added to each well. Finally, the 7
442 µL of Tagmentation mix was added and mixed. The PCR was run at 72°C for 3 minutes, 95°C
443 for 1 minute, 14 cycles of 95°C for 10s, 55°C for 20s and 72°C for 3 minutes. Following the
444 PCR reaction, the libraries from each sample were quantified using the methods described
445 earlier and the high sensitivity Quant-iT dsDNA Assay Kit. Libraries were pooled following
446 quantification in equal quantities. The final pool was double-SPRI size selected between 0.5
447 and 0.7X bead volumes using KAPA Pure Beads (Roche, Wilmington, US). The final pool was
448 quantified on a Qubit 3.0 instrument and run on a D5000 ScreenTape (Agilent, Waldbronn,
449 DE) using the Agilent Tapestation 4200 to calculate the final library pool molarity. qPCR was

450 done on an Applied Biosystems StepOne Plus machine. Samples quantified were diluted 1 in
451 10,000. A PCR master mix was prepared using 10 µL KAPA SYBR FAST qPCR Master Mix (2X)
452 (Sigma-Aldrich, Gillingham, UK), 0.4 µL ROX High, 0.4 µL 10 µM forward primer, 0.4 µL 10
453 µM reverse primer, 4 µL template DNA, 4.8 µL PCR grade water. The PCR programme was:
454 95°C for 3 minutes, 40 cycles of 95°C for 10s, 60°C for 30s. Standards were made from a 10
455 nM stock of Phix, diluted in PCR-grade water. The standard range was 20 pmol, 2 pmol, 0.2
456 pmol, 0.02 pmol, 0.002 pmol, 0.0002 pmol. Samples were then sent to Novogene
457 (Cambridge, UK) for sequencing using an Illumina NovaSeq instrument, with sample names
458 and index combinations used. Demultiplexed FASTQ's were returned on a hard drive.

459 **Nanopore library preparation and PromethION sequencing.** Library preparation was
460 performed using SQK-LSK109 (Oxford Nanopore Technologies, Oxford, UK) with barcoding
461 kits EXP-NBD104 and EXP-NBD114. The native barcoding genomic DNA protocol by Oxford
462 Nanopore Technologies (ONT) was followed with slight modifications. Starting material for
463 the End-Prep/FFPE reaction was 1 µg per sample in 48 µL volume. 3.5 µL NEBNext FFPE DNA
464 Repair Buffer (NEB, New England Biolabs, Ipswich, USA), 3.5 µL NEB Ultra II End-prep Buffer,
465 3 µL NEB Ultra II End-prep Enzyme Mix and 2 µL NEBNext FFPE DNA Repair Mix (NEB) were
466 added to the DNA (final volume 60 µL), mixed slowly by pipetting and incubated at 20°C for
467 5 minutes and then 65°C for 5 minutes. After a 1X bead wash with AMPure XP beads
468 (Agencourt, Beckman Coulter, High Wycombe, UK), the DNA was eluted in 26 µL of
469 nuclease-free water. 22.5 µL of this was taken forward for native barcoding with the
470 addition of 2.5 µL barcode and 25 µL Blunt/TA Ligase Master Mix (NEB) (final volume 50 µL).
471 This was mixed by pipetting and incubated at room temperature for 10 minutes. After
472 another 1X bead wash (as above), samples were quantified using Qubit dsDNA BR Assay Kit
473 (Invitrogen, Loughborough, UK). In the first run, samples were equimolar pooled to a total

474 of 900 ng in a volume of 65 μ L. In the second run, samples were pooled to 1700 ng followed
475 by a 0.4X bead wash to achieve the final volume of 65 μ L. 5 μ L Adapter Mix II (ONT), 20 μ L
476 NEBNext Quick Ligation Reaction Buffer (5X) and 10 μ L Quick T4 DNA Ligase (NEB) were
477 added (final volume 100 μ L), mixed by flicking, and incubated at room temperature for 10
478 minutes. After bead washing with 50 μ L of AMPure XP beads and two washes in 250 μ L of
479 Long Fragment Buffer (ONT), the library was eluted in 25 μ L of Elution Buffer and quantified
480 with Qubit dsDNA BR and TapeStation 2200 using a Genomic DNA ScreenTape (Agilent
481 Technologies, Edinburgh, UK). 470 ng of DNA was loaded for sequencing in the first run and
482 400 ng in the second run. The final loading mix was 75 μ L SQB, 51 μ L LB and 24 μ L DNA
483 library.

484 Sequencing was performed on a PromethION Beta using FLO-PRO002 PromethION
485 Flow Cells (R9 version). The sequencing runtime was 57 hours for Run 1 and 64 hours for
486 Run 2. Flow cells were refuelled with 0.5X SQB (75 μ L SQB and 75 μ L nuclease free water) 40
487 hours into both runs.

488 **Bioinformatics analysis.** The bioinformatics analysis was performed using default options
489 unless specified otherwise.

490 *Nanopore basecalling:* Basecalling was performed using Guppy version 3.0.5+45c3543 (ONT)
491 in high accuracy mode (model dna_r9.4.1_450bps_hac), and demultiplexed with qcat
492 version 1.1.0 (Oxford Nanopore Technologies, <https://github.com/nanoporetech/qcat>).

493 *Sequence quality:* For Nanopore, sequence metrics were estimated by Nanostat version
494 1.1.2[41]. In total, 22 million sequences were generated with a median read length of 4500
495 bp and median quality of 10 (phred). Quality trimming and adapter removal was performed
496 using Porechop version 0.2.3 (<https://github.com/rrwick/Porechop>). For Illumina, quality
497 control was done for paired-end reads using fastp, version 0.20.0.[42] to remove adapter

498 sequences and filter out low-quality (phred quality < 30) and short reads (length < 60 bp).

499 After quality control, the average number of reads in the samples was over 26.1 million

500 reads, with a minimum of 9.7 million reads; the average read length was 148 bp.

501 *Taxonomic profiling:* Trimmed and high-quality short reads are processed using MetaPhlAn3

502 version 3.0.2,[43] to estimate both microbial composition to species level and also the

503 relative abundance of species from each metagenomic sample. MetaPhlAn3 uses the latest

504 marker information dataset, CHOCOPhIAn 2019, which contains ~1 million unique clade-

505 specific marker genes identified from ~100,000 reference genomes; this includes bacterial,

506 archaeal and eukaryotic genomes. Hclust2 was used to plot the hierarchical clustering of

507 the different taxonomic profiles at each time point [<https://github.com/SegataLab/hclust2>].

508 The results of the microbial taxonomy were analysed in RStudio Version 1.1.453

509 (<http://www.rstudio.com/>).

510 Principle Coordinate analyses using the pcoa function in the ape package version 5.3

511 (<https://www.rdocumentation.org/packages/ape/versions/5.3>) and the vegan package was

512 used to identify differences in microbiome profiles amongst treatments.

513 *Hybrid assembly:* Trimmed and high-quality Illumina reads were merged per

514 treatment, and then used in a short-read-only assembly using Megahit version 1.1.3.[44, 45]

515 Then OPERA-MS[46] version 0.8.2, was used to combine the short-read only assembly with

516 high-quality long reads, to create high-quality hybrid assemblies. By combining these two

517 technologies, OPERA-MS overcomes the issue of low-contiguity of short-read-only

518 assemblies and the low base-pair quality of long-read-only assemblies.

519 *Genome binning, quality, dereplication and comparative genomics of hybrid*

520 *assemblies:* The hybrid co-assemblies from Opera-MS[46] were used for binning. Here,

521 Illumina reads for each time period were mapped to the co-assembled contigs to obtain a

522 coverage map. Bowtie2 version 2.3.4.1 was used for mapping, and samtools to convert SAM
523 to BAM format. MaxBin2 version 2.2.6[47] and MetaBat2 version 2.12.1[48] which uses
524 sequence composition and coverage information, was used to bin probable genomes using
525 default parameters. The binned genomes and co-assembled contigs were integrated into
526 Anvi'o version 6.1 for manual refinement and visual inspection of problematic genomes.[49]
527 In particular, we used the scripts: 'anvi-interactive' to visualise the genome bins; 'anvi-run-
528 hmms' to estimate genome completeness and contamination; 'anvi-profile' to estimate
529 coverage and detection statistics for each sample; and 'anvi-refine' to manually refine the
530 genomes. All scripts were run using default parameters. Additionally, DAS tool version 1.1.2
531 [50] was used to aggregate high-quality genomes from each treatment by using single copy
532 gene-based scores and genome quality metrics to produce a list of good quality genomes for
533 every treatment. Additionally, checkM version 1.0.18[51] was used on all final genomes to
534 confirm completion and contamination scores. In general, genomes with a 'quality satisfying
535 completeness - 5*contamination > 50 score' and/or with a '>60% completion and <10%
536 contamination' score according to CheckM, were selected for downstream analyses.

537 *Dereplication into representative clusters:* In order to produce a dereplicated set of
538 genomes across all treatments, dRep version 2.5.0[52] was used. Pairwise genome
539 comparisons or Average Nucleotide Identity (ANI) was used for clustering. dRep clusters
540 genomes with ANIs of 97% were regarded as primary clusters, and genomes with ANI of 99
541 % regarded as secondary clusters. A representative genome is provided for each of the
542 secondary clusters.

543 *Relative abundance of genomes:* Since co-assemblies were used for binning, relative
544 abundance was calculated as the proportion of reads recruited to that bin across all time
545 periods for each treatment. This provides an estimate of which time period recruited the

546 most reads. To provide this estimate in relative terms, the value is normalised to the total
547 number of reads that was recruited for that genome. As for Avicel that misses the time 0h, a
548 mean relative abundance from each MAG in the cluster at time 0h was used. The relative
549 abundance scores was provided by 'anvi-summarize' (from the Anvi'o package) as relative
550 abundance. Further, fold changes were calculated between the relative abundance at time
551 0h to the corresponding relative abundance at 6h, 12h and 24h using gtools R package
552 version 3.5.0. Fold changes provide an estimate of change in MAG abundance which might
553 be a result from utilisation of a particular carbohydrate. Fold changes were converted to log
554 ratios. MAGs with a fold change of 2x (\log_2 foldchange=1) were regarded as an active
555 carbohydrate utiliser.

556 *Metagenomic assignment and phylogenetic analyses:* Genome bins that passed
557 quality assessment were analysed for their closest taxonomic assignment. To assign
558 taxonomic labels, the genome set was assigned into the microbial tree of life using GTDB
559 version 0.3.5 and database R95 to identify the closest ancestor and obtain a putative
560 taxonomy assignment for each genome bin. For genomes where the closest ancestor could
561 not be determined, the Relative Evolutionary Distance (RED) to the closest ancestor and
562 novel taxa names were provided. Using these genome bins, a phylogenetic tree was
563 constructed using Phylophlan version 0.99 and visually inspected using iTOL version 4.3.1
564 and ggtree from package <https://github.com/YuLab-SMU/ggtree.git>. The R packages ggplot2
565 version 3.3.2, dplyr version 1.0.2, aplot, ggtree version 2.2.4 and inkscape version 1.0.1
566 were used for illustrations

567 *Carbohydrate metabolism analyses:* All representative genome clusters were
568 annotated for CAZymes using dbCAN.[53] The genome's nucleotide sequences were
569 processed with Prodigal to predict protein sequences, and then three tools were used for

570 automatic CAZyme annotation: a) HMMER[54] to search against the dbCAN HMM (Hidden
571 Markov Model) database; b) DIAMOND[55] to search against the CAZy pre-annotated
572 CAZyme sequence database; and c) Hotpep[56] to search against the conserved CAZyme
573 PPR (peptide pattern recognition) short peptide library. To improve annotation accuracy, a
574 filtering step was used to retain only hits to CAZy families found by at least two tools. The R
575 packages ggplot2, dplyr, ComplexHeatmap version 2.4.3 and inkscape were used for
576 illustrations.

