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MACHINE LEARNING ALGORITHMS FOR THE PREDICTION OF FEED EFFICIENCY BASED ON CAECAL MICROBIOTA

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ABSTRACT

This study aimed at predicting feed conversion ratio (FCR) of young rabbits from abundances of amplicon sequence variants (ASVs) to improve this trait by selecting animals with the most favorable microbiota and identifying the most relevant microorganisms involved in feed efficiency. Data come from two rabbit populations coming from paternal INRA 1001 line (the G10, selected for 10 generations for decreased residual feed intake and the G0 control produced from frozen embryos of the common ancestor line). There were 296 and 292 FCR data from G10 and G0 individuals, respectively. Phenotypic data were pre-corrected for the systematic effects of group, batch, litter size and sex and the random litter effect. Sequence quality control and chimera removal were performed with the DADA2 pipeline. Samples with less than 5,000 final sequence counts and doubleton ASV were removed. The ASV counts of the final table (including 918 ASVs) were centered log-ratio transformed and corrected for batch effects with a surrogate variable analysis. Nested resampling for hyper-parameter tuning and prediction validation was implemented leading to 25 pairs of training/test sets. Bayesian regression models (Bayesian Lasso, Bayesian Ridge Regression and Reproducing Kernel Hilbert Spaces) and machine learning algorithms (Support vector machine and Elastic net) were fitted to all ASVs leading to an almost null prediction accuracy in all cases. Then, ASVs were ranked for their prediction importance using the permutation accuracy importance score in a Random Forest algorithm based on conditional inference and, different subsets of increasing size (50, 100, 150, 200, 300, 400, 500, All) of the most important ASVs and surrogate variables were used as predictors in the machine learning algorithms. The best performance and the most stable results were obtained with machine learning using the 100 most important ASVs being most of them assigned to order Clostridiales. The medians of the Spearman correlation (interquartile range) were 0.33 (0.09) and 0.32 (0.06) for SVM and ENET, respectively.

Key words: feed efficiency, machine learning, caecal microbiota, prediction, selection.

INTRODUCTION

Rabbit gut microbiota plays an important role in production traits (Drouilhet et al., 2016) because of its effect on metabolic, nutritional, physiological, and immunological processes. Among production traits, feed efficiency (FE) is one of the most important components of productivity, profitability and sustainability of meat production and, therefore, improving this trait is a priority. One possible strategy could be to change animal’s gut microbial composition based on its effect on animal performance. In addition, recent studies indicate that gut microbiota is heritable and could be modified by selection (Velasco-Galilea et al., 2018; Crespo-Piazuelo et al., 2019). Therefore, selecting animals with the optimal microbial composition based on its effect on FE could also lead to selection of individuals with genes that promote the presence of those beneficial microorganisms. Selection would be based on the prediction of FE (previously corrected by environmental factors) obtained from high-throughput deep sequencing data of microbial composition. Machine learning (ML) algorithms can be suitable...
models because they are efficient for finding generalizable patterns from high-dimensional data in a small number of samples.

This research aimed at assessing the suitability of ML algorithms for the prediction of feed efficiency from abundances of amplicon sequence variants (ASVs) and identifying the most relevant microorganisms involved.

MATERIALS AND METHODS

Animal material and experimental design.

The experimental rabbits came from the paternal INRA 1001 line. Two populations were used in this analysis: G10, selected for 10 generations for decreased residual feed intake (RFI) (Drouilhet et al., 2016), and G0 control produced from frozen embryos of the ancestor population of the selected line. The 296 G10 and 292 G0 rabbits were produced in 3 batches with a 42 days interval. In each batch, half of the kits were fostered by G0 does and the rest by G10 does. The does adopted alternatively kits from both lines in successive batches. At weaning (32 days), kits were placed in individual cages. More details about the experiment can be found in Garreau et al., (2019). Genomic DNA of caecal samples collected from 588 kits was extracted with ZR Soil Microbe DNA MiniPrep™ kit (ZymoResearch, Freiburg, Germany). A fragment containing V4-V5 hypervariable regions of the 16 rRNA gene was amplified with the pair of primers F5 15Y/R926 (Parada et al., 2016) and re-amplified in a limited-cycle PCR to add barcodes of multiplex Nextera® XT kit (Illumina, Inc., San Diego CA, United States) following the manufacturer’s instructions. Final libraries were paired-end sequenced in parallel in a MiSeq Illumina 2x250 platform at the Autonomous University of Barcelona.

