MetaPrism: A versatile toolkit for joint taxa/gene analysis of metagenomic sequencing data

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Abstract

In microbiome research, metagenomic sequencing generates enormous amounts of data. These data are typically classified into taxa for taxonomy analysis, or into genes for functional analysis. However, a joint analysis where the reads are classified into taxa-specific genes is often overlooked. To enable the analysis of this biologically meaningful feature, we developed a novel bioinformatic toolkit, MetaPrism, which can analyze sequence reads for a set of joint taxa/gene analyses to: 1) classify sequence reads and estimate the abundances for taxa-specific genes; 2) tabularize and visualize taxa-specific gene abundances; 3) compare the abundances between groups; and 4) build prediction models for clinical outcome. We illustrated these functions using a published microbiome metagenomics dataset from patients treated with immune checkpoint inhibitor therapy and showed the joint features can serve as potential biomarkers to predict therapeutic responses. MetaPrism is a toolkit for joint taxa and gene analysis. It offers biological insights on the taxa-specific genes on top of the taxa-alone or gene-alone analysis.

MetaPrism is open-source software and freely available at https://github.com/jiwoongbio/MetaPrism. The example script to reproduce the manuscript is also provided in the above code repository.

Keywords: metagenomics sequence analysis; joint analysis; microbiome biomarker

Introduction

The human microbiome consists of ~39 trillion bacteria and influences host health (Sender et al. 2016). Recently, the use of metagenomic sequencing has become increasingly popular as a more unbiased approach to gut microbiome profiling as compared to 16S rRNA sequencing. A common approach to comparing differences in the gut microbiome between groups (cases and controls) is to identify significant differences in either taxa or microbial genes. Several popular bioinformatic tools have been developed for this purpose, including MetaPhlAn2 (Truong et al. 2015), Kraken (Wood and Salzberg 2014), HUMAnN2 (Franzosa et al. 2018), and FMAP (Kim et al. 2016b) (Table S1). However, these tools analyze either taxonomic abundances (taxonomic profiling) or gene abundances (function profiling) separately. As each microorganism carries its own genes, taxonomic and functional profiling results are not intrinsically independent. In fact, recent discoveries demonstrated that taxon-specific genes have a causative role in disease progression and treatment responses. For example, Duan et al. found that a specific Enterococcus faecalis carrying the cytolysin gene promotes alcoholic liver disease (Duan et al. 2019). Simms-Waldrip et al. found that the antibiotic resistance genes in the graft-versus-host-disease patients are enriched for Klebsiella (Simms-Waldrip et al. 2017). Therefore, a joint analysis, where taxonomy and functional features are analyzed together, could provide useful biological and clinical insights (Langille 2018). However, bioinformatics tools for joint analyses are lacking.

Our innovation in this manuscript is to define and utilize joint taxa/gene features via bioinformatics approach, with the goal of offering biologically interpretable findings. For example, our method characterizes the genes discovered for each species. This facilitates quantitative analysis of gene abundances in a species-specific manner, which is usually not readily available. Our approach is initiated from de novo assembled contigs, which are
both taxonomically and functionally annotated. Our simulations showed this method could accurately detect bacterial species and their carried genes. In a recent review article (Langille 2018), Langille prompted that understanding the gene contents at species level can offer better interpretation than using the taxon or gene content alone, and potentially improve outcome predictions. This confirmed that the joint feature is useful for general microbiome studies. Our tool provided these joint features as the first step for a wide range of downstream analysis tasks. For example, we demonstrated that the quantity of taxa-specific gene abundances is a potentially useful biomarker to predict the immunotherapy responses.

To facilitate joint analysis, we developed MetaPrism, a novel bioinformatics tool to (1) classify metagenomic sequence reads into both taxa and gene level, (2) normalize the taxa-specific gene abundances within samples, (3) tabularize or visualize these joint features, (4) perform comparative microbiome studies, and (5) build prediction models for clinical outcomes. Using simulated sequence reads, we validated that the performance of MetaPrism is accurate. We further applied the MetaPrism analysis to an immune checkpoint therapy and detected novel joint features as potential biomarkers.

MetaPrism is open-sourced and is available at https://github.com/jiwoongbio/MetaPrism. Given the advantages of joint analysis, MetaPrism is a useful tool for a wide range of microbiome-metagenomic sequence studies.

Materials and Methods

Analysis workflow

MetaPrism is a toolkit for joint analysis tasks. At its core, MetaPrism will infer the taxa and gene for each metagenome sequence read. One approach is to align each read to bacterial nucleotide reference genomes to obtain its taxonomy and align it to a protein database to obtain its gene functions. However, this approach is technically challenging: due to the short lengths of illumina sequence reads and the high sequence similarities between bacteria genomes, alignment of short reads is not feasible. We thus developed a novel algorithm (Figure 1A) in an integrated toolkit (Figure 1B) to tackle this challenge.

