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Short communication: Signs of host genetic regulation in the microbiome composition in 2 dairy breeds: Holstein and Brown Swiss

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ABSTRACT

This study aimed to evaluate whether the host genotype exerts any genetic control on the microbiome composition of the rumen in cattle. Microbial DNA was extracted from 18 samples of ruminal content from 2 breeds (Holstein and Brown Swiss). Reads were processed using mothur (https://www.mothur.org/) in 16S and 18S rRNA gene-based analyses. Then, reads were classified at the genus clade, resulting in 3,579 operational taxonomic units (OTU) aligned against the 16S database and 184 OTU aligned against the 18S database. After filtering on relative abundance (>0.1%) and penetrance (95%), 25 OTU were selected for the analyses (17 bacteria, 1 archaea, and 7 ciliates). Association with the genetic background of the host animal based on the principal components of a genomic relationship matrix based on single nucleotide polymorphism markers was analyzed using Bayesian methods. Fifty percent of the bacteria and archaea genera were associated with the host genetic background, including *Butyrivibrio*, *Prevotella*, *Paraprevotella*, and *Methanobrevibacter* as main genera. Forty-three percent of the ciliates analyzed were also associated with the genetic background of the host. In total, 48% of microbes were associated with the host genetic background. The results in this study support the hypothesis and provide some evidence that there exists a host genetic component in cattle that can partially regulate the composition of the microbiome.
Key words: genomic, Holstein, microbiome, metagenome

Short Communication

Research interest in the microbiome and its effects on complex traits in both humans (Huttenhower et al., 2014; Waldor et al., 2015) and livestock (Jewell et al., 2015; Malmuthuge and Guan, 2016) has increased recently. The microbiome plays an important role in the phenotypic expression of many traits such as feed efficiency, disease status, and methane emission (Zhang et al., 2007). Traditionally, microbes have been studied in the laboratory without considering the interaction with the host or how they can modulate the expression of complex traits such as gut metabolism or disease incidence. In the particular case of livestock, the traits of interest are usually related to productive, health, or environmental factors. In the last decade, more attention has been placed on the interactions between microbes and diets (Saro et al., 2012, 2014; Mohammed et al., 2014; Henderson et al., 2015), methane emissions (Deng et al., 2008; Yáñez-Ruiz et al., 2010; Hayes et al., 2013; Wallace et al., 2015; Wadhwa et al., 2016), and the microbiome composition across hosts, environment, and age (Henderson et al., 2015; Jewell et al., 2015; Wang et al., 2016). The microbiome has also been proposed as a predictor of complex traits (Ross et al., 2013; Wallace et al., 2015).

Therefore, there is interest in determining whether a host genetic control exists that determines the microbiome composition. Recent studies show some evidence that supports the hypothesis that there is some sort of host control over the composition of the microbiome in mammals. For instance, Weimer et al. (2010) reported that after a near-total exchange of ruminal contents, the ruminal bacterial composition returned to a status similar to that prior to the exchange. More recently, Roehe et al. (2016) showed differences between sire progeny groups on the archaea:bacteria ratio in Aberdeen Angus and Limousin cattle breeds, and Goodrich et al. (2016) reported heritabilities greater than 0.20 for the relative abundance (**RA**) of several microbes in a twin human study.

The host genetic control of the ruminal microbiome composition could be used in breeding programs to select individuals with a favorable microbiome compo-

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sition for a given breeding goal, such as the reduction of enteric methane yield or the improvement of feed efficiency. The objective of this study was to determine whether genetic differences between Holstein and Brown Swiss cows exist for the RA of the rumen microbiome.

This trial was carried out in accordance with Spanish Royal Decree 53/2013 for the protection of animals used for experimental and other scientific purposes. In this study, ruminal content was sampled from 18 dairy cows (10 Holstein and 8 Brown Swiss) from Fraisoro Farm School (Zizurkil, Gipuzkoa, Spain). These cows were undergoing a nutrition experiment. They were randomly assigned to 1 of 2 concentrate supplement treatments. Both breeds were fed both diets in a balanced design in which half the cows from each breed were assigned to each treatment. Concentrates were formulated to contain cold-pressed rapeseed cake or palm as fat sources and to provide equal amounts of CP, energy, and fat. Details on the feed formulation are provided in Supplemental Table S1 (https://doi.org/10.3168/jds.2017-13179).

