Evaluation of machine learning methods for 16S rRNA gene data

Running title: Machine learning methods in microbiome studies

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Abstract

Introduction

Advances in sequencing technology and decreasing costs of generating 16S rRNA gene sequences have allowed rapid exploration and taxonomic characterization of the human associated microbiome. Currently, the microbiome field is growing at an unprecedented rate and as a result, there is an increasing demand for reproducible methods to identify associations between members of the microbiome and human health. However, this is an undertaking as human associated microbial communities are remarkably uneven. It is unlikely that a single species can explain a disease. Instead, subsets of those communities, in relation to one another and to their host, account for the differences in the health outcomes. Therefore, researchers have started to explore the utility of machine learning (ML) models that use microbiota associated biomarkers to predict human health and to understand the microbial ecology of the human diseases such as liver cirrhosis, colorectal cancer, inflammatory bowel diseases (IBD), obesity, and type 2 diabetes (1–11). However, currently the field's use of ML lacks clarity and consistency on which methods are used and how these methods are implemented. Moreover, there is a lack of deliberation on why a particular ML model is utilized. Recently, there is a trend towards using more complex ML models such as random forest, extreme gradient boosting and neural networks (11–14) without a discussion on if and how much model interpretibility is necessary for the study. If we want to generate reproducible and robust microbiota-associated biomarker models we need to (1) implement consistent, accessible and transparent machine learning practices; (2) use ML models that reflect the goal of the study as it will inform the expectations of model accuracy, complexity, interpretibility, computational efficiency and scalability.

To shed light on how much task definition and modeling choices can affect the outcomes of ML studies, we performed an empirical analysis comparing several different modeling pipelines using the same dataset. We used a previously published colorectal cancer (CRC) study (3) which had fecal 16S rRNA gene sequences and human hemoglobin concentrations from 490 patients. We built ML models using fecal 16S rRNA gene sequences and human hemoglobin concentrations to predict patients with normal or screen relevant neoplasias (SRN) disease status. The study had 261 normal and 229 SRN samples. We established modeling pipelines for L2-regularized

logistic regression, L1 and L2 support vector machines (SVM) with linear and radial basis function kernels, a decision tree, random forest and XGBoost. These models increase in complexity while they decrease in interpretability. Our ML pipeline performed 100 data-splits and utilized held-out test data to evaluate generalization and predicton performance of each ML model. The mean AUROC varied from 0.67 (std ± 0.05) to 0.82 (std ± 0.04). Random Forest and XGBoost had the highest mean AUROC for detecting SRN. Despite the simplicity, the L1-regularized linear kernel SVM performed on par with many of the non-linear models. In terms of computational efficiency, L2 Logistic Regression trained the fastest (0.105 hours, std ± 0.013), while XGBoost took the longest (82.47 hours, std ± 17.045). We found that mean cross-validation and testing AUROC could vary by as much as 0.062, which highlights the importance of a seperate held-out test set for evaluation. Aside from evaluating generalization and classification performances for each of these models, this study established standards for modeling pipelines of microbiome-associated machine learning models.

Results and Discussion

The prediction and generalization performance of classifiers during cross-validation and when applied to the held-out test data.

We evaluated the prediction performance of seven binary classification models when applied to held-out test data using AUROC as our metric. Random Forest and XGBoost had the highest mean AUROC for detecting SRN, 0.816 (std ± 0.039) and 0.814 (std ± 0.038) respectively [Figure 2]. L1 linear SVM and decision tree had significantly lower AUROC values, 0.748 (std ± 0.043) and 0.741 (std ± 0.038) [Figure 2]. However, they had significanly higher performances than L2 linear SVM, RBF SVM and L2 logistic regression which had mean AUROC values of 0.67 (std ± 0.054), 0.684 (std ± 0.049) and 0.677 (std ± 0.053) respectively [Figure 2]. We also evaluated the generalization performance of each classifier by comparing their mean cross-validation AUROC and mean testing AUROC. We found a statistically significant difference for classifiers L2 support vector machines (SVM) with linear and radial basis function kernels and L2 logistic regression. These differences were 0.062, 0.046 and 0.061, respectively [Figure 2] .

Hyperparameter optimization for each classifier.

The interpretation of classifiers.

Conclusions

Materials and Methods

Data collection

The data used for this analysis are stool bacterial abundances, stool hemoglobin levels and clinical information of the patients recruited by Great Lakes-New England Early Detection Research Network study. These data were obtained from Sze et al (15). The stool samples were provided by recruited adult participants who were undergoing scheduled screening or surveillance colonoscopy. Colonoscopies were performed and fecal samples were collected from participants in four locations: Toronto (ON, Canada), Boston (MA, USA), Houston (TX, USA), and Ann Arbor (MI, USA). Patients' colonic disease status was defined by colonoscopy with adequate preparation and tissue histopathology of all resected lesions. Patients with an adenoma greater than 1 cm, more than three adenomas of any size, or an adenoma with villous histology were classified as advanced adenoma. Study had 172 patients with normal colonoscopies, 198 with adenomas and 120 with carcinomas. Of the 198 adenomas, 109 were identified as advanced adenomas. Stool provided by the patients was used for Fecal Immunological Tests (FIT) which measure human hemoglobin concentrations and for 16S rRNA gene sequencing to measure bacterial population abundances. The bacterial abundance data was generated by Sze et al, by processing 16S rRNA sequences in Mothur (v1.39.3) using the default quality filtering methods, identifying and removing chimeric sequences using VSEARCH and assigning to OTUs at 97% similarity using the OptiClust algorithm (16–18).

Data definitions and pre-processing

The colonic disease status is re-defined as two encompassing classes; Normal or Screen Relevant

Neoplasias (SRNs). Normal class includes patients with non-advanced adenomas or normal colons whereas SRN class includes patients with advanced adenomas or carcinomas. Colonic disease status is the label predicted with each classifier. The bacterial abundances and FIT results are the features used to predict colonic disease status. Bacterial abundances are discrete data in the form of Operational Taxonomic Unit (OTU) counts. There are 6920 OTUs for each sample. FIT levels are continuous data present for each sample. Because the data are in different scales, features are transformd by scaling each feature to a [0-1] range (Table 1).

Learning the Classifier

To train and validate our model, labeled data is randomly split 80/20 into a training set and testing set. Then, seven binary class classifiers, L2 logistic regression, L1 and L2 linear suppor vector machines (SVM), radial basis function SVM, decision tree, random forest and XGBoost, are learned. The training set is used for training purposes and validation of hyperparameter selection, and the test set is used for evaluation purposes. Hyperparameters are selected using 5-fold cross-validation with 100-repeats on the training set. Since the colonic disease status are not uniformly represented in the data, 5-fold splits are stratified to maintain the overall label distribution on the training set.

Classifier Performance

The classification performance of learned classifier is evaluated on the labeled held-out testing set. The optimal classifier with optimal hyperparameters selected in the cross-validation step is used to produce a prediction for the testing set. The performance of this prediction is measured in terms of the sensitivity and specificity, in addition to Area Under the Curve (AUC) metrics. This process of splitting the data, learning a classifier with cross-validation, and testing the classifier is repeated on 100 different splits. In the end cross-validation AUC and testing AUC averaged over the 100 different training/test splits are reported. Hyperparameter budget and performance for each split is also reported.

Figure 1. Generalization and classification performance of modeling methods AUC values of all cross validation and testing performances. The boxplot shows quartiles at the box ends and the statistical median as the horizontal line in the box. The whiskers show the farthest points that are not outliers. Outliers are data points that are not within 3/2 times the interquartile ranges.

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