First, we perform de novo assembly for each sample using metaSPAdes (Nurk et al. 2017) with all metagenomic sequence reads to obtain long contigs. These contigs are much longer than sequence reads to obtain long contigs. These contigs are much longer than metaSPAdes (Nurk et al. 2017) with all metagenomic sequence reads. To assess the accuracy of the joint features estimated by MetaPrism, we conducted a simulation study using simulated sequence reads from a collection of bacteria species. First, we selected all 118 bacterial species with complete reference genomes where the latest genome collected from June 8, 2018 (Table S3). Then, we downloaded their sequences from NCBI FTP (ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank/bacteria). These sequences include 229 contigs including both bacterial chromosomes and plasmids. Their lengths range from 1,308 bp to 10,236,715 bp (mean length is 1,969,971 bp). Finally, we simulated shotgun metagenomic sequencing reads and generated at 10X coverage to resemble typical read lengths from the illumina using ART (Huang et al. 2012). Specifically, we set read length to be 100 base pair and the mean and standard deviation of the fragment size to be 200 bp and 50 bp, respectively.

Data analysis for the microbiome in an immune checkpoint therapy

Immune checkpoint therapy is a revolutionary cancer treatment regime. Researchers realize that the gut microbiome plays an indispensable role in modulating the immune system and boost the therapy efficacy (Frankel et al. 2017). We demonstrated a joint analysis using MetaPrism to build a therapy-response prediction model. We collected stool samples of 12 melanoma patients before anti-PD1 (pembrolizumab) therapy and performed metagenomic sequencing (Frankel et al. 2017). Six patients responded to the therapy and six did not. We performed quality-control procedures on the metagenomic sequence reads. That included the removal of human contamination as previously described (Frankel et al. 2017).
Results
Joint features inferred by MetaPrism are accurate in simulation

We evaluate the gene abundances calculated by MetaPrism and other methods to the true abundances. We determined the true abundances by the multiplication of sequence depth and the depth of KEGG ortholog (KO) genes in the reference genomes. Notably, there could be more than one copy of KO genes in one contig; thus, the true abundance of KO genes can vary from 0 to 1,200. This is also verified by aligning the gene sequences to the KEGG protein database using DIAMOND (Buchfink et al. 2015).

We use the simulated reads totally 4.2 × 10^9 nucleotide bases. We ran two programs: MetaPrism and FMAP. The FMAP software used translation alignment (BLASTX) and our previous benchmarks showed it can report gene abundances accurately (Kim et al. 2016b). Another popular approach is HUMAnN2. However, our simulation showed that its performance to report KO gene abundances is not accurate (Supplementary: Simulation results using HUMAnN2). In Figure 2, we visualized the true abundances (X-axis) and the estimated abundances (Y-axis) for FMAP and MetaPrism using scatterplots. The correlation coefficient ($\rho = 1.000$) from MetaPrism is higher than that from FMAP ($\rho = 0.985$). In brief, this simulation mimics a metagenomic sequence data from known species. We inferred the gene abundances using FMAP (Kim, et al., 2016b) and MetaPrism, and the benchmark showed that gene abundances inferred by MetaPrism were accurate and achieved the highest correlation between inferred abundances and true abundances (Figure 2).

Joint features can be potential biomarkers in immune checkpoint therapy

We used MetaPrism on the remaining sequence reads (detailed data retrieval, analysis steps, and command lines were available in Supplementary: Discover species-specific biomarker in an immune checkpoint therapy study). On average, each sample has 1.2 billion reads. We profiled sequence in MetaPrism and there are on average 24,532 joint features consisting of 2,058 taxa and 3,432 KO genes per sample. Next, we used MetaPrism to normalize read counts for each sample by reporting the mean depth per assembled contig. As demonstrated in previous simulation, the inferred abundance represents the gene counts of specific taxa. These taxa-specific gene abundances were ranked using a random forest model with 500 trees and leave-one-out cross-validation. This prediction model reached 69% accuracy to predict the immunotherapy responses. It was higher than the accuracy using taxa features alone (54%), gene features alone (62%), or just random guess (50%). The prediction accuracy based on the proposed joint features achieved a 7% lead compared to the second-
best model where gene features were used. Furthermore, it detected four joint features with variable importance greater than 50%. We examined the abundances these abundances with red to green colors representing the depth values (Figure 3). We observed these joint features are more abundant in the responder group suggesting that they may improve the treatment efficacy. Among them, the most important feature is the K00826 gene (branched-chain amino acid aminotransferase, BCAT1) from the genus Eubacterium (Table 1). The average abundance of this joint feature in the response group is three-fold higher compared to that in the progression group (response = 4.70 vs progression = 1.17). Interestingly, BCAT1, as an important enzyme in branched-chain amino acid, is associated with glycolysis and oxygen consumption (Kelly and Pearce 2020). These biological procedures determine the cancer growth (Bertout et al. 2008; Yttersian Sletta et al. 2017), and they may be interfered by the high activity of BCAT of Eubacterium, the top abundant taxon in this dataset (20.9%). Although alteration of BCAA metabolism from the bacterial contributes to creating a tumor-favoring metabolic condition in the host remains a hypothesis, further mechanistic studies may investigate the K00826 genes from Eubacterium as a biomarker for cancer immune checkpoint therapy.