Bioinformatics

Sequence processing was performed using QIIME2 software (version 2018.6; Bolyen et al., 2018). Sequence quality control and chimera removal were performed in a single step with the DADA2 pipeline (Callahan et al., 2016), implemented through the q2-dada2 plugin. The output table containing the counts of unique sequences for each sample, i.e., 100% ASVs, was clustered into ASVs with 99% similarity. The ASV table was filtered at: (1) sample level by discarding samples with less than 5,000 final sequence counts and at (2) ASV level by removing the doubleton ones. The ASV counts of the final table (including 918 ASVs) were centered log-ratio transformed using the R package “chemometrics” to account for the compositional nature of microbiota data. Taxonomic assignment of ASVs was conducted by mapping them to the Greengenes reference database.

Data and Statistical Analysis

Feed efficiency was measured as feed conversion ratio (FCR), i.e., feed intake divided by body weight gain. The statistical analysis was performed in three steps. In a first step, FCR records were pre-corrected for the systematic effects of group, batch, litter size and sex, and the random effect of litter. Then, a surrogate variable analysis (Leek and Storey, 2007) was performed using the R package “SVA” to include surrogate variables (SV) in the model of prediction which allows accounting for unnoticed factors of variation affecting ASVs abundances. In a second step, the ASVs were ranked for their predictive importance using the permutation accuracy importance score in a Random Forest algorithm based on conditional inference (Strobl et al., 2007). In the last step, different subsets of increasing size (i.e., 50, 100, 150, 200, 300, 400, 500 and 918) of the most important ASVs and SV were selected as predictors of FCR using two machine learning algorithms. Support Vector Machine (SVM; Vapnik et al., 1999) and Elastic Net (ENET; Zou and Hastie, 2005) algorithms were implemented using the “mlr” R package which allows to compare results from different algorithms under the same conditions and to find the optimal hyper-parameters for each algorithm. Nested resampling for hyper-parameter tuning was implemented. It consisted of 2 nested resampling loops. In the outer resampling loop, a 5-fold cross-validation was repeated 5 times originating 25 pairs of training/testing sets. On each of those outer training sets, hyper-parameter tuning was done in an inner resampling loop of 5-fold cross-validation repeated 2 times using the R-squared performance criterion.
One set of selected hyper-parameters was obtained for each outer training set. The learner was fitted on each training set using the selected hyper-parameters and its performance was evaluated on the corresponding testing set. Predictive ability was assessed as the Spearman correlation (SC) between the observed and predicted records in the testing sets. On the other hand, Bayesian regression models (de los Campos et al., 2013) such as Bayesian Lasso (BL), Bayesian Ridge Regression (BRR) and Reproducing Kernel Hilbert Spaces (RKHS; Gianola et al., 2006) were also implemented in the same 25 pairs of training/test sets using all ASVs and SV as predictors with “BGLR” R package (Pérez & de los Campos, 2014).

RESULTS AND DISCUSSION

Using as predictors all ASVs and SV (Figure 1, panel A), ENET was not able to fit a model because of lack of convergence and SVM had a null prediction ability with a very large variability among sets (the median and interquartile range (IQR) of the SC were -0.07 and 0.14, respectively). Predictive performance was slightly better but still very low for BL, BRR and RKHS algorithms being the median of the SC (IQR) 0.11 (0.13), 0.11 (0.13) and 0.12 (0.08), respectively. When feature selection was performed (Figure 1, panel B), the predictive performance improved significantly. The best performances and the most stable results were obtained with SVM and ENET using the 100 most important ASVs. The medians of the SC (IQR) were in this case 0.33 (0.09) and 0.32 (0.06) for SVM and ENET, respectively.

Taxonomic assignment of representative sequences revealed that most (74) of the ASVs belong to order Clostridiales. In animals with low FCR performances, 32 ASVs belonging to order Clostridiales (families Lachnospiraceae (8), Ruminococcaceae (8), Clostridiaceae (1) and unknown (15)), 6 ASVs belonging to order Bacteroidales (families Bacteroidaceae (1), Rikenellaceae (2), S24-7 (2) and unknown (1)) and 2 ASVs belonging to phylum Tenericutes (orders RF39 and ML615J-28) were overrepresented. In addition, two completely unknown ASVs were also associated with high efficient animals. For animal with high FCR, 42 ASVs belonging to order Clostridiales (families Lachnospiraceae (14), Ruminococcaceae (15) and unknown (13)), 10 ASVs belonging to order Bacteroidales (families Bacteroidaceae (3), Rikenellaceae (3), S24-7 (3) and unknown (1)), 2 ASVs belonging to phylum Tenericutes (order RF39), 2 ASVs belonging to order Verrucomicrobiales (genus Akkermansia) and 2 ASVs belonging to phylum Proteobacteria (families Oxalabacteraceae and Desulfovibrionales) were overrepresented.
CONCLUSIONS

Support Vector Machine and Elastic net algorithms enabled the best prediction of FCR when the abundances of the 100 most important ASVs were used as predictive variables. Taxonomic assignment of the representative sequences of these selected ASVs revealed that different species belonging to order Clostridiales are involved in feed efficiency.

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