577 **Acknowledgements**

578 We thank Dave J. Baker for assisting with sequencing and the anonymous donor who
579 provided faecal material for this study. We thank Dr. Judith Pell for assistance with editing
580 the manuscript. We acknowledge the kind assistance of Prof. Aharon Oren for checking and
581 correcting the grammar of the protologue species names.

582 **Author contributions**

583 All authors read and contributed to the manuscript. AR, PR and JAJ are joint first authors.
584 FJW conceived and designed the study. AR led on the preparation of the manuscript. AA and
585 GLK prepared the sequencing libraries and did the sequencing. AR and PR did the sequence
586 and bioinformatics analysis. TLV did the post-sequencing analysis. JAJ, KC and SH did the
587 model colon experiments and DNA extractions. HH enumerated the bacterial cells. RG and
588 MJP assisted with bioinformatic analysis and taxonomic descriptions. JOG provided long-
589 read sequencing and molecular biology expertise; AJP provided bioinformatics expertise;
590 and FJW provided expertise in carbohydrate structure and model colon protocols. FJW, JOG,
591 AJP secured funding, provided management oversight and scientific direction.

592 **Ethical approval**

593 Ethical approval was granted by the Human Research Governance Committee at the
594 Quadram Institute (IFR01/2015) and the London - Westminster Research Ethics Committee
595 (15/LO/2169). The trial is registered on clinicaltrials.gov (NCT02653001). A signed informed
596 consent was obtained from the participant prior to donation.

597 **Funding statements**

598 The authors gratefully acknowledge the support of the Biotechnology and Biological
599 Sciences Research Council (BBSRC). This research was funded by: the BBSRC Institute
600 Strategic Programme (ISP) Food Innovation and Health BB/R012512/1 and its constituent
601 projects (BBS/E/F/000PR10343, BS/E/F/000PR10346); the BBSRC ISP Microbes in the Food
602 Chain BB/R012504/1 and its constituent projects (BBS/E/F/000PR10348,
603 BBS/E/F/000PR10349, BBS/E/F/000PR10352); and the BBSRC Core Capability Grant
604 (BB/CCG1860/1). The funders had no role in study design, data collection and analysis,
605 decision to publish, or preparation of the manuscript.

606 **Availability of data and materials**

607 Raw read data from the PromethION and NovoSeq sequencing runs can be accessed
608 through the NCBI SRA project number PRJNA722408 and can be accessed at
609 <https://dataview.ncbi.nlm.nih.gov/object/PRJNA722408?reviewer=ts65d8lkvj8nbv4mpfsar7>
610 [sv3g](#). GenBank accession numbers for individual MAG's within this ProjectID can be found in
611 Supplementary Table 5.

612 **Competing interests**

613 The authors declare that they have no competing interests

614 **Figure legends**

615 **Figure 1.** Workflow for bioinformatics analysis of combined Illumina NovoSeq and Oxford
616 Nanopore PromethION metagenomics data collected in a model colon study of the
617 fermentation of different carbohydrate substrates with contrasting structures (Avicel, Inulin,
618 Normal maize (N.maize), Retrograded maize (R.maize), Potato and Hylon) by the gut
619 microbiota present in a human stool sample.

620 **Figure 2. Hierarchical clustering** of the top 30 selected gut microbial species present after
621 fermentation of Avicel, Inulin, N.maize, R.maize, Potato and Hylon at 0h, 6h, 12h and 24h in
622 the model colon. The hierarchical clustering also includes a water sample (“the kitome”).

623 **Figure 3: Comparison of Illumina short read assemblies and hybrid assemblies:** a) shows
624 the number of contigs per treatment, b) shows the N50, c) statistics on the largest contig, d)
625 size of the total assembly for each carbohydrate treatment.

626 **Figure 4: MAG quality.** Dots represent each MAG. Completeness and contamination scores
627 were estimated using CheckM. Colours are based on the MAG standards (high quality as
628 >90% completeness & <5% contamination; good quality as <90%- 60% completeness and
629 >5% - 10% contamination. The horizontal and vertical bar charts provide the number of
630 genomes with high completeness and low contamination scores.

631 **Figure 5: Phylogenomic tree and fold changes.** The phylogenetic tree was **constructed from**
632 **concatenated protein sequences using PhyloPhlAn and illustrated using ggtree.** Clades
633 belonging to similar bacterial family and bacterial genus were collapsed. The colour strips
634 represent the phylum-level distribution of the phylogenetic tree. Dot plot shows the
635 decrease (negative \log_2 fold change; blue shades) and increase (positive \log_2 fold change;
636 red shades) of read proportions from 0h to 6h, 0h to 12h and 0h to 24h for all treatments.

637 **Figure 6: CAZyme profiles of selected-MAGs.** The colour strip represents the phylum-based
638 taxonomy annotation. The heat map represents the number of proteins identified for each
639 CAZy protein family.

640 **Supplementary Files**

641 **Supplementary Table 1:** Read stats and quality metrics for PromethION and Illumina
642 sequence data

643 **Supplementary Table 2:** Taxonomy profiles of relative abundances for all treatments using
644 MetaPhlAn3.

645 **Supplementary Table 3:** Assembly stats for short read assemblies using Megahit and hybrid
646 assemblies using OPERA-MS

647 **Supplementary Table 4:** MAG genomic stats, assembly features, closest taxonomy
648 annotation and relative evolutionary distance for novel genus and species.

649 **Supplementary Table 5:** Dereplicated MAGs with representative cluster names and their
650 taxonomy annotations

651 **Supplementary Table 6:** Stats showing the diversity of GTDb taxonomy within MAGs.

652 **Supplementary Table 7:** Novel latin binomials for MAGs and taxa names submitted to
653 Genbank

654 **Supplementary Table 8:** Comparison of genome stats between MAGs from this study and
655 GTDb corresponding representative MAG cluster

656 **Supplementary Table 9:** Relative abundance, fold change and log ratio foldchange for all
657 MAGs

658 **Supplementary Table 10:** Genomes depicted as early and late degraders according to the
659 time the genomes showed a 2x fold change.

660 **Supplementary Table 11:** MAGs and their CAZyme profiles.

661 **Supplementary Table 12:** CaZymes counts for selected MAG clusters

662 **Supplementary Table 13:** media preparation materials, sources and quantity

663

664 **Supplementary Figure 1:** Principle Component Analysis (PCoA) showing the dynamics of the
665 microbiome during the different time points and between the Carbohydrate treatment. PC1
666 and PC2 represent the percentage of variance explained by Principle Component (PC) 1 and
667 2.

668 **Supplementary Figure 2:** Changes in inverse Simpson index between time periods of the
669 substrates.

670 **Supplementary figure 3: Box plots showing the dynamic shifts in read proportions for all**
671 **binned MAGs after 0h, 6h, 12h and 24h fermentation in the model colon.** The box
672 represents the interquartile range (IQR) (25th and 75th percentile); the median is shown
673 within the box. The whiskers indicate minimum and maximum Inter Quartile Range (IQR);
674 dots represent outliers.

675 **Supplementary Figure 4:** Distribution of CAZy families per substrate and in all the genome