In terms of computation, all the above analyses can be accomplished on a standard computation cluster (e.g., 128 GB memory with 2 GB hard drive space per sample).

**Discussion**

We present a novel bioinformatics tool, MetaPrism. It implements functions to quantify the joint features (both taxonomic and functional) from metagenomic sequence reads, as well as other functions for downstream data analyses including comparative studies and prediction modeling. We demonstrate that the joint features can provide novel insights to understand the microbial role in a cancer immunotherapy study.
Table 1. Prediction models and performances for taxonomical analysis, functional analysis, and joint analysis. We tabularized the details of prediction models used in three types of analyses and their prediction performances.

| Model               | Taxonomic profiling | Functional profiling | Joint profiling |
|---------------------|---------------------|----------------------|-----------------|
| Number of trees     | 500                 | 500                  | 500             |
| Number of features  | 1,048               | 5,227                | 62,086          |
| Top features (if variable importance > 50%) Therefore | Chondromyces (100) | K07705 (100) | K00826 Eubacterium (100) |
| 1st feature         |                     |                      |                 |
| 2nd feature         | Roseovarius (65)    | –                    | K02305 Streptococcus (89) |
| 3rd feature         | –                   | –                    | K01006 Flavonifractor (81) |
| 4th feature         | –                   | –                    | K06187 Thermoan aerobacter (74) |
| Accuracy*           | 53.8%               | 61.5%                | 69.2%           |

* : The variable importance values are listed in parentheses.
* : Prediction accuracy was evaluated using leave-one-out cross-validations.

MetaPrism is flexible and can be customized. For example, we can prepare a specific gene database to investigate taxa-specific antibiotic resistance genes (ARGs). We have used reference protein databases with ARGs, such as ARDB (Liu and Pop 2009) or CARD (McArthur et al. 2013). In a graft-versus-host disease (GVHD) study, we used MetaPrism with the ARDB to infer taxa-specific ARGs for joint resistome profiling. Then we correlated patients’ resistome to the outcome of GVHD. We found increased abundances of antibiotic-resistance genes (e.g., mdtG, AcrA, AcrB, and TolC) in Klebsiella and E. coli in the GVHD patients compared with the abundances in non-GVHD patients. This finding may hint optimal antibiotic prescription for better management of GVHD.

MetaPrism characterizes the joint features based on the contigs that are de novo assembled from metagenomic sequence reads. This is a distinct feature compared with other software. For example, HUMAnN2 used a tiered search strategy that relied on a curated reference database for organism-specific genes (Franzosa et al. 2018). However, many bacterial genes are shared across organisms and can be missed by the organism-specific gene database. Thus, we designed the MetaPrism to reduce the dependency on curated reference databases. The tradeoff for this decision is that MetaPrism requires more computational resources for the de novo assembling step.

In all, MetaPrism is free and useful software to facilitate joint analyses and it is suitable for general microbiome studies. Researchers can expect MetaPrism to quantify species-specific gene abundances and use these interpretable features in association studies and prediction tasks.

Data Availability

The metagenomic shotgun sequence dataset in the immune checkpoint therapy is available from the NCBI BioProject PRJNA397906. The treatment responses for the 12 patients as well as the analysis codes were available in the Supplementary: Discover species-specific biomarker in an immune checkpoint therapy study. The source codes of MetaPrism software are available at: https://github.com/jiwoongbio/MetaPrism. That resource contains the software requirements, usage example, and documentations for all MetaPrism components (e.g., download bacterial database, quantify species-specific gene abundances, build association models and prediction models, tabularize results, and visualize results in heatmap plots). Supplemental material is available at figshare DOI: https://doi.org/10.25387/g3.13944521.

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Authors’ contributions

JK, SJ, and XZ conceived of the project and wrote the first draft of the manuscript. GX, YX, AY, and XZ coordinated and oversaw the study. QL and DL provided critical inputs for the study. JK and SJ developed the software and associated databases. JK, SJ, and YW perform statistical analysis. All authors contributed to the review of the manuscript before submission for publication. All authors read and approved the final manuscript.

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