

1 **Title:** *Prevotella copri*, a potential indicator for high feed efficiency in western steers.

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21 **Abstract:**

22 There has been a great interest to identify a microbial marker that can be used to predict
23 feed efficiency of beef cattle. Such a marker, specifically one that would allow an early
24 identification of animals with high feed efficiency for future breeding efforts, would facilitate
25 increasing the profitability of cattle operations and simultaneously render them more sustainable
26 by reducing their methane footprint. The work presented here suggests that *Prevotella copri*
27 might be an ideal microbial marker for identifying beef cattle with high feed efficiency early in
28 their life span and in the production cycle. Developing more refined quantification techniques
29 that allow correlation of *P. copri* to feed efficiency of beef cattle that can be applied by lay
30 people in the field holds great promise to improve the economy of cattle operations while
31 simultaneously reducing their environmental impact by mitigating methane production from
32 enteric fermentation.

33

34 **Key words:** animal microbiome, cattle, fecal microbiome, feed efficiency, microbial ecology,
35 molecular marker

36

37 INTRODUCTION

38 Beef cattle is a significant source of protein in industrialized countries and feed
39 constitutes up to 70% of the cost associated with meat production (1). In order to optimize these
40 costs, there has been a great effort to identify and breed animals that possess higher feed
41 efficiency. A common and widely accepted method of quantifying feed efficiency is to determine
42 residual feed intake (RFI), which is a measure of the difference between an animal's expected
43 and actual feed intake (2) and is negatively correlated with the feed efficiency of an animal.
44 Whereas methods for determining the RFI are well established, the biological and biochemical
45 parameters that determine an animal's RFI and therefore its feed efficiency phenotype are still
46 poorly understood.

47 The rumen is host to a complex consortium of archaea, bacteria, fungi, and protozoa that
48 work synergistically to degrade plant material and generate metabolites that deliver up to 80% of
49 the energy of the host animal (3). Host genetics and diet impact the rumen microbiome of
50 ruminant animals (4, 5) and even changes in the assemblage of the fiber-adherent population in
51 response to the accumulation of metabolic intermediates has been reported (6). It has also been
52 proposed that feed efficiency is linked to the host genetics and the microbiome assemblage
53 within the gastrointestinal system of the animals (4, 7). Findings from studies investigating the
54 correlation between microbiome members vary, which is not surprising since the experimental
55 setup and analyses usually differ on multiple levels. A recent study focusing on the feed
56 efficiency of dairy cows reported an inverse relationship between bacterial richness and feed
57 efficiency and an increased abundance of *Megasphaera* sp in the highly efficient animals (8). A
58 positive correlation ($p < 0.02$) between *Eubacterium* sp. and low feed efficiency was reported by

59 Hernandez-Sanabria *et al.* for Hereford ×Aberdeen Angus steers that were fed a high energy diet
60 (9), and Jami *et al.* reported a positive correlation between RFI and the uncultured rumen
61 bacterium *RF39* [$R = 0.51$, $p = 0.055$,(10)]. Taxonomic markers at the family level associated
62 with low efficient steers were identified recently (11), but the authors were not able to identify a
63 taxonomic marker at higher resolution which could be used to predict the feed efficiency of the
64 animals.

65 Since a microbial marker with sufficient resolution to enable a quick classification of
66 animals into either feed efficiency category would be of great value to the cattle industry and
67 have a direct positive impact on the environment by reducing the negative impact of cattle
68 operations on methane emission, as well as reducing other waste products that are associated
69 with maintaining large numbers of cattle, we investigated if such a marker would exist within
70 samples found further along the gastrointestinal tract. Although collecting rumen fluid from
71 fistulated cows or via tubulation is performed on a regular basis for scientific purposes, it is
72 either not possible or practical on a cattle operation for the purpose of assigning feed efficiency
73 to every animal that is considered for breeding. Since microbial community dynamics in
74 response to changing diets in the reticulum have been reported to accurately reflect shifts within
75 the rumen microbiome (12), we assessed if the fecal microbiome might contain microbial marker
76 taxa that could be used for assigning feed efficiency to steers without investing significant
77 amounts of resources. To identify potential microbial markers associated with feed efficiency
78 phenotypes, we explored the fecal microbiome of low and high efficiency steers (based on RFI
79 scores) by targeting the V4 region of the 16S rRNA gene.