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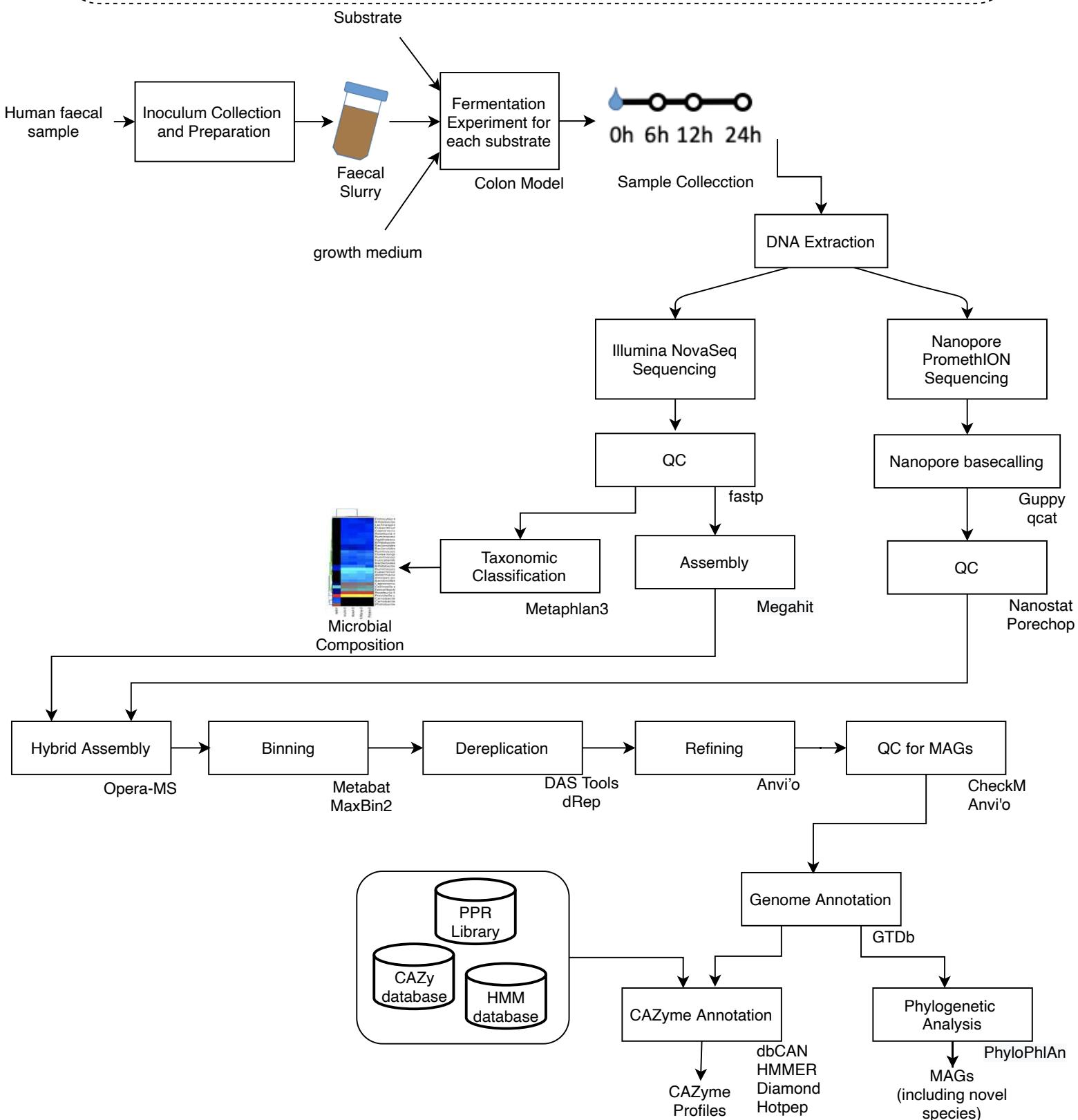
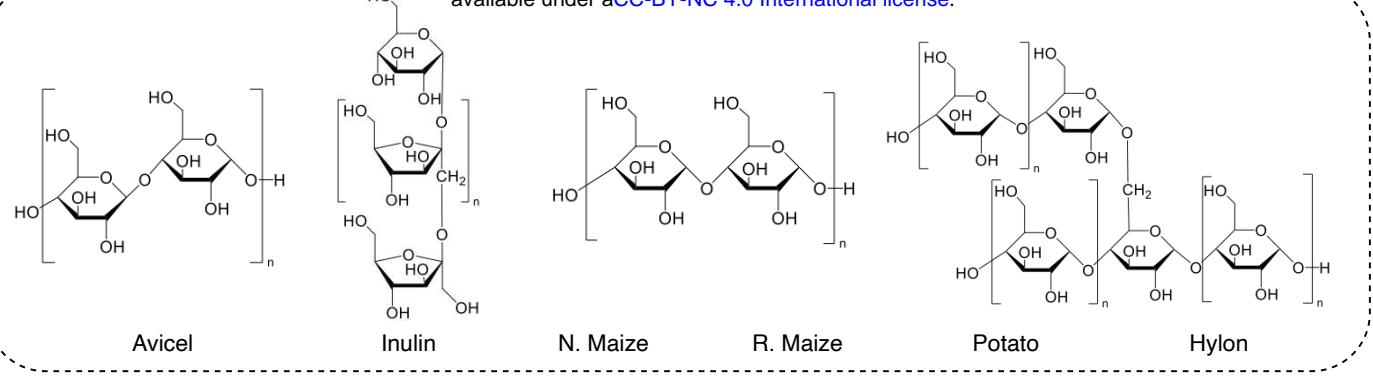
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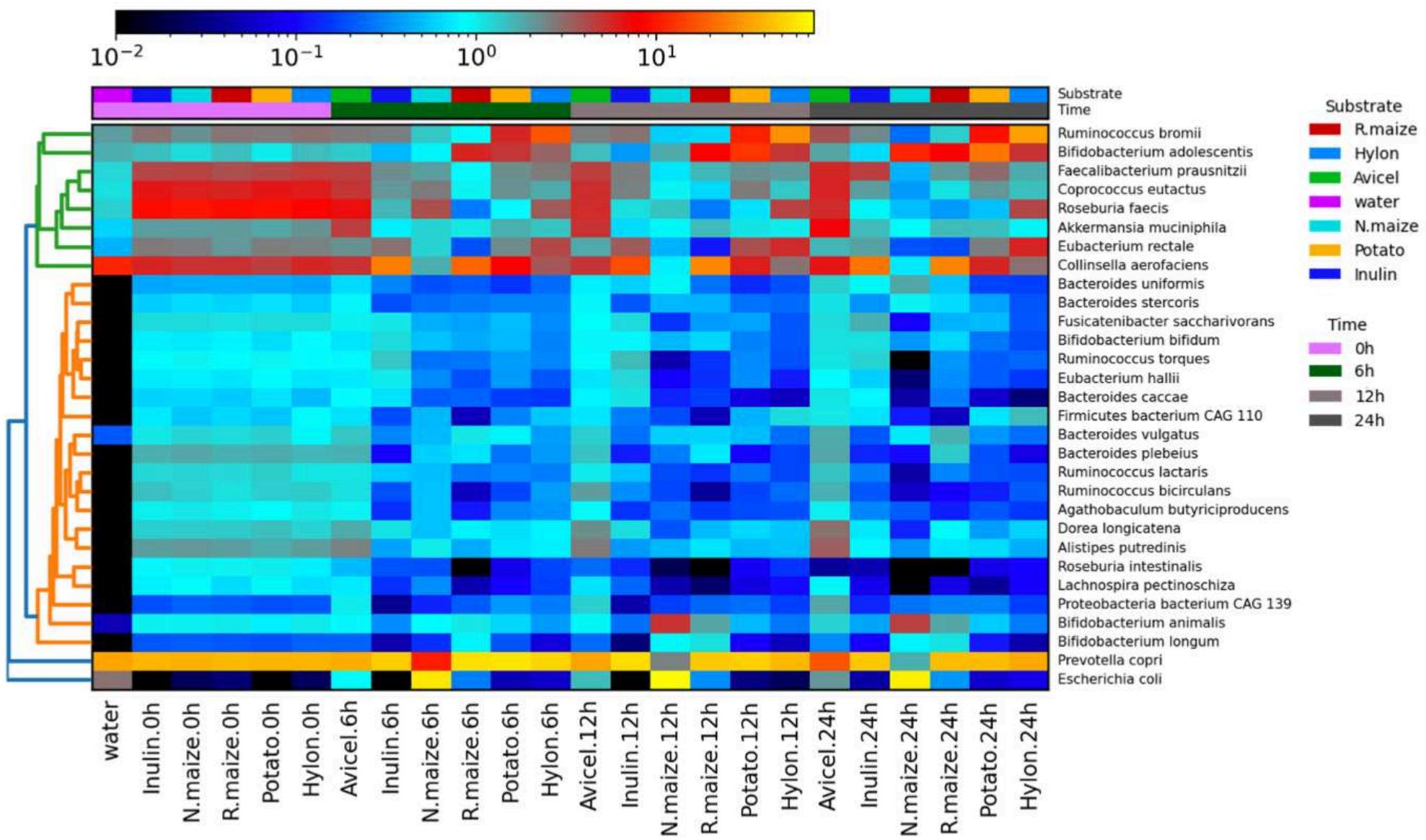
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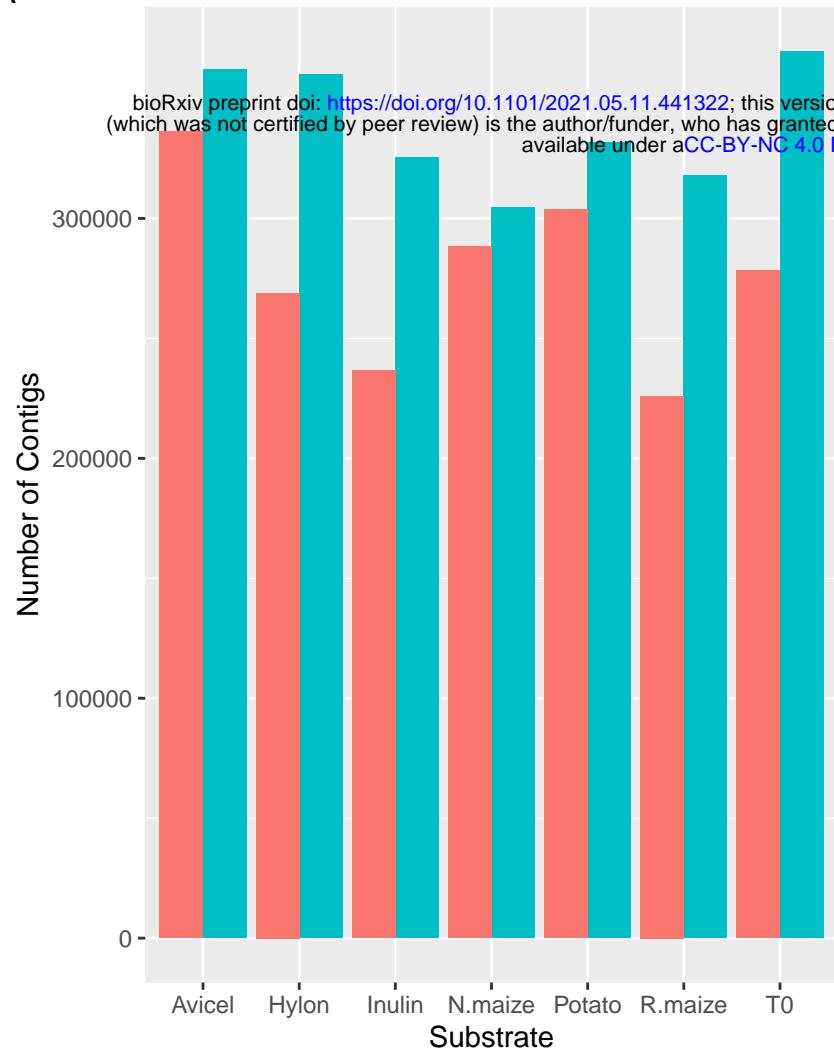
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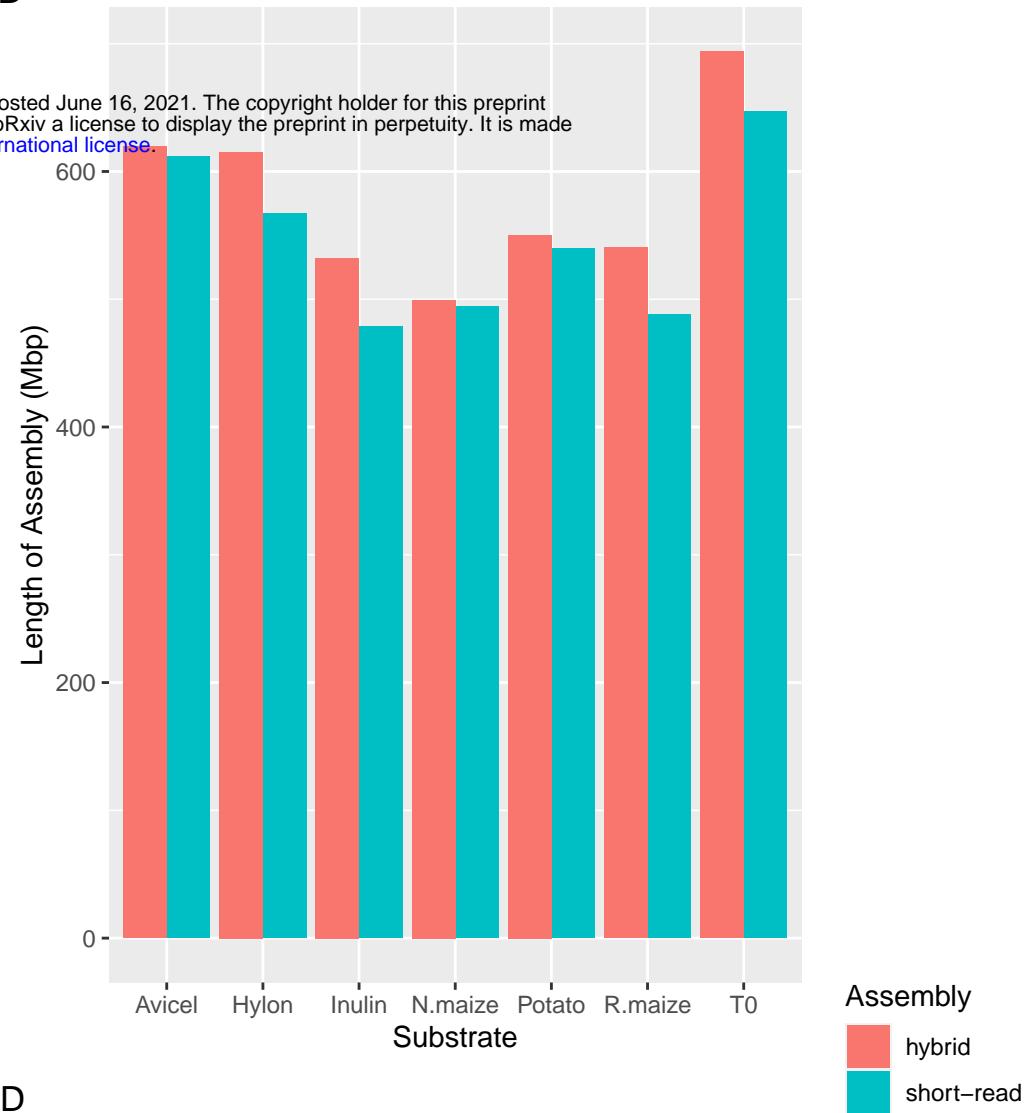




