Rough Set Microbiome Characterisation

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Abstract—Microbiota profiles measure the structure of microbial communities in a defined environment (known as microbiomes). In the past decade, microbiome research has focused on health applications as a result of which the gut microbiome has been implicated in the development of a broad range of diseases such as obesity, inflammatory bowel disease, and major depressive disorder. A key goal of many microbiome experiments is to characterise or describe the microbial community. High-throughput sequencing is used to generate microbiota profiles, but data gathered via this method are extremely challenging to analyse, as the data violate multiple strong assumptions of standard models. Rough Set Theory (RST) has weak assumptions that are less likely to be violated, and offers a range of attractive tools for extracting knowledge from complex data. In this paper we present the first application of RST for characterising microbiomes. We begin with a demonstrative benchmark microbiota profile and extend the approach to gut microbiomes gathered from depressed subjects to enable knowledge discovery. We find that RST is capable of excellent characterisation of the gut microbiomes in depressed subjects and identifying previously undescribed alterations to the microbiome-gut-brain axis. An important aspect of the application of RST is that it provides a possible solution to an open research question regarding the search for an optimal normalisation approach for microbiome census data, as one does not currently exist.

I. INTRODUCTION

High-throughput DNA sequencing has enabled culture-independent profiling of complex microbial communities. A microbiota is defined as the assemblage of micro-organisms present in a defined environment [1]. Over the past decade interest about the role microbiota play in both health and disease, across the human body, has rapidly grown. Microbes directly and indirectly interact with human physiology through a variety of mechanisms, including protecting against pathogenic infections, contributing to normal metabolic functions, and by training the immune system [2]. Alterations to the gut microbiota have been implicated in the pathogenesis of a number of diseases, including Inflammatory Bowel Disease, diabetes, obesity, and depression [3], [4], [5]. Typically a microbiota profile is created by collecting an environmental sample (e.g. stool to study the human gut microbiota), extracting the DNA present, and sequencing the 16S ribosomal RNA (16S rRNA) marker gene. Put simply, the 16S rRNA marker gene is similar to a barcode: different bacterial species have different marker gene sequences. Marker gene sequences can be matched to reference databases to discover the taxonomy of a given sequence (e.g. ACTG . . . → E. coli). A high-throughput DNA sequencer will create millions of discrete DNA sequence reads, often between 200 – 400 nucleotides long. Microbiota profiles (also known as microbiome census data) are extremely challenging to analyse: they are highly dimensional, sparse, noisy, and compositional. These properties violate many assumptions of standard models that have been widely applied to analyse microbiota profiles [6] (described further in Section II). A broad range of complex normalisation algorithms (data transformations) has been developed and applied to resolve the problems inherent to such data [7]. However, identifying which normalisation algorithm is optimal remains an open question in the microbiome research community. In contrast, data-driven computational intelligence paradigms have minimal or weak prior assumptions and offer powerful tools for extracting knowledge from problematic data such as microbiota profiles [8]. As such, their application to microbiome census data may offer the potential for more robust analysis.

Among computational intelligence/AI approaches, Rough Set Theory (RST) is a topic of great interest amongst the research community and has been applied to a variety of domains for the purpose of data analysis [9], including many areas of computational biology [10]. Many microbiome experiments aim to identify correlations between the characterised microbial community and disease. Only a small part of this process is concerned with evaluating predictive power: the process of determining elements of a microbial community that have predictive power is described as biological marker (biomarker) analysis by molecular biologists. RST can elegantly address both characterisation and prediction. Firstly, by identifying a minimal knowledge representation (a reduct), redundant or irrelevant bacterial species can be discarded, simplifying analysis. Additionally, transparent rules can be induced to describe the minimal knowledge representation, enabling knowledge discovery. If additional data are available the rules can be used to evaluate the predictive power of putative biomarkers. A combination of both approaches allows domain experts to interpret a model and gain an understanding of the underlying biological processes involved. Additionally, RST does not require parameters to be set, which eliminates a source of potential human bias.