80

81 **METHODS**

82

83 **Feed Trial**

84 A feeding trial involving 98 beef steers born fall 2014 was performed at UC Davis, CA. Animal
85 procedures were performed under an animal care protocol IACUC # 17888. Residual feed
86 efficiency was determined for each animal during a period of 56 days (07/06-08/31/15). Animals
87 were fed *ad libitum* a diet composed of 69.8% flaked corn grain, 7.9% corn distillers dried
88 grains, 5.9% wheat hay, 5.9% sudan hay, 5.6% liquid molasses, 1.17% urea, 1.8% yellow grease,
89 0.13% magnesium oxide, 1.42% calcium carbonate, 0.32% trace mineral salt, and 0.015%
90 Rumensin® (Elanco, Greenfield, IN). On August 31st, 36 animals were identified as either high
91 or low RFI performers and will henceforth be referred to as low and high-efficiency steers
92 respectively. Feed trial-meta data for sampled animals is provided in Supplementary Table S1.

93

94 **Sampling**

95 Fecal samples were collected from 3 high efficiency and 3 low efficiency steers.
96 Approximately 50 grams of fecal material was collected manually from 10-20 cm in the rectum
97 and immediately flash frozen in liquid nitrogen. Samples were transported to the laboratory and
98 stored at -20°C until further analysis.

99

100 **DNA extraction**

101 DNA extraction was performed using the FastDNA SPIN Kit for Soil (MP Biomedicals,
102 Solon, OH) according to the manufacturer's protocol.

103

104 **PCR amplification, library preparation, and sequencing**

105 The V4 region of the 16S rRNA gene was amplified using the 515F/806R primer set (13).
106 For sequencing, forward and reverse sequencing oligonucleotides were designed. A detailed
107 description of these oligonucleotide constructs and the conditions of the PCR reaction for
108 amplification of the target region is provided in the Technical Appendix. Amplicon quantity was
109 determined using a Qubit instrument with the Qubit High Sensitivity DNA kit (Invitrogen,
110 Carlsbad, CA). Individual amplicon libraries were pooled, cleaned with Ampure XP beads
111 (Beckman Coulter, Brea, CA), and sequenced using a 250 bp paired-end method (14) on an
112 Illumina MiSeq at the DNA Technologies Core in the UC Davis Genome Center. Raw sequence
113 reads were submitted to NCBI's SRA under project accession number: PRJNA420377.

114

115 **Sequence Analysis**

116 Forward and reverse sequencing oligonucleotides were designed to contain a unique 8 nt barcode
117 (N), a primer pad (underlined), and the Illumina adaptor sequences (**bold**). Each sample was
118 barcoded with a unique forward and reverse barcode combination. (Forward primer construct:

119 **AATGATACGGCGACCACCGAGATCTACAC**-NNNNNNNN-TATGGTAATT-

120 GTGCCAGCMGCCGCGGTAA; reverse primer construct:

121 **CAAGCAGAAGACGGCATAACGAGAT**-NNNNNNNN-AGTCAGTCAG-

122 GGACTACHVGGGTWTCTAAT.) Barcode combinations for each sample are provided in

123 Supplementary Table S2. Each PCR reaction contained 1 Unit Kapa2G Robust Hot Start

124 Polymerase (Kapa Biosystems, Boston, MA), 1.5 mM MgCl₂, 10 pmol of each primer, and 1μL

125 of DNA. The PCR was performed using the following conditions: 95°C for 2 min, followed by
126 30 cycles of 95°C for 10 s, 55°C for 15 s, 72°C for 15 s and a final extension of 72°C for 3 min.
127 Sequencing resulted in a total of 298,445 reads, which were analyzed using Mothur v1.39.5
128 following the MiSeq SOP accessed on 09/01/2017. Using the *make.contigs* command, raw
129 sequences were combined into contiguous sequences, which were filtered using *screen.seqs* to
130 remove sequences that were >270 bp or contained ambiguous base calls to reduce PCR and
131 sequencing error. Duplicate sequences were merged with *unique.seqs*, and the resulting unique
132 sequences were aligned to the V4 region of the SILVA SEED alignment reference v123 (15)
133 using *align.seqs*. Sequences were removed if they contained homopolymers longer than 8 bp or
134 did not align to the correct region in the SILVA SEED alignment reference using *screen.seqs*. To
135 further de-noise the data, sequences were pre-clustered within each sample allowing a maximum
136 of 3 base pair differences between sequences using the *pre.cluster* command. Finally, chimeric
137 sequences were identified using VSEARCH (16) and removed.