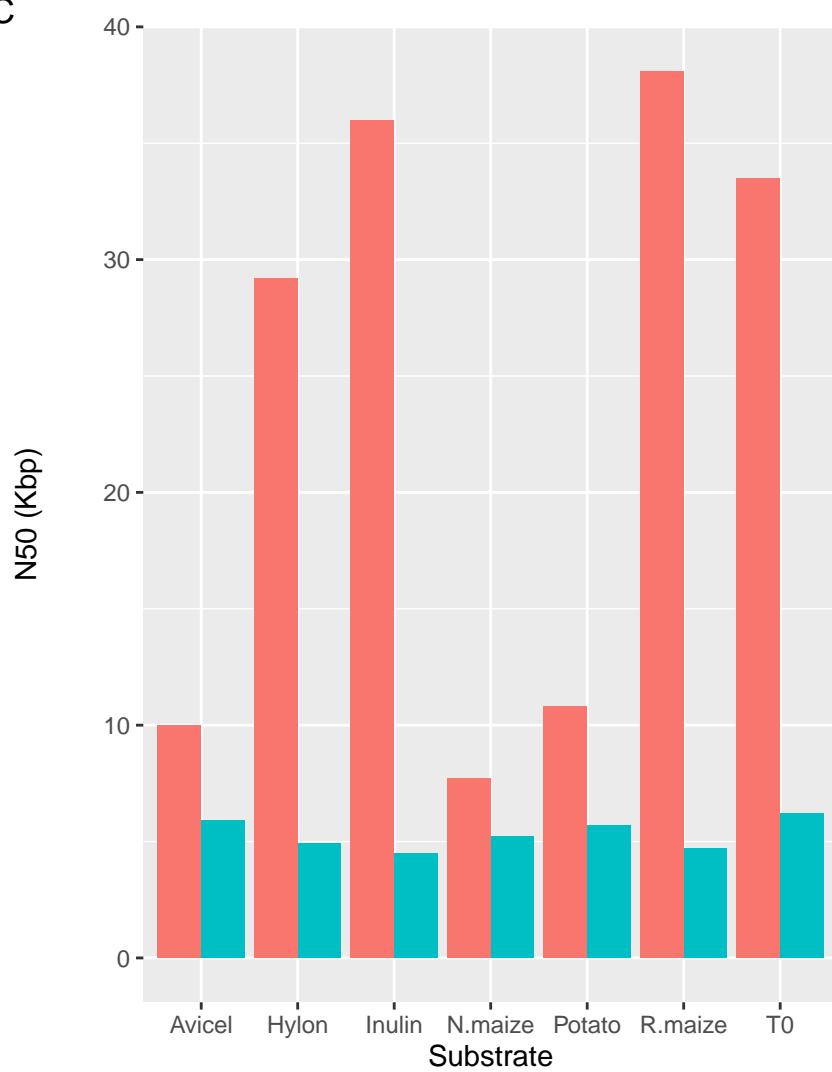
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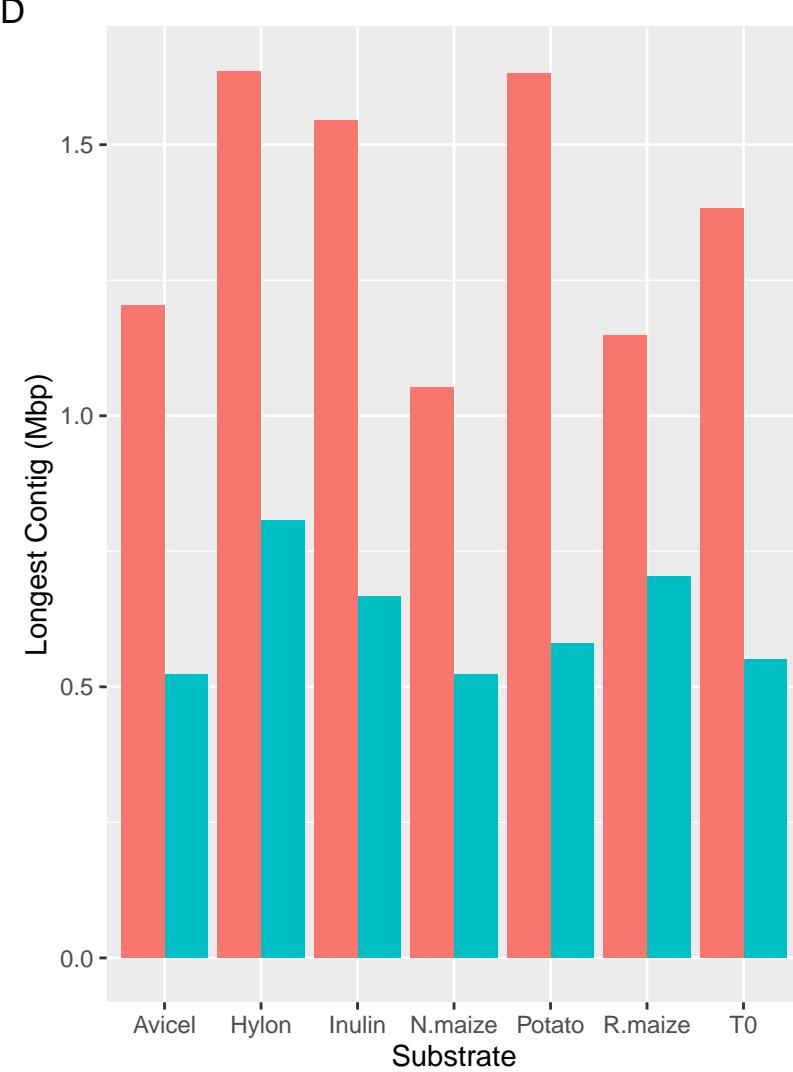
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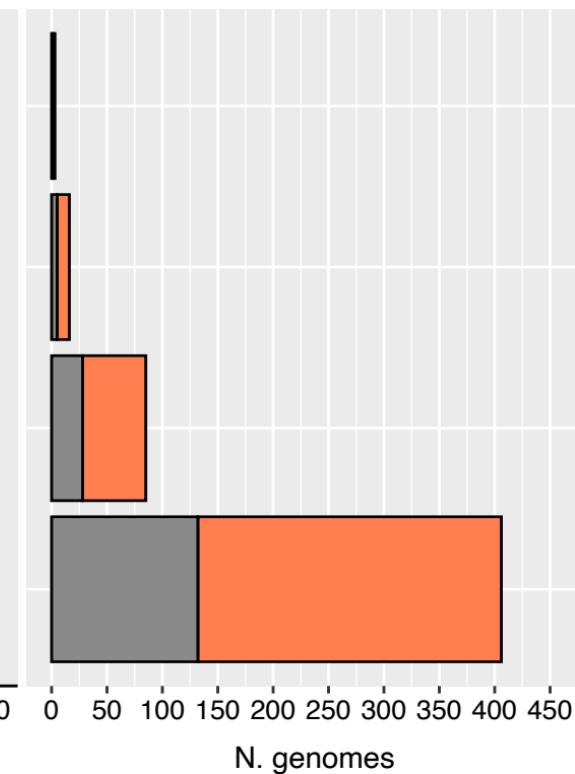
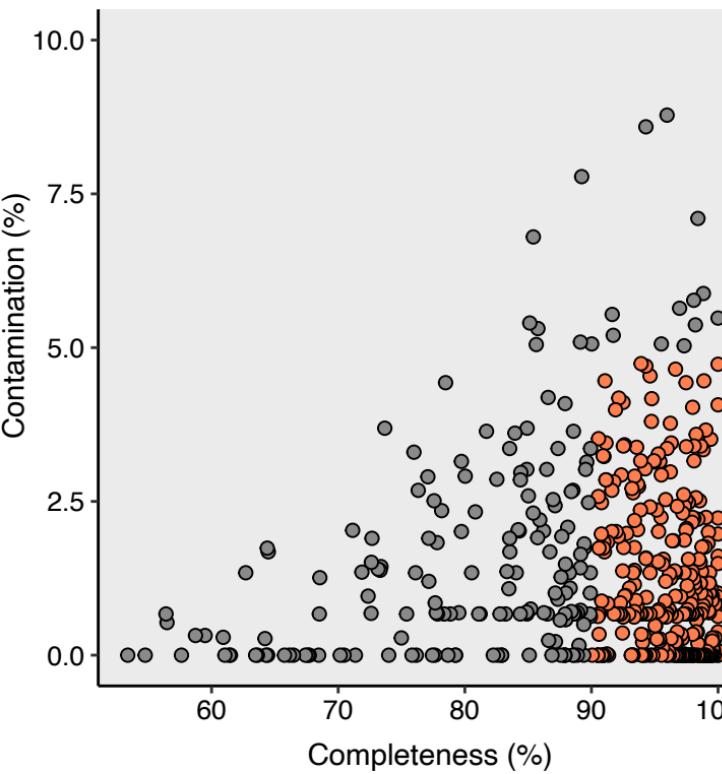
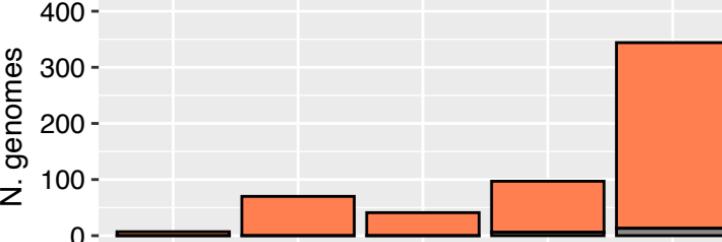
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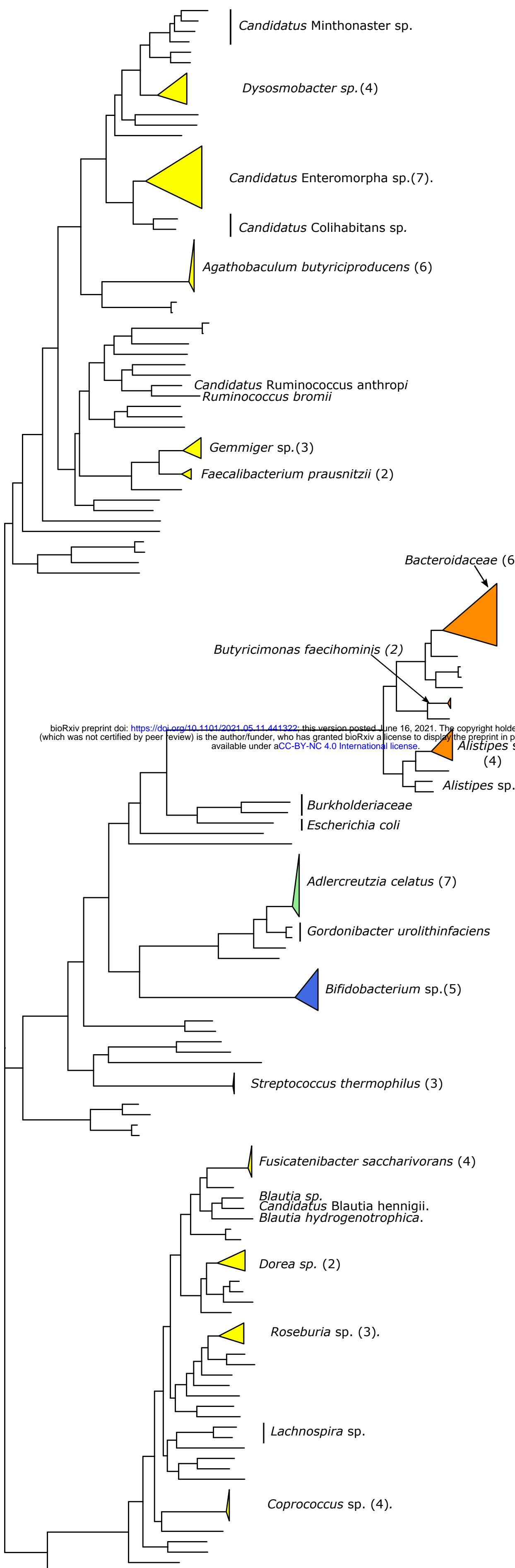
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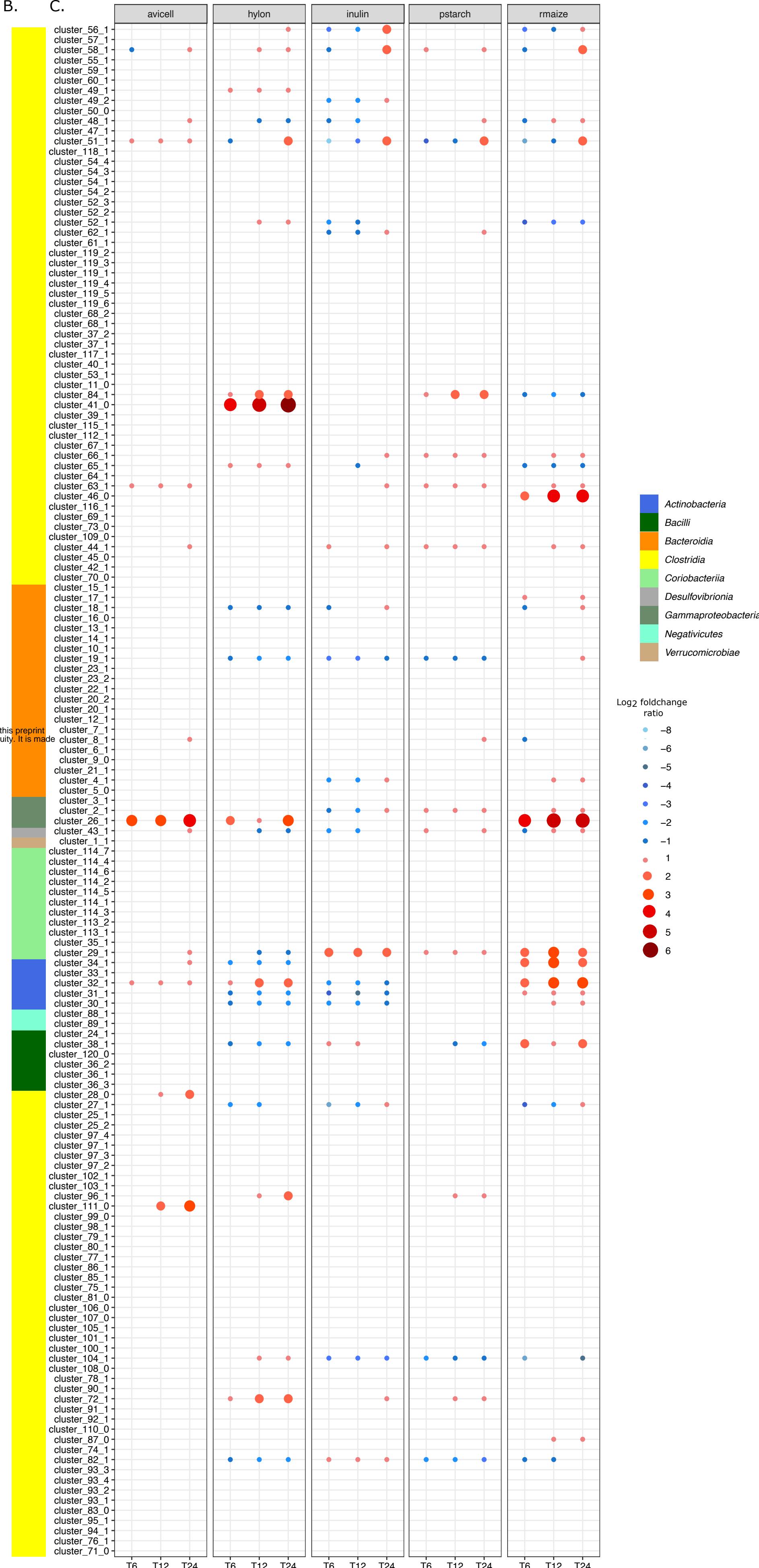
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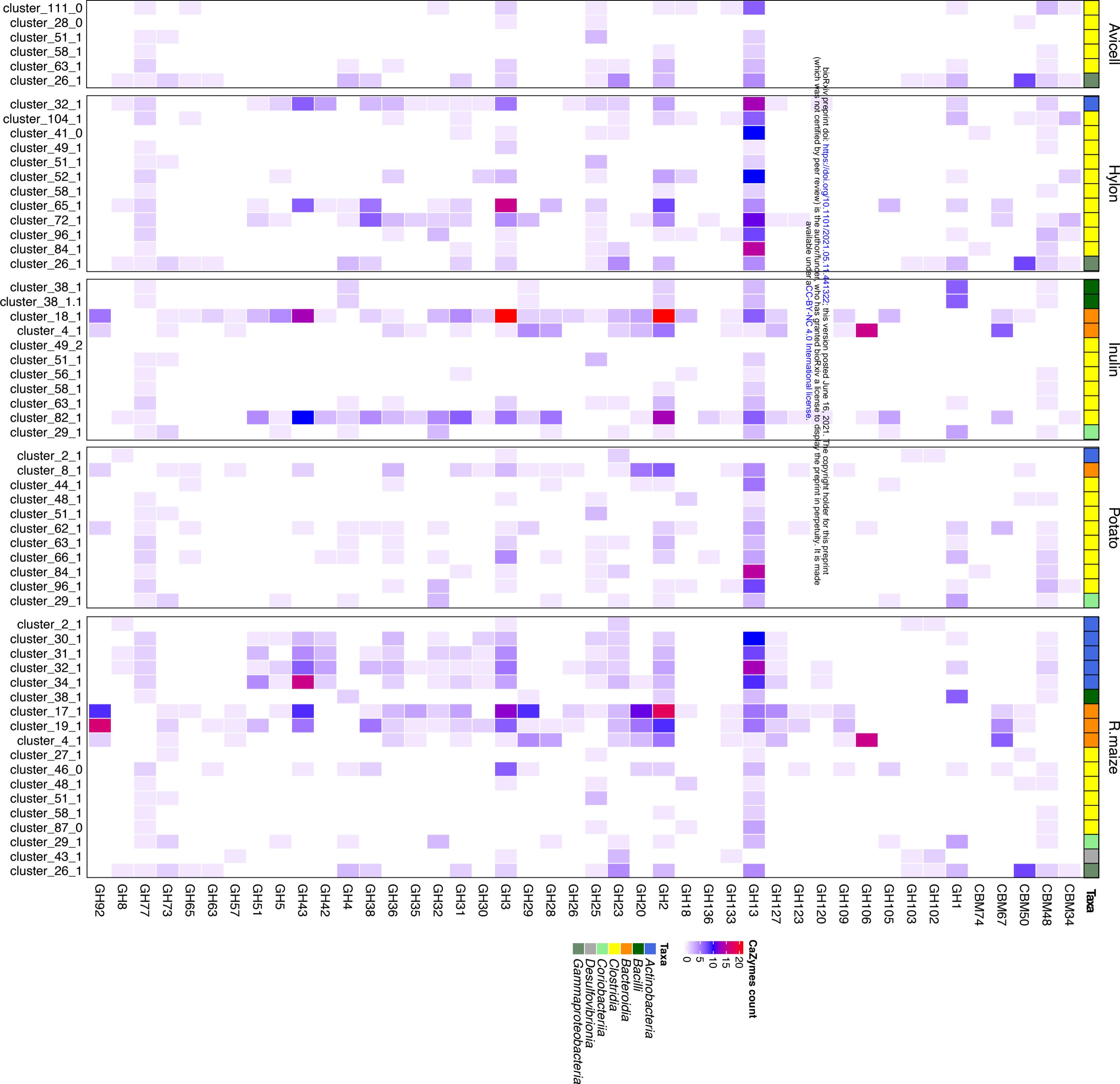


