

1 Novel insights into the pig gut microbiome using metagenome-assembled genomes

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16 **Running title:** Swine gut-derived metagenome-assembled genomes

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24 **Abstract**

25 Pigs are among the most numerous and intensively farmed food-producing animals in the world.
26 The gut microbiome plays an important role in the health and performance of swine and changes
27 rapidly after weaning. Here, fecal samples were collected from pigs at 7 different times points
28 from 7 to 140 days of age. These swine fecal metagenomes were used to assemble 1,150
29 dereplicated metagenome-assembled genomes (MAGs) that were at least 90% complete and had
30 less than 5% contamination. These MAGs represented 472 archaeal and bacterial species, and
31 the most widely distributed MAGs were the uncultured species *Collinsella* sp002391315,
32 *Sodaliphilus* sp004557565, and *Prevotella* sp000434975. Weaning was associated with a
33 decrease in the relative abundance of 69 MAGs (e.g. *Escherichia coli*) and an increase in the
34 relative abundance of 140 MAGs (e.g. *Clostridium* sp000435835, *Oliverpabstia intestinalis*).
35 Genes encoding for the production of the short-chain fatty acids acetate, butyrate, and propionate
36 were identified in 68.5%, 18.8%, and 8.3% of the MAGs, respectively. Carbohydrate-active
37 enzymes associated with the degradation of arabinose oligosaccharides and mixed-linkage
38 glucans were predicted to be most prevalent among the MAGs. Antimicrobial resistance genes
39 were detected in 327 MAGs, including 59 MAGs with tetracycline resistance genes commonly
40 associated with pigs such as *tet(44)*, *tet(Q)*, and *tet(W)*. Overall, 82% of the MAGs were
41 assigned to species that lack cultured representatives indicating that a large portion of the swine
42 gut microbiome is still poorly characterized. The results here also demonstrate the value of
43 MAGs in adding genomic context to gut microbiomes.

44 **Importance**

45 Many of the bacterial strains found in the mammalian gut are difficult to culture and isolate due
46 to their various growth and nutrient requirements that are frequently unknown. Here, we

47 assembled strain-level genomes from short metagenomic sequences, so-called metagenome-
48 assembled genomes (MAGs), that were derived from fecal samples collected from pigs at
49 multiple time points. The majority of these MAGs represented bacterial species that have yet to
50 be cultured or described thus underlining the need for cultivation studies that isolate and describe
51 novel bacterial species. The genomic context of a number of antimicrobial resistance genes
52 commonly detected in swine was also determined. In addition, our study connected taxonomy
53 with potential metabolic functions such as carbohydrate degradation and short-chain fatty acid
54 production.

55 **Introduction**

56 Pork production continues to increase globally (1) despite serious challenges, such as
57 antimicrobial use and resistance (2) and infectious disease (3), that threaten its profitability and
58 sustainability. Microbiome research has the potential to contribute solutions to some of these
59 issues, given a better understanding of the swine gut microbiome. The pig gut microbiome, as in
60 most mammals, provides the host with numerous benefits including protection against pathogen
61 colonization, aiding immune system development and maturation, and production of certain
62 vitamins and metabolites (4). The number of unique genes within the gut microbiome also
63 greatly exceeds those encoded within the pig genome thereby providing the host with an
64 expanded repertoire of genes that can degrade dietary substrates (5).

65 Cultivation of many of the microbes found in the swine gastrointestinal tract remains
66 difficult due to their unique, and often unknown, growth requirements. Consequently this has
67 traditionally limited study of the mammalian gut microbiome to those microbes that can be easily
68 grown and characterized in the laboratory (6). However, this often represents only a small
69 fraction of the microbial diversity in the gut. In recent years, metagenome-assembled genomes
70 (MAGs) recovered from shotgun metagenomic sequences have greatly expanded the number of

71 microbial genomes in reference databases (7-10). Although the quality of these MAGs varies,
72 they enable researchers to connect functional potential with microbial species and strains lacking
73 cultured representatives.

74 Previously, we assessed the effects of varying weaning ages on the development of the
75 swine gut microbiome using shotgun metagenomic sequences generated from fecal samples
76 collected from pigs throughout the swine production cycle (11). Here, we assembled those
77 sequences and binned the assembled contigs into MAGs, retaining only those MAGs that were at
78 least 90% complete and had less than 5% contamination. Our main objective was to characterize
79 the functional potential represented by those MAGs, including carbohydrate-active enzymes
80 (CAZymes) and antimicrobial resistance genes (ARGs) and to associate those functions with
81 individual taxa. In addition, we aimed to determine if MAGs assembled in this study are
82 representative of the pig gut microbiome in general by using metagenomic sequences from other
83 publicly-available swine studies.