138 Quality filtered sequences were grouped into operational taxonomic units (OTUs) based
139 on 97% sequence identity and classified using the Bayesian classifier and the Greengenes
140 database (August 2013 release of *gg_13_8_99*) (17) with *classify.seqs*. Sequences that classified
141 as mitochondria, chloroplasts, eukaryotes, or of unknown origin were removed using
142 *remove.lineage*. Samples were rarefied to 7,012 sequences per sample, the smallest number of
143 sequences across all collected samples. After pooling samples by feed efficiency, singleton and
144 doubleton abundances were calculated with *filter.shared*. Chao1 diversity indices (18), Good's
145 coverage (19), Shannon indices (20), and inverse Simpson indices were calculated using
146 *summary.single* to quantify coverage and alpha diversity for individual and pooled samples.

147 Analysis of molecular variance (AMOVA) (21) was used to identify significant differences in
148 community structure between feed efficiency phenotypes using the θ_{YC} distance matrix for the
149 *amova* command (22), while linear discriminant analysis (LDA) effect size (LEfSe) (23) was
150 used to identify indicator taxa that were significantly enriched in their respective groups.

151

152 **Statistical analysis**

153 A two-tailed t-test was used to determine differences in diversity indices between high
154 and low efficiency groups via SigmaPlot (SigmaPlot 11.0, Systat Software, Inc. San Jose, CA).
155 Significant differences were defined as $p < 0.05$.

156

157 **RESULTS**

158 **Sequencing and Quality Filtering**

159 A total of 298,445 reads were generated from the fecal samples of 6 steers, with a mean
160 of 21,170 reads per sample. After quality filtering and pooling the remaining 127,061 high
161 quality reads into the two efficiency (high and low) groups, OTU based analysis (at 97%
162 sequence identity) revealed 2,201 unique OTUs across the 6 samples. Of these 2201 OTUs,
163 1,510 (68.6%) were found in Low-efficiency steers and 1,471 (66.8%) were found in High-
164 efficiency steers. Low and High-efficiency steers contained 730 and 691 exclusive OTUs
165 respectively. Low and High-efficiency steers contained 469 and 534 singletons, and 224 and 234
166 doubletons, respectively. Singletons contributed to 0.56% (Low-efficiency) and 1.06% (High-
167 efficiency) and doubletons contributed 0.27% and 0.53% of the quality-filtered reads
168 respectively (Table 1).

169 **Table 1: Statistics of 16S rRNA gene data.** Data of steers were pooled based on their feed
 170 efficiency with n = 3 for each efficiency group.

	Low-efficiency	High-efficiency	Total
Raw Reads	191,530 (100%)	106,915 (100%)	298,445 (100%)
Quality Filtered Reads	82,851 (43%)	44,172 (41%)	127,061 (42%)
Doubletons	224	234	
Singletons	469	534	
Observed OTUs	1,510	1,471	2,201
Unique OTUs	730	691	
Shared OTUs			780
Good's Coverage	97%	97%	

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174 **Alpha and Beta Diversity Analysis**

175 To estimate the microbial diversity within each group, rarefaction analyses were
 176 performed (Supplementary Fig. S1) and species richness as well as diversity indices were
 177 calculated (Table 2). No difference in Chao1, inverse Simpson or Shannon indices were detected
 178 ($p > 0.05$) between low and high efficiency groups. Good's coverage estimates were $\geq 97\%$ in all
 179 samples (Table 2), suggesting that sequencing efforts were sufficient to recover a large
 180 proportion of the microbial diversity in each of the samples under investigation.

181 **Table 2:** Diversity indices of 16S rRNA gene sequences from
 182 animals of low and high efficiency groups (n = 3 for each group)

	Mean (SD)	
	Low-efficiency	High-efficiency
Chao1	748.94 (30)	739.15 (33)
Shannon	4.15 (0.19)	3.9 (0.54)
Simpson-1	14.16 (2.82)	13.37 (7.8)

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187 Variance of the microbial community between and among low and high efficiency steers
 188 were quantified using a θ_{YC} distance matrix (22) and subsequent AMOVA and HOMOVA

189 analyses. The microbial community did not differ significantly between the two groups of steers
190 (AMOVA, $p > 0.05$), and the variance between the two groups was not significantly different
191 (HOMOVA, $p > 0.05$).