A.



B.





Description of *Candidatus Acetatifactor hominis* sp. nov.

Candidatus Acetatifactor hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier rmaize_MAXBIN_038 and which is available via NCBI BioSample SAMN18871269. This is a new name for the alphanumeric GTDB species sp900066565. The GC content of the type genome is 47.74 % and the genome length is 3.05 Mbp.

Description of *Candidatus Aphodonaster* gen. nov.

Candidatus Aphodonaster (Aph.od.o.nas'ter. Gr. fem. n. *aphodos* dung; Gr. masc. n. *naster* an inhabitant; N.L. masc. n. *Aphodonaster* a microbe associated with faeces).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Aphodonaster merdae*. This is a new name for the GTDB alphanumeric genus SFFH01. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Christensenellales* and to the family CAG-74

Description of *Candidatus Aphodonaster intestinalis* sp. nov.

Candidatus Aphodonaster intestinalis (in.tes.ti.na'lis. N.L. masc. adj. *intestinalis*, pertaining to the intestines).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_97 and which is available via NCBI BioSample SAMN18871333. This is a new name for the alphanumeric GTDB species sp900548125. The GC content of the type genome is 55.44 % and the genome length is 2.54 Mbp.

Description of *Candidatus Aphodonaster merdae* sp. nov.

Candidatus Aphodonaster merdae (mer'dae. L. gen. fem. n. *merdae*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier psstarch_METABAT_69 and which is available via NCBI BioSample SAMN18871262. This is a new name for the alphanumeric GTDB species sp900542395. The GC content of the type genome is 59.61 % and the genome length is 2.66 Mbp.

Description of *Candidatus Avimicrobium caecorum* sp. nov.

Candidatus Avimicrobium caecorum (cae.co'rum. N. L. gen. pl. n. *caecorum*, of caeca).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *avicell_METABAT_34* and which is available via NCBI BioSample SAMN18871193. This is a new name for the alphanumeric GTDB species *sp900547185*. This genus was named by Glendinning et al. (2020). The GC content of the type genome is 56.83 % and the genome length is 2.20 Mbp.