84 **Results**

85 **Metagenome-assembled genomes**

86 From 738 Gb of shotgun metagenomic sequencing data, 87,472 MAGs with greater than
87 90% completeness and less than 5% contamination were recovered. After dereplication at 99%
88 ANI (average nucleotide identity), the remaining 1,150 non-redundant MAGs represented
89 potentially unique strains that were assigned to 360 and 472 archaeal and bacterial genera and
90 species, respectively (Supplementary Table S1). The MAGs ranged in size from 0.74 to 6.14 Mb
91 with an average size of 2.28 Mb (SEM \pm 0.02). The 358 MAGs that were not assigned to a
92 species in the Genome Database Taxonomy (GTDB) at a 95% ANI threshold may represent
93 potentially novel species, 32 of which also had no genus designation. When 95% ANI was used

94 for secondary clustering, 758 dereplicated MAGs (putative species) were produced from the
95 original 87,472 MAGs. The vast majority of the MAGs classified using GTDB were bacteria and
96 only 10 MAGs were assigned to the archaea, all of which were identified as methanogens. In
97 total, 19 unique phyla were represented among the MAGs (Fig. 1). The most common species
98 designation was *Collinsella* sp002391315 (22 MAGs), followed by *Sodaliphilus* sp004557565
99 (19 MAGs), *Prevotella* sp000434975 (17 MAGs), and UBA3388 sp004551865 (13 MAGs).
100 Overall, 938 MAGs were assigned to archaeal or bacterial species that lack cultured
101 representatives.

102 **Functional analysis of MAGs**

103 Functional profiling of the dereplicated MAGs using CAZymes and Kyoto Encyclopedia
104 of Genes and Genomes (KEGG) pathways was carried out using the Distilled and Refined
105 Annotation of Metabolism (DRAM) package. There were 6,656 unique KOs (KEGG Orthology)
106 and 155,297 CAZymes within 281 unique CAZyme families identified among the MAGs. The
107 average number of CAZymes per MAG was 135.0 ± 2.9 within 33.3 ± 0.5 CAZyme families
108 (Supplementary Table S2). The glycoside hydrolases (GHs; $n = 122$) were most prevalent among
109 the unique CAZyme families followed by carbohydrate-binding modules (CBMs; $n = 58$),
110 glycosyltransferases (GTs; $n = 55$), polysaccharide lyases (PLs; $n = 24$), carbohydrate esterases
111 (CEs; $n = 14$), and auxiliary activities (AAs; $n = 8$). The GH families GH2, GH3, GH13, GH23,
112 GH25, GH31, GH32, GH36, GH73, and GH77 were most widely distributed. Many of the
113 MAGs encoding the greatest number of CAZymes and CAZyme families belonged to the
114 *Bacteroidaceae* family including *Bacteroides fragilis*, *Bacteroides heparinolyticus*, *Bacteroides*
115 *stercoris*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides xylanisolvens*, and
116 *Phocaeicola vulgatus*. CAZymes involved in the degradation of arabinose oligosaccharides and

117 mixed-linkage glucans were found in at least 50% of the MAGs and CAZymes predicted to be
118 involved in the digestion of amorphous cellulose, arabinan, beta-mannan, fucose
119 oligosaccharides, pectin, rhamnose oligosaccharides, starch, xylan, and xyloglucan were encoded
120 by 25% or more of the MAGs (Supplementary Table S3). Mucin-degrading CAZymes were
121 identified in only 26 MAGs, with four of these classified as *Pauljensenia hyovaginalis* and
122 another three as *Tractidigestivibacter* sp004557505.

123 The production of SCFAs from carbohydrates is an important function of the gut
124 microbiome from the perspective of the host. The most significant of these are acetate, butyrate,
125 and propionate. Here, 68.5% of the MAGs encoded acetate-producing enzymes (acetate kinase
126 [K00925] and phosphate acetyltransferase enzymes [K00625 or K13788]) and 8.3% had the
127 propionate CoA-transferase gene (K01026) (Supplementary Table S3). Genes for butyrate
128 production via the butyryl-CoA:acetate CoA-transferase (K01034, K01035) or butyrate kinase
129 (K00634, K00929) pathways were identified in 18.8% of MAGs and included known butyrate
130 producers, such as *Anaerostipes hadrus*, *Butyricimonas virosa*, *Butyrivibrio crossotus*,
131 *Cloacibacillus porcorum*, *Coprococcus catus*, *Gemmiger formicilis*, *Faecalibacterium*
132 *prausnitzii*, *Flavonifractor plautii*, and *Megasphaera elsdenii*.

133 Succinate is a propionate precursor that is produced by certain bacteria species. Genes
134 encoding a fumarate reductase/succinate hydrogenase (K00239, K00240, K00241 and K00244,
135 K00245, K00246) were identified in 28.9% of the MAGs. Known succinate producers among the
136 MAGs with these genes included *Akkermansia muciniphila*, *Anaerobiospirillum*
137 *succiniciproducens*, *Mitsuokella jalaludinii*, *Parabacteroides distasonis*, and *P. vulgatus*. The
138 potential for either D-lactate or L-lactate production via lactate dehydrogenase (K00016, K03778)
139 was detected in 55.3% of the MAGs. Genes for the production of both enantiomers of lactate

140 were carried by 172 MAGs and over half (n = 92) of these were members of the *Treponema*
141 genus or *Lachnospiraceae* or *Lactobacillaceae* families.