192
193 **Microbial Community Structure**

194 Across all samples, 2,201 OTUs were classified into one archaeal and 21 bacterial phyla.
195 The 10 most abundant phyla recruited >98% of the reads generated from the microbial
196 communities of both low and high efficiency groups (Fig. 1). *Firmicutes* dominated the
197 microbial communities of both phenotypes and recruited on average 67.3% ($\pm 6\%$) and 51%
198 ($\pm 27\%$) of the reads in the low and high efficiency groups respectively. *Bacteroidetes* was the
199 second most dominant phylum in both groups with a similar average percentage of 20% ($\pm 5\%$)
200 and 19.3% ($\pm 3\%$) of the total reads. The remaining sequences from the low and high efficiency
201 groups were assigned to the *Proteobacteria* [0.4% ($\pm 0.08\%$) and 5% ($\pm 7\%$)], *Tenericutes*
202 [0.5% ($\pm 0.32\%$) and 0.3% ($\pm 0.18\%$)], *Euryarcheota* [3% ($\pm 4\%$) and 3% ($\pm 4\%$)], *Actinobacteria*
203 [0.5% ($\pm 0.42\%$) and 0.6% ($\pm 0.37\%$)], *Spirochetes* [4.6% ($\pm 6\%$) and 2.4% ($\pm 3\%$)] and
204 *Verrucomicrobia* [0.7% ($\pm 1\%$) and 0.8% ($\pm 0.7\%$)]. *Fusobacteria* were largely associated with the
205 high efficiency phenotype, with an average of 16% of the total reads, with an increased
206 proportion (46%) of reads in one subject (animal #61) of the high efficiency phenotype. This
207 portion of reads was attributed to a single OTU that was classified as *Cetobacteria somerae*. The
208 average relative abundance of *Proteobacteria* was higher in the high efficiency group recruiting
209 0.4% ($\pm 0.08\%$) and 5% ($\pm 7\%$) in low and high efficiency animals respectively. The amplicon
210 library of animal #61 drove this difference as well, with *Proteobacteria* representing 13% of the

211 total reads from this sample. The differential abundance of proteobacteria in this sample can also
212 be attributed to a single OTU, classified to the *Succinovibrionaceae* family.

213

214 **Indicator taxa**

215 Overall, 24 taxa at various phylogenetic levels were differentially abundant in their
216 respective samples (LDA scores >2.0 , p value ≤ 0.05 , Table 3). To facilitate data interpretation
217 and subsequent discussion we focused on the most highly enriched taxa, reporting only taxa with
218 LDA scores >3 . Four OTUs with LDA scores >3 were identified as indicators of the low
219 efficiency group and three were identified in the high efficiency group. An OTU in the
220 *Ruminococcaceae* family generated the highest LDA score (4.67) in the low efficiency group
221 with a mean read percentage of 23.5% ($\pm 1.6\%$) compared to the high efficiency group with 14%
222 ($\pm 5.4\%$) of the total reads. An OTU that mapped to the genus *Clostridium* of the *Firmicutes*
223 phylum was assigned the second highest LDA score within the low efficiency group (3.63)
224 accounting for an average of 1.32% ($\pm 0.6\%$) of the reads for the low efficiency group and 0.5%
225 ($\pm 0.05\%$) of the high efficiency reads. The remaining two OTUs that generated LDA scores >3
226 and classified as indicators of the low efficiency group were: An OTU that classified to the
227 *Oscillispira* genera [LDA score = 3.31, low efficiency group abundance = 0.48% ($\pm 0.12\%$),
228 High-efficiency = 0.27% ($\pm 0.14\%$)] and an OTU that was classified to the *Bacteriodaceae*
229 family [LDA score = 3.01, low efficiency abundance = 0.44% ($\pm 0.23\%$), low efficiency group
230 abundance = 0.31% ($\pm 0.21\%$)]. Three OTUs with LDA scores >3 were more abundant in the
231 high efficiency group. *Prevotella copri* was assigned an LDA score of 4.14 and on average,
232 accounted for 3% ($\pm 0.6\%$) of the reads in the high efficiency group, and 0.14% ($\pm 0.02\%$) in the