Description of *Candidatus Blautia hennigii* sp. nov.

Candidatus Blautia hennigii (hen.ni'gi.i. N.L. gen. masc. n. *hennigii* derived from the Latinised family name for Willi Hennig, 1913-1976, the East German scientist who founded phylogenetic systematics or cladistics).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *hylon_METABAT_127* and which is available via NCBI BioSample SAMN18871203. This is a new name for the alphanumeric GTDB species *sp900066505*. GTDB has assigned this species to genus with an alphabetic suffix which cannot be incorporated into a well-formed binomial, so in naming this species, we have used the basonym for the genus. The GC content of the type genome is 43.26 % and the genome length is 2.93 Mbp.

Description of *Candidatus Caccadaptatus* gen. nov.

Candidatus Caccadaptatus (Cacc.ad.ap.ta'tus. Gr. fem. n. *kakké* dung; L. masc. part. adj. *adaptatus* adapted to; N.L. masc. n. *Caccadaptatus* a microbe associated with faeces).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show ≥60% average aminoacid identity to the genome of the type strain from the type species, *Candidatus Caccadaptatus darwinii*. This is a new name for the GTDB alphanumeric genus *NK3B98*. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Oscillospiraceae*

Description of *Candidatus Caccadaptatus darwinii* sp. nov.

Candidatus Caccadaptatus darwinii (dar.wi'ni.i. N.L. gen. masc. n. *darwinii* derived from the Latinised family name for Charles Darwin, 1809-1882, the British scientist who proposed the theory of evolution by natural selection).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *rmaize_METABAT_56* and which is available via NCBI BioSample SAMN18871284. This is a new name for the alphanumeric GTDB species

sp900545815. The GC content of the type genome is 56.11 % and the genome length is 2.31 Mbp.

Description of *Candidatus Chesmatocola* gen. nov.

Candidatus Chesmatocola (Ches.ma.to'co.la. Gr. neut. n. *chesma* dung; N.L. masc./fem. suffix *cola* an inhabitant of; N.L. fem. n. *Chesmatocola* a microbe associated with faeces).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Chesmatocola anthropi*. This is a new name for the GTDB alphanumeric genus CAG-354. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *TANB77* and to the family CAG-508

Description of *Candidatus Chesmatocola anthropi* sp. nov.

Candidatus Chesmatocola anthropi (an.thro'pi. Gr. masc. n. *anthropos*, a human being; N.L. gen. masc. n. *anthropi*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier hylon_METABAT_79 and which is available via NCBI BioSample SAMN18871215. This is a new name for the alphanumeric GTDB species sp001915925. The GC content of the type genome is 28.31 % and the genome length is 1.38 Mbp.

Description of *Candidatus Cholicomonas* gen. nov.

Candidatus Cholicomonas (Cho.li.co.mo'nas. Gr. fem. n. *cholix*, *cholikos* guts; L. fem. n. *monas* a monad; N.L. fem. n. *Cholicomonas* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Cholicomonas copri*. This is a new name for the GTDB alphanumeric genus CAG-628. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *RF39* and to the family UBA660

Description of *Candidatus Cholicomonas copri* sp. nov.

Candidatus Cholicomonas copri (cop'ri. Gr. masc. n. *kópros*, faeces; N.L. gen. n. *copri*; of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier

T0_METABAT_30 and which is available via NCBI BioSample SAMN18871328. This is a new name for the alphanumeric GTDB species sp000438415. The GC content of the type genome is 27.35 % and the genome length is 0.62 Mbp.

Description of *Candidatus Choliconaster* gen. nov.

Candidatus Choliconaster (Cho.li.co.nas'ter. Gr. fem. n. *cholix*, *cholikosguts*; Gr. masc. n. *naster* an inhabitant; N.L. masc. n. *Choliconaster* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show ≥60% average aminoacid identity to the genome of the type strain from the type species, *Candidatus Choliconaster caccae*. This is a new name for the GTDB alphanumeric genus ER4. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Oscillospiraceae*

Description of *Candidatus Choliconaster caccae* sp. nov.

Candidatus Choliconaster caccae (cac'cae. Gr. fem. n. *kakkē*, faeces; N.L. gen. n. *caccae*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_MAXBIN_028 and which is available via NCBI BioSample SAMN18871294. This is a new name for the alphanumeric GTDB species sp000765235. The GC content of the type genome is 57.68 % and the genome length is 2.86 Mbp.

Description of *Candidatus Choliconaster merdae* sp. nov.

Candidatus Choliconaster merdae (mer'dae. L. gen. fem. n. *merdae*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_240 and which is available via NCBI BioSample SAMN18871322. This is a new name for the alphanumeric GTDB species sp900317525. The GC content of the type genome is 60.79 % and the genome length is 1.92 Mbp.

Description of *Candidatus Clostridium faecihominis* sp. nov.

Candidatus Clostridium faecihominis (fae.ci.ho'mi.nis. L. fem. n. *faex*, *faecis* faeces; L. gen. masc. n. *hominis*, of a human being; N.L. gen. n. *faecihominis*, of human faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_195 and which is available via NCBI BioSample SAMN18871315.

This is a new name for the alphanumeric GTDB species sp003024715. GTDB has assigned this species to genus with an alphabetic suffix which cannot be incorporated into a well-formed binomial, so in naming this species, we have used the basonym for the genus. The GC content of the type genome is 49.01 % and the genome length is 2.60 Mbp.

Description of *Candidatus Colibacterium* gen. nov.

Candidatus Colibacterium (Co.li.bac.te'ri.um. L. neut. n. *colon* large intestine; N.L. neut. n. *bacterium* a bacterium; N.L. neut. n. *Colibacterium* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Colibacterium hominis*. This is a new name for the GTDB alphanumeric genus SFEL01. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Christensenellales* and to the family CAG-138

Description of *Candidatus Colibacterium hominis* sp. nov.

Candidatus Colibacterium hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_MAXBIN__134 and which is available via NCBI BioSample SAMN18871298. This is a new name for the alphanumeric GTDB species sp004557245. The GC content of the type genome is 54.28 % and the genome length is 1.56 Mbp.

Description of *Candidatus Colihabitans* gen. nov.

Candidatus Colihabitans (Co.li.ha'bi.tans. L. neut. n. *colon* large intestine; L. masc./fem. adj. part. *habitans* an inhabitant; N.L. fem. n. *Colihabitans* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Colihabitans norwichensis*. This is a new name for the GTDB alphanumeric genus CAG-170. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Oscillospiraceae*

Description of *Candidatus Colihabitans hominis* sp. nov.

Candidatus Colihabitans hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT__180 and which is available via NCBI BioSample SAMN18871312. This is a new name for the alphanumeric GTDB species sp900549635. The GC content of the type genome is 56.47 % and the genome length is 2.50 Mbp.

Description of *Candidatus Colihabitans norwichensis* sp. nov.
Candidatus Colihabitans norwichensis (nor.wich.en'sis. N.L. fem. adj. *norwichensis* pertaining to English city of Norwich).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier hylon_METABAT__172 and which is available via NCBI BioSample SAMN18871205. This is a new name for the alphanumeric GTDB species sp000432135. The GC content of the type genome is 57.56 % and the genome length is 3.16 Mbp.

Description of *Candidatus Dysosmobacter stercoris* sp. nov.
Candidatus Dysosmobacter stercoris (ster'co.ris. L. gen. neut. n. *stercoris*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier hylon_METABAT__95 and which is available via NCBI BioSample SAMN18871217. This is a new name for the alphanumeric GTDB species sp900542115. The GC content of the type genome is 58.43 % and the genome length is 1.44 Mbp.

Description of *Candidatus Eisenbergiella faecalis* sp. nov.
Candidatus Eisenbergiella faecalis (fae.ca'lis. N.L. fem. adj. *faecalis*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier pstarch_METABAT__22 and which is available via NCBI BioSample SAMN18871259. This is a new name for the alphanumeric GTDB species sp900066775. The GC content of the type genome is 48.80 % and the genome length is 2.82 Mbp.

Description of *Candidatus Enteromorpha* gen. nov.
Candidatus Enteromorpha (En.te.ro.mor'pha. Gr. neut. n. *enteron* the gut; Gr. fem. n. *morphe* a form, shape; N.L. fem. n. *Enteromorpha* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the

genome of the type strain from the type species, *Candidatus Enteromorpha quadrami*. This is a new name for the GTDB alphanumeric genus CAG-110. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Oscillospiraceae*

Description of *Candidatus Enteromorpha barnesiae* sp. nov.

Candidatus Enteromorpha barnesiae (bar.ne'si.ae. N.L. gen. fem. n. *barnesiae*, of Barnes, named after Ella M. Barnes, a British microbiologist).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier avicell_METABAT_70, hylon_METABAT_9, inulin_METABAT_82 and rmaize_METABAT_177 and which is available via NCBI BioSample SAMN18871197. This is a new name for the alphanumeric GTDB species sp003525905. The GC content of the type genome is 61.70 %, 61.32 %, 61.45 % and 62.01 % and the genome length is 1.70 Mbp, 2.26 Mbp, 2.12 Mbp and 1.81 Mbp.

Description of *Candidatus Enteromorpha quadrami* sp. nov.

Candidatus Enteromorpha quadrami (quad.ra'mi. N.L. gen. n. *quadrami* of the Quadram Institute).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifiers avicel_MAXBIN_045, inulin_METABAT_94 and pstarch_METABAT_151 and which is available via NCBI BioSample SAMN18871185. This is a new name for the alphanumeric GTDB species sp000434635. The GC content of the type genome is 57.48 %, 57.06 % and 57.30 % and the genome length are 2.09 Mbp, 2.31 Mbp and 2.27 Mbp.

Description of *Candidatus Enteronaster* gen. nov.

Candidatus Enteronaster (En.ter.o.nas'ter. Gr. neut. n. *enteron* the gut; Gr. masc. n. *naster* an inhabitant; N.L. masc. n. *Enteronaster* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Enteronaster faecalis*. This is a new name for the GTDB alphanumeric genus CAG-103. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Oscillospiraceae*

Description of *Candidatus Enteronaster faecalis* sp. nov.

Candidatus Enteronaster faecalis (fae.ca'lis. N.L. masc. adj. *faecalis*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier inulin_METABAT_98 and which is available via NCBI BioSample SAMN18871242. This is a new name for the alphanumeric GTDB species sp000432375. The GC content of the type genome is 61.98 % and the genome length is 1.97 Mbp.

Description of *Candidatus Enteroplasma* gen. nov.

Candidatus Enteroplasma (En.te.ro.plas'ma. Gr. neut. n. *enteron* the gut; L. neut. n. *plasma* a form; N.L. neut. n. *Enteroplasma* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Enteroplasma stercoris*. This is a new name for the GTDB alphanumeric genus CAG-115. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Ruminococcaceae*

Description of *Candidatus Enteroplasma stercoris* sp. nov.

Candidatus Enteroplasma stercoris (ster'co.ris. L. gen. neut. n. *stercoris*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier inulin_MAXBIN_035 and which is available via NCBI BioSample SAMN18871220. This is a new name for the alphanumeric GTDB species sp003531585. The GC content of the type genome is 52.91 % and the genome length is 2.79 Mbp.