142 The 10 archaeal MAGs all carried the genes encoding the methyl-coenzyme M reductase
143 complex (K00399, K00400, K00401, K00402, K03421, K03422) involved in methanogenesis
144 (Supplementary Table S3). However, only the *Methanobacteriaceae* MAGs had the genes for the
145 formylmethanofuran dehydrogenase complex (K00200, K00201, K00202, K00203, K00204,
146 K00205, K11260, K11261) that is necessary for the reduction of carbon dioxide to methane.
147 Hydrogen sulfide production in swine manure slurry has been linked to *Desulfovibrio* spp. (12)
148 and here genes encoding the dissimilatory sulfite reductase and involved in the metabolism of
149 sulfate were only identified in the eight *Desulfovibrionaceae* MAGs. This included *Desulfovibrio*
150 *piger* and *Desulfovibrio* sp900556755 as well as one MAG classified as *Bilophila wadsworthia*.
151 These *Desulfovibrionaceae* MAGs also carried the gene for thiosulfate reductase (K08352)
152 which produces sulfide and sulfite through the reduction of thiosulfate.

153 **Antimicrobial resistance genes**

154 The 1,150 dereplicated MAGs were screened for ARGs using the comprehensive
155 antibiotic resistance database (CARD). A total of 327 MAGs carried at least one ARG
156 (Supplementary Table S4), and together they accounted for 115 unique ARGs, excluding those
157 due to point mutations. The six *Escherichia coli* MAGs contained the greatest number of ARGs
158 (52 to 60) by a large margin. However, this is expected given that the vast majority of these
159 ARGs are widespread within this species. ARGs conferring resistance to tetracycline (*tet* genes)
160 are frequently among the most abundant in the gastrointestinal tract of conventionally-raised pigs
161 and here, 59 MAGs carried at least one *tet* gene. Among the MAGs with at least one *tet* gene and
162 an overall relative abundance of at least 0.1%, were those identified as *B. fragilis* [*tet*(Q)], *B.*

163 *stercoris* [*tet(Q)*], CAG-873 sp001701165 [*tet(Q)*], *Campylobacter coli* [2 MAGs; *tet(W/N/W)*,
164 *tet(O)*], *Limosilactobacillus reuteri* [*tet(B)*], *P. vulgatus* [*tet(Q)*], *Prevotella* sp000434975
165 [*tet(Q)*], *Prevotella* sp000436915 [*tet(37)*], and *Streptococcus pasteurianus* [*tet(M)*].

166 Resistance to macrolide-lincosamide-streptogramin B (MLS_B) antimicrobials is also often
167 detected in the swine gut microbiome and 48 MAGs were carried at least one MLS_B resistance
168 gene. Relatively abundant ($\geq 0.1\%$) MAGs carrying one or more MLS_B resistance genes included
169 *B. fragilis* [*mef(En2)*], *Catenibacterium mitsuokai* [*erm(G)*], *Clostridium* sp000435835 [*erm(Q)*],
170 *Fusobacterium mortiferum* [*lnu(C)*], *Lactobacillus johnsonii* [2 MAGs; *erm(B)*, *erm(G)*], *L.*
171 *reuteri* [*erm(B)*], *Parabacteroides merdae* [*mef(En2)*], *P. vulgatus* [*mef(En2)*], *Treponema*
172 *succinifaciens* [*erm(F)*], *Schaedlerella* sp004556565 [*lnu(C)*], SFDP01 sp004558185 [*erm(B)*],
173 and *S. pasteurianus* [*lnu(A)*, *lnu(C)*, *erm(B)*].

174 The *vanC* cluster genes, (*vanC*, *vanR_C*, *vanS_C*, *vanT_C*, *vanXY_C*), which confer resistance
175 to vancomycin were found in one MAG classified as *Enterococcus gallinarum*. In this species
176 low-level vancomycin resistance is intrinsic due to this gene cluster (13). The beta-lactamase
177 resistance gene *cfxA2* was identified in 13 MAGs, including six given the taxonomic designation
178 *Sodaliphilus* sp004557565. Many of the other beta-lactamase genes detected were associated
179 with only one bacterial species: *bla_{OXA-61}* (three *C. coli* MAGs), *bla_{TEM-1}* (one *E. coli* MAG),
180 *cblA-1* (three *Bacteroides uniformis* MAGs), and *cepA* (one *B. fragilis* MAG). Aminoglycoside
181 resistance genes among the relatively abundant MAGs ($> 0.1\%$) included *aph(3')-IIIa* in three
182 MAGs (*C. mitsuokai*, *F. mortiferum*, SFDP01 sp004558185), *aac(6')-Im* (*Blautia* spp.), *aad(6)*
183 (SFDP01 sp004558185), *aadA* (*E. coli*), *ant(6)-Ib* (*L. johnsonii*), *aph(2'')-IIa* (*Blautia* spp.), and
184 *aph(6)-Id* (*E. coli*).