Rumen content was sampled 4 times within 48 h from each cow to cover the whole circadian cycle (0000 h on d 1, 1200 h on d 1, 0600 h on d 2, and 1800 h on d 2). Ruminal samples were collected from each dairy cow using a stomach tube connected to a mechanical pumping unit. About 100 mL of ruminal extraction from each of the 4 time points was placed in a unique container per animal to obtain a single mixed sample per animal. All samples were frozen immediately after the extraction and then stored at -20 ± 5°C until analysis.

Then, all samples were gradually thawed overnight at refrigeration temperature (5 ± 3°C), mixed, and squeezed through 4 layers of sterile cheesecloth to separate solid (solids with a particle size smaller than the diameter of the tube) from liquid digesta phases. This latter phase was subsequently separated into planktonic organisms and bacteria associated with the liquid fraction. The solid phase was separated into associated and adherent fractions. Fractionation procedures were carried out following the methodology described in Yu and Foster (2005). The 4 fractions were lyophilized and composited to obtain a unique sample with the 4 fractions represented proportionally (on a DM basis).

After composition, DNA extraction was performed using the commercial Power Soil DNA isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA) following the manufacturer's instructions. The extracted DNA was subjected to paired-end Illumina sequencing of the V4 hypervariable region of the 16S rRNA (Caporaso et al., 2011) and of the V7 region of the 18S rRNA genes. The libraries were generated by means of a Nextera kit (Illumina Inc., San Diego, CA). The 250-bp paired-end sequencing reactions were carried out on a MiSeq platform (Illumina Inc.).

Sequence data were processed using mothur version 1.38.1.1 (Schloss et al., 2009; Kozich et al., 2013). Sequences below 220 bp in length and Phred score below 20 were discarded. After joining forward and backward reads, 3,261,168 and 3,431,242 reads remained for the analysis from the 16S and 18S rRNA regions, respectively. Chimeras and unique sequences were removed. Sequences were then preclustered and finally classified using the default method (Wang et al., 2007) on *classify.seqs()* using the GreenGenes database (May 2013 version; http://greengenes.secondgenome.com/) for the bacterial and archaeal 16S rRNA genes, whereas protozoal and fungi 18S rRNA genes were aligned against the 18S SILVA database (March 2015 version; https://www.arb-silva.de/). The reads clustered to 3,579 operational taxonomic units (OTU) after being aligned against the 16S database and to 184 OTU against the 18S SILVA database. Data were summarized at the genus level, with 287 known genera for 16S (Supplemental Table S2; https://doi.org/10.3168/jds.2017-13179) and 49 genera for fungi and protozoa (Supplemental Table S3; https://doi.org/10.3168/jds.2017-13179). The RA of genera in each animal was calculated after excluding those genera that appeared in <0.1% proportion in both breeds and in at least 17 animals. In total, 18 bacteria and archaea genera and 7 ciliate genera were kept for final analyses.

Genotypes from animals under study were also obtained with the Illumina 9K chip (Illumina Inc.). A total of 9,146 SNP with call rate >95% and minor allele frequency >0.05 in the whole genotyped Spanish population were kept (data from more than 3,000 individuals provided by the Spanish Holstein association CONAFE; Madrid, Spain). Less than 2% of SNP were not under Hardy–Weinberg equilibrium but still remained in the analyses.

The background genetic effect was analyzed for each of the ruminal microbes selected with their RA as a dependent covariate (phenotype). A logarithmic transformation $[1 + \ln(x)]$ was applied if the phenotypic distribution of microbial RA did not visually approximate to a Gaussian distribution. The statistical model was adjusted by diet treatment (2 groups: with or without cold-pressed rapeseed cake), age (primiparous or multiparous) groups, and DIM as a covariate. It also included the first 2 principal components (**PC**) of a genomic relationship matrix that contains the genomic relationship between individuals i and j (Yang et al., 2011), which was calculated as