Description of *Candidatus Enterovivens* gen. nov.

Candidatus Enterovivens (En.te.ro.vi'vens. Gr. neut. n. *enteron* the gut; N.L. masc./fem. adj. part. *vivens* living; N.L. fem. n. *Enterovivens* a microbe living in the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Enterovivens caccae*. This is a new name for the GTDB alphanumeric genus CAG-127. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Lachnospirales* and to the family *Lachnospiraceae*

Description of *Candidatus Enterovivens caccae* sp. nov.

Candidatus Enterovivens caccae (cac'cae. Gr. fem. n. *kakkē*, faeces; N.L. gen. n. *caccae*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_183 and which is available via NCBI BioSample SAMN18871313. This is a new name for the alphanumeric GTDB species sp900319515. The GC content of the type genome is 44.48 % and the genome length is 2.61 Mbp.

Description of *Candidatus Eubacterium caccanthonri* sp. nov.

Candidatus Eubacterium caccanthonri (cacc.an.thro'pi. Gr. fem. n. *kakkē*, faeces; Gr. masc. n. *anthropos*, a human being; N.L. gen. masc. n. *caccanthonri*, of human faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_MAXBIN_022 and which is available via NCBI BioSample SAMN18871293. This is a new name for the alphanumeric GTDB species sp000434995. GTDB has assigned this species to genus with an alphabetic suffix which cannot be incorporated into a well-formed binomial, so in naming this species, we have used the basonym for the genus. The GC content of the type genome is 36.52 % and the genome length is 1.94 Mbp.

Description of *Candidatus Eubacterium colihabitans* sp. nov.

Candidatus Eubacterium colihabitans (co.li.ha'bi.tans. L. neut. n. *colum*, colon; L. pres. part. *habitans*, inhabiting; N.L. part. adj. *colihabitans*, inhabiting the colon).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_220 and which is available via NCBI BioSample SAMN18871319. This is a new name for the alphanumeric GTDB species sp003491505. GTDB has assigned this species to genus with an alphabetic suffix which cannot be incorporated into a well-formed binomial, so in naming this species, we have used the basonym for the genus. The GC content of the type genome is 41.07 % and the genome length is 2.49 Mbp.

Description of *Candidatus Gallacutalibacter hominis* sp. nov.

Candidatus Gallacutalibacter hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifiers avicel_METABAT_20 and inulin_METABAT_180 and which is available via NCBI BioSample SAMN18871192. This is a new name for the alphanumeric GTDB species sp003477405. This genus was named by Gilroy et al. (2021). The GC content of the type genomes is 56.15 % and 56. 25 % and the genome lengths are 2.33 Mbp and 1.92 Mbp.

Description of *Candidatus Gemmiger merdicola* sp. nov.

Candidatus Gemmiger merdicola (mer.di'co.la. L. gen. fem. n. *merda*, faeces; L. masc./fem. suff. *-cola*, inhabitant of; N.L. fem. n. *merdicola* inhabitant of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *inulin_METABAT_93* and which is available via NCBI BioSample SAMN18871240. This is a new name for the alphanumeric GTDB species *sp900539695*. The GC content of the type genome is 58.43 % and the genome length is 2.39 Mbp.

Description of *Candidatus Holdemanella enterica* sp. nov.

Candidatus Holdemanella enterica (en.ter'i.ca. Gr. neut. n. *enteron*, gut; L. fem. adj. suff. *-ica*, pertaining to; N.L. fem. adj. *enterica*, pertaining to the gut).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *T0_METABAT_130* and which is available via NCBI BioSample SAMN18871306. This is a new name for the alphanumeric GTDB species *sp002299315*. The GC content of the type genome is 34.07 % and the genome length is 2.18 Mbp.

Description of *Candidatus Huxleyella* gen. nov.

Candidatus Huxleyella (Hux.ley.el'la. L. fem. dim. suff. *-ella* diminutive ending; N.L. fem. n. *Huxleyella* named in honour of the British scientist Thomas Henry Huxley (1825-1895), known for his advocacy of Charles Darwin's theory of evolution).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Huxleyella fimi*. This is a new name for the GTDB alphanumeric genus *UMGS1071*. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Acutalibacteraceae*

Description of *Candidatus Huxleyella fimi* sp. nov.

Candidatus Huxleyella fimi (fi'mi. L. neut. gen. n. *fimi*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *T0_METABAT_173* and which is available via NCBI BioSample SAMN18871311. This is a new name for the alphanumeric GTDB species *sp900542375*. The GC content of the type genome is 38.84 % and the genome length is 1.60 Mbp.

Description of *Candidatus Minthomorpha* gen. nov.

Candidatus Minthomorpha (Min.tho.mor'pha. Gr. masc. n. *minthos* dung; Gr. fem. n. *morphe* a form, shape; N.L. fem. n. *Minthomorpha* a microbe associated with faeces).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Minthomorpha faecalis*. This is a new name for the GTDB alphanumeric genus CAG-81. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Lachnospirales* and to the family *Lachnospiraceae*

Description of *Candidatus Minthomorpha faecalis* sp. nov.

Candidatus Minthomorpha faecalis (fae.ca'lis. N.L. fem. adj. *faecalis*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier rmaize_METABAT_174 and which is available via NCBI BioSample SAMN18871281. This is a new name for the alphanumeric GTDB species sp900066535. The GC content of the type genome is 49.05 % and the genome length is 2.98 Mbp.

Description of *Candidatus Minthonaster* gen. nov.

Candidatus Minthonaster (Min.tho.nas'ter. Gr. masc. n. *minthos* dung; Gr. masc. n. *naster* an inhabitant; N.L. masc. n. *Minthonaster* a microbe associated with faeces).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Minthonaster faecium*. This is a new name for the GTDB alphanumeric genus CAG-83. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Oscillospiraceae*

Description of *Candidatus Minthonaster anthropi* sp. nov.

Candidatus Minthonaster anthropi (an.thro'pi. Gr. masc. n. *anthropos*, a human being; N.L. gen. masc. n. *anthropi*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_167 and which is available via NCBI BioSample SAMN18871310. This is a new name for the alphanumeric GTDB species sp900552475. The GC content of the type genome is 61.38 % and the genome length is 2.18 Mbp.

Description of *Candidatus Minthonaster faecium* sp. nov.

Candidatus Minthonaster faecium (fae'ci.um. L. fem. gen. pl. n. *faecium*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier hylon_METABAT_44 and which is available via NCBI BioSample SAMN18871213. This is a new name for the alphanumeric GTDB species sp003539495. The GC content of the type genome is 57.03 % and the genome length is 2.06 Mbp.

Description of *Candidatus Minthonaster hominis* sp. nov.

Candidatus Minthonaster hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier inulin_METABAT_175 and which is available via NCBI BioSample SAMN18871228. This is a new name for the alphanumeric GTDB species sp900545585. The GC content of the type genome is 60.55 % and the genome length is 2.24 Mbp.

Description of *Candidatus Minthonaster merdae* sp. nov.

Candidatus Minthonaster merdae (mer'dae. L. gen. fem. n. *merdae*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_66 and which is available via NCBI BioSample SAMN18871332. This is a new name for the alphanumeric GTDB species sp000431575. The GC content of the type genome is 59.89 % and the genome length is 2.00 Mbp.

Description of *Candidatus Minthoplasma* gen. nov.

Candidatus Minthoplasma (Min.tho.plas'ma. Gr. masc. n. *minthos* dung; L. neut. n. *plasma* a form; *Minthoplasma* a microbe associated with faeces).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Minthoplasma entericum*. This is a new name for the GTDB alphanumeric genus GCA-900066135. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Lachnospirales* and to the family *Lachnospiraceae*

Description of *Candidatus Minthoplasma copri* sp. nov.

Candidatus Minthoplasma copri (cop'ri. Gr. masc. n. *kópros*, faeces; N.L. gen. n. *copri*; of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT__206 and which is available via NCBI BioSample SAMN18871318. This is a new name for the alphanumeric GTDB species sp900543575. The GC content of the type genome is 49.81 % and the genome length is 3.26 Mbp.

Description of *Candidatus Minthoplasma enterica* sp. nov.

Candidatus Minthoplasma entericum (en.te'ri.cum. Gr. neut. n. *enteron*, gut; L. neut. adj. suff. *-icum*, pertaining to; N.L. neut. adj. *entericum*, pertaining to the gut).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT__122 and which is available via NCBI BioSample SAMN18871303. This is a new name for the alphanumeric GTDB species sp900066135. The GC content of the type genome is 47.02 % and the genome length is 1.90 Mbp.

Description of *Candidatus Minthovivens* gen. nov.

Candidatus Minthovivens (Min.tho.viv'ens. Gr. masc. n. *minthos* dung; N.L. masc./fem. part. adj. *vivens* living; N.L. fem. n. *Minthovivens* a microbe living in faeces).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Minthovivens enterohominis*. This is a new name for the GTDB alphanumeric genus KLE1615. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Lachnospirales* and to the family *Lachnospiraceae*

Description of *Candidatus Minthovivens enterohominis* sp. nov.

Candidatus Minthovivens enterohominis (en.te.ro.ho'mi.nis. Gr. neut. n. *enteron*, gut; L. gen. masc. n. *hominis*, of a human being; N.L. gen. masc. n. *enterohominis*, of the human gut).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier inulin_METABAT__130 and which is available via NCBI BioSample SAMN18871226. This is a new name for the alphanumeric GTDB species sp900066985. The GC content of the type genome is 40.97 % and the genome length is 3.77 Mbp.

Description of *Candidatus Negativibacillus quadrami* sp. nov.

Candidatus Negativibacillus quadrami (quad.ra'mi. N.L. gen. n. *quadrami* of the Quadram Institute).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT__114 and which is available via NCBI BioSample SAMN18871301. This is a new name for the alphanumeric GTDB species sp000435195. The GC content of the type genome is 51.95 % and the genome length is 2.20 Mbp.

Description of *Candidatus Neoacutalibacter* gen. nov.

Candidatus Neoacutalibacter (Ne.o.a.cu.ta.li.ibac'ter. Gr. masc. adj. *neos* new; N.L. masc. n. *Acutalibacter* an existing genus name; N.L. masc. n. *Neoacutalibacter* a bacterial genus related to but distinct from the existing named genus).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Neoacutalibacter hominis*. This is a new name for the GTDB alphanumeric genus CAG-177. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Acutalibacteraceae*

Description of *Candidatus Neoacutalibacter hominis* sp. nov.

Candidatus Neoacutalibacter hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier inulin_MAXBIN__022 and which is available via NCBI BioSample SAMN18871219. This is a new name for the alphanumeric GTDB species sp003514385. The GC content of the type genome is 51.47 % and the genome length is 2.22 Mbp.

Description of *Candidatus Neoanaerovorax* gen. nov.

Candidatus Neoanaerovorax (Ne.o.an.ae.ro.vo'rax. Gr. masc. adj. *neos* new; N.L. masc. n. *Anaerovorax* an existing genus name; N.L. masc. n. *Neoanaerovorax* a bacterial genus related to but distinct from the existing named genus).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Neoanaerovorax merdae*. This is a new name for the GTDB alphanumeric genus CAG-238. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Peptostreptococcales* and to the family *Anaerovoracaceae*

Description of *Candidatus Neoanaerovorax merdae* sp. nov.