185 The location of ARGs within the MAGs was also determined to identify those ARGs co-
186 located on the same contig as other ARGs and/or integrase/transposase sequences
187 (Supplementary Fig. S2). The *aac(6')-Im* and *aph(2'')-IIa* genes were adjacent to each other in
188 three MAGs classified as *Blautia* sp018919065, *Ruminococcus gnavus*, and CAG-238 sp. In one
189 *T. succinifaciens* MAG, *erm(F)* and *tet(X)* were also found on the same contig as were *tet(M)*
190 and *tet(W/N/W)* and *tet(44)* and *ant(6)-Ib* in a CAG-877 sp. MAG. In several MAGs, *lnu(C)* was
191 co-located on the same contig as a putative transposase or integrase gene. Other ARGs
192 potentially associated with transposases included *tet(44)* in two MAGs assigned to *Onthovivens*
193 sp016302065 and CAG-1000 sp004552445, *tet(M)* in *Erysipelatoclostridium ramosum* and *S.*
194 *pasteurianus*, and *tet(Q)* in *Onthomorpha* sp004551865 and *Prevotella* sp900548195.

195 **Pre- vs. post-weaning changes**

196 As these MAGs were assembled from fecal samples taken before and after weaning it
197 was possible to identify MAGs that were differentially abundant in the fecal microbiome of pigs
198 immediately before weaning and 7 days post-weaning. There were 69 MAGs that were more
199 relatively abundant in samples taken just prior to weaning, the most differentially abundant of
200 which were those classified as *Limousia pullorum*, *B. fragilis*, *E. coli*, *P. hyovaginalis* (*Schaalia*
201 *hyovaginalis* in NCBI), and *P. vulgatus* (Supplementary Table S5). There were also six MAGs
202 with a relative abundance greater than 0.1% in the fecal microbiomes of nursing piglets that were
203 not detected in samples from these same pigs 7 d later. These MAGs were classified as *B.*
204 *thetaitaomicron*, *Bulleidia* sp., *Enterococcus faecalis*, *Mediterraneibacter torques*, *Parvimonas*
205 sp., and *P. hyovaginalis*. Among the 140 MAGs that were most enriched in the post-weaning
206 samples were MAGs assigned to *Copromorpha* sp., *Clostridium* sp000435835, *Fusicatenibacter*
207 *saccharivorans*, *Intestinibacter* sp., *Oliverpabstia intestinalis*, *Phascolarctobacterium*

208 sp004558595, *Prevotella* sp002251295, *Prevotella* sp004556065, and *Ruminococcus*
209 sp003011855.

210 **Presence of MAGs in publicly available datasets**

211 To determine how widely distributed the species/strains represented by the MAGs in the
212 present study are among pigs from other studies in different geolocations, the presence and
213 relative abundance of these MAGs within publicly available swine gut metagenomic datasets
214 was assessed. These metagenomic sequences were from 626 fecal and cecal content samples
215 within nine studies representing 13 different counties (Supplementary Table S6). On average,
216 $45.5\% \pm 0.4\%$ SEM of these metagenomic sequences mapped to one of the MAGs from the
217 present study (Supplementary Table S7). Two MAGs classified as *Lactobacillus amylovorus*
218 were the most relatively abundant overall. Other relatively abundant MAGs ($>0.25\%$) included
219 those identified as *B. fragilis*, *C. mitsuokai*, *L. reuteri*, *Phascolarctobacterium succinatutens*,
220 *Prevotella pectinovora*, *Prevotella* sp002251295, *Prevotella* sp002300055, *Streptococcus*
221 *alactolyticus*, and VUNA01 sp002299625. Metagenomic sequences from 96 MAGs were
222 detected in 90% of these publicly available samples. Thirty-three of these MAGs were classified
223 within the *Oscillospiraceae* family including 12 as co-abundance gene groups (CAGs), and 8
224 each as *Dysosmobacter* spp. and *Faecousia* spp. An additional 19 MAGs were assigned to
225 *Sodaliphilus* sp004557565 and 8 as *Cryptobacteroides* spp.

226 The samples from these studies were all collected from post-weaned pigs and therefore
227 on a non-metric multidimensional scaling (NMDS) plot of the Bray-Curtis dissimilarities the pre-
228 weaned pig samples from the present study appear separate from the other samples
229 (Supplementary Fig. S1). Only eight MAGs were not detected in at least one sample among all
230 of the publicly available metagenomic samples and three of these MAGs (*Clostridium* sp.,

231 *Erysipelotrichaceae* sp., and *Negativicoccaceae* sp.,) were not identified in any of the post-
232 weaned samples in the present study either. Overall, including the samples from the current
233 study, there were 71 MAGs that were found in 85% of all samples (Fig. 2).