233 low efficiency group. An OTU classified as member of the genus *Prevotella* had the second
 234 highest LDA score within the high efficiency group [LDA score = 3.32, high efficiency group
 235 abundance = 0.53% ($\pm 0.2\%$), low efficiency abundance = 0.35% ($\pm 0.2\%$)]. The Last OTU in the
 236 high efficiency group with an LDA score >3 classified to the *Paraprevotella* genus [LDA score
 237 = 3.28, high efficiency = 0.09% ($\pm 0.09\%$), low efficiency = 0.07% ($\pm 0.07\%$)]

238 **Table 3.** Operational Taxonomic Units (OTUs) identified as indicator taxa for low and high
 239 efficiency group based on linear discriminant analysis

OTU	Taxonomic Classification	LDA Score	% of Total Reads (\pm STD)		Efficiency Group
			Low-efficiency	High-efficiency	
Otu0001	f__Ruminococcaceae_unclassified(100)	4.67	23.56 (1.6)	14.22 (5.4)	Low
Otu0015	g__Prevotella; s__copri(100)	4.14	0.14 (0.02)	3.01 (0.6)	High
Otu0016	g__Clostridium_unclassified(60)	3.63	1.33 (0.63)	0.46 (0.06)	Low
Otu0028	g__Prevotella_unclassified(100)	3.32	0.35 (0.20)	0.53 (0.2)	Low
Otu0034	g__Oscillospira_unclassified(100)	3.31	0.48 (0.12)	0.27 (0.14)	Low
Otu0114	g__Paraprevotella_unclassified(100)	3.28	0.07 (0.07)	0.09 (0.09)	High
Otu0032	f__Bacteroidaceae_unclassified(100)	3.01	0.44 (0.23)	0.31 (0.21)	Low
Otu0128	f__Lachnospiraceae_unclassified(77)	2.98	0.06 (0.02)	0.10 (0.14)	High
Otu0087	g__Ruminococcus; s__bromii(99)	2.93	0.18 (0.01)	0.15 (0.07)	Low
Otu0109	g__5-7N15_unclassified(100)	2.78	0.14 (0.06)	0.10 (0.06)	Low
Otu0170	g__Prevotella_unclassified(100)	2.78	0.09 (0.05)	0.02 (0.02)	Low
Otu0191	g__Dorea_unclassified(99);	2.71	0.05 (0.03)	0.05 (0.03)	Low
Otu0180	g__Ruminococcus_unclassified(100)	2.67	0.10 (0.05)	0.02 (0.02)	Low
Otu0240	f__Lachnospiraceae_unclassified(100)	2.64	0.03 (0.02)	0.06 (0.05)	High
Otu0164	f__Lachnospiraceae_unclassified(100)	2.59	0.10 (0.04)	0.05 (0.03)	Low
Otu0236	C__L635J-21_unclassified(100)	2.53	0.01 (0.01)	0.13 (0.11)	High
Otu0264	g__Oscillospira_unclassified(100)	2.51	0.02 (0.004)	0.05 (0.03)	Low
Otu0417	f__Ruminococcaceae_unclassified(100)	2.40	0 (0)	0.02 (0.02)	Low
Otu0280	f__Lachnospiraceae_unclassified(98)	2.34	0.08 (0.04)	0.005 (0.004)	Low
Otu0267	g__Anaerovorax_unclassified(100)	2.34	0.06 (0.03)	0.01 (0.005)	High
Otu0310	f__Lachnospiraceae_unclassified(85)	2.31	0.03 (0.02)	0.01 (0.01)	Low
Otu0552	g__Prevotella_unclassified(100)	2.18	0 (0)	0.05 (0.05)	Low
Otu0487	g__Anaeroplasmata_unclassified(100)	2.16	0.005 (0.005)	0 (0)	Low
Otu0382	p__Bacteroidetes_unclassified(100)	2.08	0.02 (0.01)	0.01 (0.01)	Low

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242 DISCUSSION

243 Results obtained from 16S rRNA-based amplicon sequencing suggests that the fecal
 244 microbiomes of steers with low and high feed efficiency are not significantly different at the
 245 community level ($p > 0.05$). This coincides with the previous observations by Myer et al (2015)