Candidatus Neoanaerovorax merdae (mer'dae. L. gen. fem. n. *merdae*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifiers rmaize_METABAT_46_sub and T0_METABAT_161 and which is available via NCBI BioSample SAMN18871283. This is a new name for the alphanumeric GTDB species sp900542245. The GC content of the type genome are 52.09 % and 51.52 % and the genome lengths are 1.57 Mbp and 2.01 Mbp.

Description of *Candidatus Neoeggerthella* gen. nov.

Candidatus Neoeggerthella (Ne.o.eg.ger.thel'la. Gr. masc. adj. *neos* new; N.L. fem. n. *Eggerthella* an existing genus name; N.L. fem. n. *Neoeggerthella* a bacterial genus related to but distinct from the existing named genus).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Neoeggerthella hominis*. This is a new name for the GTDB alphanumeric genus CAG-1427. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Coriobacteriales* and to the family *Eggerthellaceae*

Description of *Candidatus Neoeggerthella hominis* sp. nov.

Candidatus Neoeggerthella hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier nmaize_METABAT_52 and which is available via NCBI BioSample SAMN18871250. This is a new name for the alphanumeric GTDB species sp900554685. The GC content of the type genome is 45.89 % and the genome length is 1.92 Mbp.

Description of *Candidatus Pararuminococcus* gen. nov.

Candidatus Pararuminococcus (Pa.ra.ru.mi.no.coc'cus. Gr. pref. *para-* beside; N.L. masc. n. *Ruminococcus* an existing genus name; N.L. masc. n. *Pararuminococcus* a bacterial genus related to but distinct from the existing named genus).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Parauminococcus sangeri*. This is a new name for the GTDB alphanumeric genus UBA1417. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Acutalibacteraceae*

Description of *Candidatus Parasutterella caccanthropi* sp. nov.

Candidatus Parasutterella caccanthropi (cacc.an.thro'pi. Gr. fem. n. *kakkē*, faeces; Gr. masc. n. *anthropos*, a human being; N.L. gen. masc. n. *caccanthropi*, of human faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *psstarch_METABAT_57* and which is available via NCBI BioSample SAMN18871261. This is a new name for the alphanumeric GTDB species *sp000980495*. The GC content of the type genome is 49.32 % and the genome length is 2.19 Mbp.

Description of *Candidatus Parauminococcus sangeri* sp. nov.

Candidatus Parauminococcus sangeri (san'ge.ri. N.L. masc. n. *sangeri* derived from the Latinised family name for Frederick Sanger, 1918-2013, the British scientist; awarded the 1958 Nobel Prize in Chemistry for his work on the structure of protein and the 1980 Nobel Prize in Chemistry for inventing dideoxy sequencing).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *T0_METABAT_250* and which is available via NCBI BioSample SAMN18871325. This is a new name for the alphanumeric GTDB species *sp003531055*. The GC content of the type genome is 53.40 % and the genome length is 2.30 Mbp.

Description of *Candidatus Pearsonella* gen. nov.

Candidatus Pearsonella (Pear.son.el'la. L. fem. dim. suff. *-ella* diminutive ending; N.L. fem. n. *Pearsonella* named in honour of the British scientist Bruce Pearson, known for his contributions to the study of *Campylobacter*).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Pearsonella faecalis*. This is a new name for the GTDB alphanumeric genus *UBA1822*. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Veillonellales* and to the family *Dialisteraceae*

Description of *Candidatus Pearsonella faecalis* sp. nov.

Candidatus Pearsonella faecalis (fae.ca'lis. N.L. fem. adj. *faecalis*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *hylon_MAXBIN_006* and which is available via NCBI BioSample SAMN18871199. This is a new name for the alphanumeric GTDB species *sp002314995*. The GC content of the type genome is 56.55 % and the genome length is 1.81 Mbp.

Description of *Candidatus Physcomorpha* gen. nov.

Candidatus Physcomorpha (Phys.co.mor'pha. Gr. fem. n. *physke* large intestine; Gr. fem. n. *morphe* a form, shape; N.L. fem. n. *Physcomorpha* a microbe associated with the large intestine).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Physcomorpha faecium*. This is a new name for the GTDB alphanumeric genus UBA11524. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Christensenellales* and to the family CAG-74

Description of *Candidatus Physcomorpha faecium* sp. nov.

Candidatus Physcomorpha faecium (fae'ci.um. L. fem. gen. pl. n. *faecium*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier rmaize_MAXBIN_031 and which is available via NCBI BioSample SAMN18871268. This is a new name for the alphanumeric GTDB species sp000437595. The GC content of the type genome is 57.78 % and the genome length is 3.22 Mbp.

Description of *Candidatus Physconaster* gen. nov.

Candidatus Physconaster (Phys.co.nas'ter. Gr. fem. n. *physke* large intestine; Gr. masc. n. *naster* an inhabitant N.L. masc. n. *Physconaster* a microbe inhabiting the large intestine).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Physconaster merdicola*. This is a new name for the GTDB alphanumeric genus UBA11774. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Lachnospirales* and to the family *Lachnospiraceae*

Description of *Candidatus Physconaster merdicola* sp. nov.

Candidatus Physconaster merdicola (mer.di'co.la. L. gen. fem. n. *merda*, faeces; L. masc./fem. suff. -cola, inhabitant of; N.L. fem. n. *merdicola* inhanbitant of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT_254 and which is available via NCBI BioSample SAMN18871326. This is a new name for the alphanumeric GTDB species sp003507655. The GC content of the type genome is 41.91 % and the genome length is 2.16 Mbp.

Description of *Candidatus Ruminococcus anthropi* sp. nov.

Candidatus Ruminococcus anthropi (an.thro'pi. Gr. masc. n. *anthropos*, a human being; N.L. gen. masc. n. *anthropi*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *hylon_METABAT_215* and which is available via NCBI BioSample SAMN18871209. This is a new name for the alphanumeric GTDB species *sp900314705*. GTDB has assigned this species to genus with an alphabetic suffix which cannot be incorporated into a well-formed binomial, so in naming this species, we have used the basonym for the genus. The GC content of the type genome is 33.46 % and the genome length is 1.46 Mbp.

Description of *Candidatus Ruminococcus faecihominis* sp. nov.

Candidatus Ruminococcus faecihominis (fae.ci.ho'mi.nis. L. fem. n. *faex*, *faecis* faeces; L. gen. masc. n. *hominis*, of a human being; N.L. gen. n. *faecihominis*, of human faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *T0_METABAT_198* and which is available via NCBI BioSample SAMN18871316. This is a new name for the alphanumeric GTDB species *sp003011855*. GTDB has assigned this species to genus with an alphabetic suffix which cannot be incorporated into a well-formed binomial, so in naming this species, we have used the basonym for the genus. The GC content of the type genome is 44.64 % and the genome length is 2.86 Mbp.

Description of *Candidatus Ruminococcus hominis* sp. nov.

Candidatus Ruminococcus hominis (ho'mi.nis. L. gen. masc. n. *hominis*, of a human being).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show ≥95% average nucleotide identity to the type genome for the species to which we have assigned the genome identifier *rmaize_MAXBIN_013* and which is available via NCBI BioSample SAMN18871266. This is a new name for the alphanumeric GTDB species *sp00433635*. GTDB has assigned this species to genus with an alphabetic suffix which cannot be incorporated into a well-formed binomial, so in naming this species, we have used the basonym for the genus. The GC content of the type genome is 45.98 % and the genome length is 2.41 Mbp.

Description of *Candidatus Sangerella* gen. nov.

Candidatus Sangerella (San.ger.el'la. L. fem. dim. suff. *-ella* diminutive ending; N.L. fem. n. *Sangerella* named in honour of Frederick Sanger (1918-2013), British scientist; awarded the 1958 Nobel Prize in Chemistry for his work on the structure of protein and the 1980 Nobel Prize in Chemistry for inventing dideoxy sequencing).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Sangerella faecicola*. This is a new name for the GTDB alphanumeric genus UBA737. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family *Acutalibacteraceae*

Description of *Candidatus Sangerella faecicola* sp. nov.

Candidatus Sangerella faecicola (fae.ci'co.la. L. fem. n. *faex*, *faecis* faeces; L. suff. -*cola* inhabitant of; N.L. fem. n. *faecicola* a microbe inhabitating faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifiers rmaize_MAXBIN_077 and T0_METABAT_221 and which is available via NCBI BioSample SAMN18871271. This is a new name for the alphanumeric GTDB species sp900549055. The GC content of the type genome are 47.36 % and 46.01 % and the genome lengths are 2.02 Mbp and 2.89 Mbp.

Description of *Candidatus Splanchousia* gen. nov.

Candidatus Splanchousia (Splanch.ou'si.a. Gr. neut. n. *splanchnon* guts; L. fem. n. *ousia* an essence; *Splanchousia* a microbe associated with the intestines).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Splanchousia colicola*. This is a new name for the GTDB alphanumeric genus UBA1191. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Peptostreptococcales* and to the family *Anaerovoracaceae*

Description of *Candidatus Splanchousia colicola* sp. nov.

Candidatus Splanchousia colicola (co.li'co.la. L. neut. n. *colum*, colon; L. masc./fem. suff. -*cola*, inhabitant of; N.L. fem. n. *colicola* inhabitant of the colon).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier avicell_METABAT_39 and which is available via NCBI BioSample SAMN18871194. This is a new name for the alphanumeric GTDB species sp900066305. The GC content of the type genome is 49.21 % and the genome length is 2.02 Mbp.

Description of *Candidatus Splanchousia faecium* sp. nov.

Candidatus Splanchousia faecium (fae.ci.um. L. fem. gen. pl. n. *faecium*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier rmaize_METABAT__166 and which is available via NCBI BioSample SAMN18871279. This is a new name for the alphanumeric GTDB species sp900549125. The GC content of the type genome is 47.53 % and the genome length is 2.11 Mbp.

Description of *Candidatus Wallaceimonas* gen. nov.

Candidatus Wallaceimonas (Wal.lace.i.mo'nas. L. fem. n. *monas* a monad; N.L. fem. n. *Wallaceimonas* named in honour of British naturalist Alfred Russel Wallace (1823-1913), co-discoverer of evolution by natural selection).

A bacterial genus identified by metagenomic analyses of human faeces. The genus includes all bacteria with genomes that show $\geq 60\%$ average aminoacid identity to the genome of the type strain from the type species, *Candidatus Wallaceimonas faecalis*. This is a new name for the GTDB alphanumeric genus UMGS1696. This genus has been assigned by GTDB-Tk v1.5.0 working on GTDB R06-RS202 reference data (Chaumeil et al., 2019; Parks et al., 2020) to the order *Oscillospirales* and to the family CAG-272

Description of *Candidatus Wallaceimonas faecalis* sp. nov.

Candidatus Wallaceimonas faecalis (fae.ca'lis. N.L. fem. adj. *faecalis*, of faeces).

A bacterial species identified by metagenomic analyses. This species includes all bacteria with genomes that show $\geq 95\%$ average nucleotide identity to the type genome for the species to which we have assigned the genome identifier T0_METABAT__60 and which is available via NCBI BioSample SAMN18871330. This is a new name for the alphanumeric GTDB species sp900753285. The GC content of the type genome is 49.14 % and the genome length is 1.81 Mbp.