234 **Discussion**

235 Here we assembled and analyzed 1,150 high-quality MAGs, including 358 that could not
236 be assigned to a species and thus some of these may represent novel species. Clearly, there still
237 exists a large fraction of the swine gut microbiome that has yet to be cultivated as demonstrated
238 by the 938 MAGs that were not associated (>95% ANI) with a phenotypically characterized
239 archaeal or bacterial species. Previously, we reported on changes in the pig gut microbiome in
240 response to different weaning ages using the unassembled short reads from this study (11). We
241 were able to recover MAGs from many of the relatively abundant species in our earlier work
242 including *Anaeromassilibacillus senegalensis* (An172 in GTDB), *B. fragilis*, *E. coli*, *L.*
243 *johnsonii*, *L. reuteri*, *P. succinatutens*, *P. pectinovora*, and *Subdoligranulum variabile*
244 (*Gemmiger variabilis* in the GTDB). The exceptions were *Prevotella copri* and *Clostridioides*
245 *difficile*; however, this may have been due to differences in how taxonomy was assigned to the
246 unassembled reads vs. the MAGs as several closely related species were identified here.

247 After weaning, pigs are typically fed a diet that is rich in cereal grains such as corn,
248 barley, and/or wheat which, in addition to high levels of starch, contain other polysaccharides
249 such as cellulose, hemicellulose (e.g., mannan, mixed-linkage glucan, xylan, and xyloglucan),
250 and pectin. These polysaccharides escape digestion by the host and are therefore available as
251 substrates for the gut microbiome (14). As such, the pig gut microbiome carries a large repertoire
252 of genes encoding enzymes called CAZymes that can breakdown and metabolize these
253 polysaccharides. The CAZymes are grouped into families based on sequence similarity, although

254 CAZymes within the same family may have different substrate specificities (15). The AAs, CEs,
255 GHs, and PLs are the CAZyme families involved in the degradation of glycans and typically
256 multiple CAZymes are required for the digestion of specific glycans.

257 Many of the MAGs encoding the greatest number of CAZymes and with the potential
258 capacity to degrade multiple types of glycans were classified within the Bacteroidales order
259 including *Alistipes senegalensis*, *Alistipes shahii*, *B. thetaiotaomicron*, *B. uniformis*, *B.*
260 *xylanisolvens*, *Parabacteroides* spp., *P. vulgatus*, and *Prevotella* spp. Bacteria within this order
261 are well documented as having a diverse and rich set of CAZymes which may be organized into
262 groups of genes termed polysaccharide utilization loci (PUL) (16, 17). These CAZymes and
263 PULs likely confer an advantage to these bacteria within a highly competitive ecosystem like the
264 mammalian gastrointestinal tract. The other taxonomic group of MAGs encoding a large number
265 of CAZymes was the *Lachnospiraceae* family that included *Acetatifactor* sp., *Blautia*
266 sp001304935, COE1 sp., *Eisenbergiella massiliensis*, *Hungatella* sp005845265, and *Roseburia*
267 sp. Some members of this family have also been reported to have gene clusters of CAZymes,
268 regulators, and transporters that are similar to PULs (18).

269 The potential for the degradation of arabinan, amorphous cellulose, arabinose
270 oligosaccharides, mixed-linkage glucans, xyloglucan, and xylan was relatively widespread
271 among the MAGs. In pigs, diets supplemented with xylan, mixed-linkage glucans, and resistant
272 starch have been shown to increase the relative abundance of *Blautia* spp., *Prevotella* spp. and
273 *Lachnospiraceae* spp. (19-21). Monosaccharides produced through the action of CAZymes can
274 then be used by the CAZyme-producer or other bacteria in the gut to generate various
275 metabolites. In particular, the potential for short-chain fatty acid (SCFA) production through the
276 fermentation of monosaccharides is frequently a focus of many mammalian gut microbiome

277 studies as even in monogastric animals like pigs, up to 25% of daily energy requirements are met
278 by SCFAs (22). Butyrate is often the SCFA of most interest as it is the primary energy source for
279 mammalian colonic epithelial cells and can regulate apoptosis, enhance barrier function, and
280 reduce inflammation in these cells (23, 24).

281 Here, 216 MAGs carried genes for butyrate production through either the butyryl-
282 CoA:acetate CoA-transferase (*but*) or butyrate kinase (*buk*) pathways. Although several known
283 butyrate producers are included among these MAGs such as *B. virosa*, *F. prausnitzii* and *M.*
284 *elsdenii*, certain MAGs were assigned to bacterial species (e.g., *E. coli*, *E. faecalis*) that do not
285 typically produce butyrate. Instead, these genes are likely involved in other metabolic functions
286 or two component systems in these species. Typically, the *but* gene is more prevalent than the
287 *buk* gene among gut bacteria (25); however, here the number of MAGs carrying either of these
288 genes was nearly the same. There were also 17 MAGs with both *but* and *buk* genes, including *C.*
289 *porcorum*, *F. plautii*, and *Intestinimonas massiliensis*.