246 who investigated the fecal microbiome of 32 steers classified by a Cartesian coordinate system of
247 average daily gain and average daily feed intake (24). Taking our findings and results from
248 previous studies into consideration, it appears 16S rRNA amplicon sequencing does not lend
249 itself as a rapid screening tool to classify individual animals regarding their feed efficiency at the
250 phylum level. However, when comparing rumen samples of steers with high and low feed
251 efficiency at the family and genus level, differences in their microbiome assemblage can be
252 detected (24, 25). Although these differences might be of great value to the scientific community
253 for efficiency related studies, acquiring rumen samples from a large number of animals and
254 performing time consuming 16S rRNA sequencing appears unlikely to be an option for the
255 average cattle producer who needs reliable information regarding the feed efficiency of an
256 individual animal within a few days if not even within hours. Microorganisms classified at the
257 species level could provide such a screenable marker, if they are enriched significantly enough in
258 one of the efficiency groups and found in sufficient abundance in the feces of a steer to warrant
259 detection via basic but still reliable molecular techniques such as qPCR. Our data indicate that
260 *Prevotella copri* was enriched in the fecal microbiome of high-efficiency steers. This data
261 suggests that *P. copri* might be a potential marker for increased feed efficiency in the feces of
262 beef cattle. It should be noted that while Shabat et al (2016) did not specifically associate *P.copri*
263 with high efficiency dairy cattle, sequencing data from high efficiency animals contained reads
264 derived from *P. copri* (11). The finding that *Prevotella copri* is associated with animals of high
265 feed efficiency is not surprising, since *P. copri* is capable of utilizing a wide variety of
266 carbohydrates, and has been identified before as potential key factor in shaping gut function and
267 host health. In a study encompassing both humans and mice, *P.copri* was shown to enhance the

268 ability to utilize complex polysaccharides (26), increase glycogen storage capability, augment
269 glucose tolerance in mice and to be associated with insulin resistance (27, 28), a major factor in
270 weight gain. Also Myer et al (24) detected a significant enrichment of *P. copri* in fecal samples
271 of steers with high daily gain and intake, but the relative abundance of *P. copri* within the
272 microbiome from these steers was reflecting the relative abundance (<0.01%) we observed in
273 steers with low feed efficiency. This might be attributed to the choice of primer set that was used
274 to generate the data or to the fact that steers deemed high efficiency would have been classified
275 as low efficiency animals in our study. Here we utilized primers targeting the V4 region, which
276 have shown to enable microbiome profiles at an increased resolution (29) and improved
277 reproducibility (30). Our analysis revealed a 20-fold increase in the abundance of *P. copri*, with
278 0.14% and 3% of the reads from the low and high feed efficient microbiomes respectively.
279 Recently, it was shown that the methods for DNA preparation have a significant affect on
280 microbiome data (31). Specifically, the kit utilized for the study presented here had a positive
281 association with the Prevotellaceae family, which may have aided in the identification of *P.*
282 *copri*.

283 In addition to increasing the profitability of a cattle operation from the standpoint of
284 increased weight gain per unit of feed, increased feed efficiency also decreases the
285 environmental footprint of a cattle operation by reducing methane production and release from a
286 feed efficient animal due to reduced dry matter intake (32). The exact molecular mechanisms of
287 reduced methane emission in more efficient ruminants are still not fully understood, but it is
288 possible that hydrogen redistribution plays a significant role, since methane production can be
289 depleted in the presence of a competing hydrogen sink such as the volatile fatty acid propionate

290 (33). Some *Bacteroidetes*, like *P. copri*, produce succinate, the precursor to propionate, as their
291 predominate fermentation byproduct, which in addition to its potential impact on
292 gluconeogenesis (27), may inhibit methane synthesis. Whether animal genetics impact the
293 relative abundance of this organism is yet to be determined. Future work investigating the
294 abundance dynamics of *P.copri* throughout the life of animals with different genetic
295 backgrounds may provide valuable data to fill this knowledge gap. It would be beneficial to
296 develop approaches that would allow the classification of animals with low or high feed
297 efficiency in an early life stage so that informed decisions could be made regarding breeding
298 practices. This study characterizes *Prevotella copri* as a potential indicator of feed efficiency
299 from the fecal microbiome of western steers and should stimulate directed research into the
300 dynamics of this organism through the life of beef cattle as well as the fecal microbiome as a
301 whole.

302
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306

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395 **Figure 1: Phylum level composition of fecal microbiome**

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