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Characterization of two divergent lines through functional inference of ruminal microbiota

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The ruminal microbiota plays an important role in the nutrition of its host, having a direct impact on milk production and animal health. However, as the links between bacteria abundances and host's traits are often difficult to interpret, we proposed to infer the abundances of bacteria functions to characterize divergent lines on somatic cell score (SCS) or on milk persistency (PERS). From 2015 to 2019, we sampled the rumen juice of 700 adults dairy Lacaune ewes belonging to either SCS line (94 SCS+/204 SCS-) or PERS line (200 PERS+/202 PERS-). DNA was extracted from ruminal juice and sequenced for the 16s rRNA gene. We analyzed microbiota sequences with FROGS pipeline to obtain relative abundances of bacteria and then to infer functions. The inference consisted of using PICRUSt2 bioinformatic approach to predict the functional composition of a metagenome using marker gene sequences. For the 700 ewes, abundances of 2059 ASVs were computed. After filtering to ensure a good quality of inference (Nearest Sequenced Taxon Index<0.4 and identity/coverage percentage>90%), abundances of 1146 ASVs were kept enabling us to quantify abundances of 330 bacterial functions. A mixed model was computed on the function's abundances (after GBM imputation and CLR transformation to consider their compositional nature) within SCS and PERS lines respectively, with fixed effects such as the line, run of sequencing, number of sequences, date of sampling, lactation stage and, for SCS line only, litter size. This analysis revealed functions significantly different for either the SCS (n=14) or PERS (n=60) lines ($pval<0.05$), responsible for branched amino acids (SCS) or glycan/lipopolysaccharide (PERS) biosynthesis. Finally, a sparse PLS discriminant analysis (with MixOmics R package) was performed on residual of functional abundances obtained from the previous model (without line effect), considering the divergent line as discriminant factor. While balanced error rates (BER) were poor for both SCS (0.52 ± 0.08) and PERS (0.49 ± 0.04) lines, interesting discriminant functions, needed for vitamin (SCS) or glycan (PERS) biosynthesis, were found.