290 Acetate and propionate, the two other physiologically important SCFAs in the
291 mammalian gut, also have anti-inflammatory effects in addition to providing an energy source
292 for the host (26). In the swine lower gastrointestinal tract the concentration ratio of
293 acetate:propionate:butyrate is approximately 65:25:10 (27, 28) and here the number of MAGs (n
294 = 788) encoding the acetate kinase and phosphate acetyltransferase genes involved in acetate
295 production outnumbered those carrying genes for producing butyrate (n = 216) and propionate (n
296 = 95). Bacterial species represented by these MAGs are therefore attractive targets for
297 microbiome manipulation studies and culturing work to obtain isolates of these species for
298 further characterization.

299 There were also significant shifts in the relative abundance of a large number of MAGs 7
300 days post-weaning. As discussed, the diet of the pigs is abruptly changed at weaning from one
301 that is liquid and milk-based to one that is solid and based on cereal grains. This often results in a
302 decrease in the relative abundance of *Bacteroides* and *Escherichia* spp. and an increase in the
303 relative abundance of *Blautia*, *Prevotella*, and *Roseburia* spp. (29-31). Many of the differentially
304 abundant MAGs pre- and post-weaning were assigned to these genera; however, there were also
305 several MAGS classified as bacterial species or genera that are not known to be associated with
306 weaning. These included MAGs enriched in post-weaning pigs that were assigned to uncultured
307 genera or species and were also identified as potential butyrate producers such as CAG-83 sp.,
308 *Aphodosoma* sp900769035, *Copromorpha* sp., *Egerieousia* sp004561775, and UMGS1668
309 sp004556975. Some of these placeholder names represent bacterial taxa that have been
310 previously reported in swine gut metagenomes and await further characterization (32, 33). One
311 MAG classified as *E. faecalis* was relatively abundant in the nursing pig samples ($0.25 \pm 0.07\%$)
312 but was not detected in any of the post-weaning fecal samples. *E. faecalis* was previously
313 identified among the unassembled reads post-weaning so this MAG may represent a strain of *E.*
314 *faecalis* that is unique to nursing piglets.

315 Binning ARGs into MAGs generated from short reads is extremely challenging as they
316 are often flanked by repeat sequences and located on mobile genetic elements such as plasmids
317 which have different properties (e.g., G+C content) than the chromosomal DNA of their host
318 (34). Therefore, one can assume that ARGs identified in the MAGs here are located on the
319 bacterial chromosome. This also explains why the number (115 ARGs) and diversity of ARGs
320 detected in the present study was much lower than in a previous study (250 ARGs) using the
321 same short reads that were used to assemble the MAGs here (11) as well as in the metagenome

322 co-assembly (897 ARGs; data not shown). Despite the limitations associated with ARG binning
323 we were able to provide genomic context for 115 ARGs including several that are relatively
324 abundant in the swine gut such as *erm*(B), *tet*(44), *tet*(Q), and *tet*(W) (35-38).

325 A number of the *tet* (tetracyclines) and *erm* (MLS_B) genes were linked to bacterial
326 species or genera that are considered to be commensal members of the pig gut microbiome such
327 as *Bacteroides* spp., *Clostridium* spp., *L. johnsonii*, *L. reuteri*, *Prevotella* spp., *Roseburia* spp.,
328 *Ruminococcus bromii*, and *Succinivibrio* spp. (39, 40). This may explain the extensive
329 background level of resistance to tetracyclines and MLS_B antimicrobials in swine gut bacteria
330 even in the absence of exposure to these antimicrobials, as observed here and reported in many
331 previous studies (37, 41-43). Until relatively recently in North America, antimicrobials were
332 often administered to all pigs in a herd for non-therapeutic purposes, namely for growth
333 promotion (44). The gut microbiome is vertically transferred from sow to piglet and so it highly
334 plausible that this microbiome would have been exposed to antimicrobials at some point in the
335 past even if the pigs used in this study were not.

336 Several MAGs also carried ARGs conferring resistance to two or more antimicrobials.
337 Most notable among these were a *C. coli* MAG encoding *bla*_{OXA-61}, *tet*(O), and *tet*(W/N/W) and a
338 *S. pasteurianus* MAG with *erm*(B), *lnu*(A), *lnu*(C), and *tet*(M). Both of these MAGs were also
339 enriched in fecal samples of pre-weaned piglets. *C. coli* can be a cause of foodborne illness in
340 humans (45) and carried by healthy pigs while *S. pasteurianus* is an opportunistic pathogen in
341 humans and has been associated with meningitis in piglets (46). In addition, certain MAGs
342 contained more than one ARG on the same contig, suggesting that the ARGs are linked. ARGs
343 linked together in this manner are more likely to be co-selected and maintained within the
344 bacterium. The aminoglycoside resistance genes *aac*(6')-*Im* and *aph*(2'')-*Iia* (also known as

345 *aph(2'')-Ib*) were adjacent to each other in three MAGs within the Clostridia class. These ARGs
346 have previously been reported together in *Enterococcus faecium* and *E. coli* strains (47). A
347 contig with *erm(F)* and *tet(X)* was also binned into a MAG classified as *T. succinifaciens*. These
348 two ARGs confer resistance to macrolides and tetracyclines, respectively, and were originally
349 described on a transposon in *B. fragilis*, although the *tet(X)* gene was reported to be inactive in
350 this species and under anaerobic conditions (48). The *tet(44)* and *ant(6)-Ib* ARGs found here
351 together on the same contig in a *Clostridium* sp. MAG have also been co-located on a transposon
352 in *C. difficile* (49) and a pathogenicity island in *Campylobacter fetus* (50).

353 There were also a number of ARGs co-located with putative transposase or integrase
354 genes. Transposases and integrases are enzymes that can transfer DNA segments, including
355 ARGs, within and between bacterial genomes (51). Here, the lincosamide resistance genes
356 *lnu(C)* and *lnu(P)* were co-located with putative transposase genes in eight different MAGs. Both
357 *lnu(C)* and *lnu(P)* have been previously identified in *Streptococcus agalactiae* (52) and
358 *Clostridium perfringens* (53), respectively, where they were located on the same genomic region
359 as transposase genes. The *tet(44)*, *tet(M)*, *tet(Q)*, and *tet(W/N/W)* genes were also detected on
360 the same contig as putative transposase genes in certain MAGs. If these ARGs are indeed able to
361 move between bacterial genomes it may also explain their ubiquity in swine gut metagenomes. It
362 is possible that some of the contigs with ARGs may have been binned incorrectly given the
363 difficulties in assembly and binning of ARGs discussed above. However, many of the ARGs
364 were found in MAGs that were closely related to the known species range for the ARG. The use
365 of long-read sequencing would likely increase the number of ARGs binned as well as improve
366 the resolution of their genomic context.

367 We also evaluated the presence of the 1,150 MAGs from the present study within 626
368 swine gut metagenomes that were publicly available. Sequences aligning to 96 MAGs were
369 identified in 90% or greater of all these samples and included 8 MAGs that were classified as
370 *Dysosmobacter* spp. and 19 as *Sodaliphilus* sp004557565. *Dysosmobacter* is a new genus most
371 closely related to *Oscillibacter* (54), thus explaining the absence of previous reports of this genus
372 in the swine gut microbiome. The type species of this genus, *Dysosmobacter welbionis*, has
373 recently been shown to partially protect against some of the negative effects of a high-fat diet
374 when administered to mice (55). Similar to *Dysosmobacter*, *Sodaliphilus* is a newly described
375 genus whose type species, *Sodaliphilus pleomorphus*, was first isolated from pig feces. Swine-
376 derived MAGs classified as *Sodaliphilus* sp004557565 have also been recently reported (33).
377 These results suggest that members of these genera are widespread among pigs and may
378 represent previously unreported bacterial taxa.

379 **Conclusions**

380 We recovered 1,150 high-quality MAGs from fecal metagenomes of pre- and post-
381 weaned pigs. The MAGs described here demonstrate the vast potential of the pig gut microbiome
382 to degrade and metabolize various glycans and of certain members to provide beneficial SCFAs
383 to the host. In addition, the significant number of ARGs found associated with MAGs assigned
384 to bacterial species that are typically commensals in the gut, may explain why resistance to
385 macrolides and tetracyclines persists in the absence of antimicrobial selective pressure. The large
386 majority of the MAGs were assigned to poorly characterized taxa and thus, there still exists a
387 large fraction of the swine gut microbiome that has yet to be cultured. This included many
388 bacterial species that appear to be widely disseminated among pigs from different geolocations.

389 Future efforts focused on expanding the number of known bacterial species would greatly
390 improve on efforts to manipulate the gut microbiome to improve production and health.

391 **Materials and Methods**

392 **Experimental design**

393 The study design and fecal sampling were previously described in Holman et al. (11). Briefly,
394 piglets (n = 15) were assigned to be weaned at one of three ages; 14, 21, or 28 days of age. Fecal
395 swabs were collected from the piglets at d 7, 14, 21, 28, 35, 70, and 140 days of age (n = 179).
396 DNA was extracted using the QIAamp BiOstic Bacteremia DNA Kit (Qiagen, Mississauga, ON,
397 Canada) and shotgun metagenomic sequencing carried out on an Illumina NovaSeq 6000
398 instrument (Illumina Inc., San Diego, CA, USA) with a SP flowcell (2 x 250 bp) as per Holman
399 et al. 2021 (11).

400 **Bioinformatics**

401 Metagenomic sequences were trimmed (quality score < 15 over a sliding window of 4 bp;
402 minimum length of 50 bp) and sequencing adapters removed using Trimmomatic v. 0.38 (56).
403 Host sequences were removed by alignment to the *Sus scrofa* genome (Sscrofa11.1) (57) using
404 Bowtie2 v. 2.4.2-1 (58). MEGAHIT v. 1.29.0 (59) was used to co-assemble and individually
405 assemble metagenomes. Prior to co-assembly, all metagenomic samples were normalized using
406 BBNorm in BBTools v. 38.79 (<https://sourceforge.net/projects/bbmap/>). For the co-assembled
407 metagenome, the metagenomic sequences from each sample were mapped to the co-assembly
408 using Bowtie2 and for individual assemblies each sample was aligned to its own metagenomic
409 assembly. These contigs in each sample with a minimum length of 2,000 bp were then binned
410 using MetaBAT 2 (60) . These bins or MAGs were assessed for quality and completeness using
411 CheckM v. 1.1.2 (61) and those MAGs that were > 90% complete and had < 5% contamination

412 were retained. This resulted in 2,327 MAGs from the individually assembled metagenomes and
413 85,145 MAGs from the co-assembled metagenomes. These MAGs were then dereplicated using
414 dRep v. 3.2.2 (62) with primary clustering at 90% and secondary clustering at 99% ANI. These
415 1,150 MAGs were then used for all subsequent analyses.

416 Taxonomy was assigned to each MAG using GTDB-tk 2.0.0 (63) and the GTDB release
417 207. CoverM v. 0.6.1 (<https://github.com/wwood/CoverM>) (parameters: --min-read-aligned-
418 percent 75% --min-read-percent-identity 95% --min-covered-fraction 0) was used to determine
419 the relative abundance (coverage) of each MAG within in each metagenomic sample. A
420 phylogenetic tree of the MAGs was constructed from the alignment of 399 marker genes in
421 PhyloPhlAn v. 3.0.60 (64) (parameters: min_num_markers=100; f = supermatrix_aa.cfg) and
422 visualized using iTol v6. (65). DRAM v. 1.2.4 (66) together with the KEGG (release 100,
423 October 1, 2021) and dbCAN2 (67) databases was used to annotate the MAGs. The MAGs were
424 also screened for ARGs using the CARD-RGI v. 5.2.0 (68). Proksee v. 1.0.0a1
425 (<https://proksee.ca>) was used to visualize the location of the ARGs within each MAG as well as
426 potential integrases and transposases as annotated by Prokka v. 1.14.6 (69) . MaAsLin2 v. 1.8.0
427 (70) was used to identify MAGs that were differentially abundant immediately before weaning
428 and 7 days post-weaning. Only those MAGs with a relative abundance greater than 0.05% in
429 these samples were included in this analysis.

430 Publicly available metagenomic sequences from other swine gut microbiome studies
431 published since 2016 were downloaded and aligned to the MAGs in the present study with
432 CoverM to assess their presence in pigs from other studies in different geographic locations. The
433 unassembled reads as well as the MAGs from the present study are available under BioProject
434 PRJNA629856.

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686

687 **Figure legends**

688 **Figure 1.** Maximum likelihood phylogenetic tree of 1,150 MAGs based on the alignment of 399
689 marker genes in PhyloPhlAn. MAGs colored by GTBD-tk assigned phyla are labelled in the
690 inner ring. The outer ring indicates the number of carbohydrate-active enzymes (CAZymes) per
691 MAG and the outer bars display the percent relative abundance (minimum = 0%; maximum =
692 2.73%) of each MAG in the pre- and post-weaning fecal samples.

693 **Figure 2.** Metagenome-assembled genomes (MAGs) that were identified in 85% or more of all
694 samples from this study and publicly available metagenome samples. The relative abundance
695 within all of the MAGs within these samples (n = 805) is displayed as a heat map and the
696 presence of genes encoding for pathways involved in selected short-chain fatty acid and other
697 organic acid production, as well as polysaccharide degradation (carbohydrate-active enzymes
698 [CAZymes]) is indicated by a dot. The total number of MAGs (n = 1,150) that encode these
699 pathways are displayed on the top of the plot.

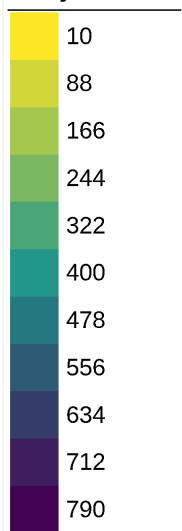
700

701

702

Tree scale: 1

CAZyme count



Relative abundance