This paper presents an approach to characterise microbial communities using RST to simultaneously transform data into knowledge and to resolve an open research question regarding normalising microbiome census data (described further in Section II). This approach is first demonstrated on a small benchmark dataset to characterise the microbial communities present across different human body sites. This serves as a simple demonstration of RST, because it is well known that microbial communities significantly differ across the human body. The application of RST is then expanded to cover a microbiome.
experiment that investigates the link between microbiomes and depression (see Section [I] to enable knowledge discovery. It is important to note that this paper focuses on characterisation and does not address prediction from the induced rules (i.e. the rules are descriptive and reveal underlying patterns in the data). The rationale for this is that characterisation enables the transformation of data into knowledge. From this knowledge insights about microbial communities can be gained and future experiments planned. The quality of characterisation can be measured by a variety of measures, discussed in Section [II].

The application of RST enables microbial ecologists to understand better what is happening in a microbial community — by removing the consideration of superfluous bacterial species and the requirement for destructive data transformations — and why bacterial species are associated with phenotypes, via the analysis of transparent induced rules.

The remainder of this paper is structured as follows: Section [II] describes some problematic properties of microbiome census data that make analysis challenging, the links between the microbiome and depression, and core RST concepts applied throughout this work. Section [III] introduces the rough set microbiota model and describes the datasets used to benchmark the model. An evaluation of the rough microbiota model that demonstrates the potential of applying RST to microbiota profiles is provided in Section [IV]. Finally, conclusions and an outline for future work are presented in Section [V].

II. BACKGROUND

Machine learning and computational intelligence approaches are often applied to microbiome census data for the purpose of predicting a categorical or numeric variable from a set of input data (e.g. predicting disease). However, classification and regression are only a subset of data mining and knowledge discovery tasks. Popular tasks for data mining and knowledge discovery include [11]:

- Describing patterns and trends in data;
- Approximating a categorical target variable from a larger data set (classification);
- Approximating a numeric target variable from a larger data set (regression);
- Predicting future events (e.g. the share price of a company in 3 months);
- Clustering observations into similar groups;
- Identifying association rules (finding features that co-occur).

Describing patterns and trends in data is the most common aim of microbiome experiments. Many microbiome experiments aim to identify correlations between the characterised microbial community and disease. The process of determining elements of a microbial community that have predictive power is described as biological marker (biomarker) analysis by molecular biologists. When attempting to describe patterns and trends in data, models that offer transparency provide significant benefits [11]. RST provides a suite of tools that allows the transparent description of data, which could help to fulfil an important objective for molecular ecologists.

A. Why RST? Problematic properties of microbiota profiles

Microbiome census data produced by high-throughput sequencing are extremely challenging to analyse: they are highly dimensional [12], noisy [13], variably sparse across different environments [14], compositional [15], and have an uneven mean-to-variance relationship [6]. Most of these properties will violate the assumptions of standard analysis models, such as normality or homoscedasticity. For example, investigating if certain bacterial species are more or less abundant in certain environments is difficult because varying sparsity across different environments can violate probability distribution assumptions. As microbiome census data are not normally distributed and are heteroscedastic it is not appropriate to model differential abundance with popular approaches such as t-tests. Microbiologists interested in examining bacterial co-occurrence relationships will have problems computing correlation coefficients [16]. A thorough review discussing these problems is available [17]. After initial quality control and clustering preprocessing steps [17] (or alternatively denoising [18]) microbial community sequencing data are typically organised into large matrices where rows represent samples and columns represent counts of clustered sequence reads that constitute different types of bacteria. The number of discrete sequence reads per sample (the sum of each row) can differ by orders of magnitude. This uneven sampling effort does not reflect true biological variation and is an artefact of the sequencing process. The uneven sampling effort will bias the estimates of bacterial abundance and should be normalised to allow fair comparison between samples. Normalisation procedures can also mitigate the other types of bias present in microbial community sequencing data described above. However, recommended normalisation procedures that aim to mitigate such complex problems are often difficult for microbiologists to incorporate (e.g. applying a variance stabilising transformation based on Gamma-Poisson mixture models [19]) and can destroy the semantics of the original data. A widely used normalisation strategy is to convert counts into relative abundances per sample (simple proportions). However, as relative abundances are constrained by an artificial limit (1) they represent compositional data. Compositional data have an arbitrary or non-informative sum [20]. Additionally, relative abundance data can be skewed by the presence of highly abundant species, and the transformation does not resolve important problems with the data such as heteroscedasticity [6]. However, it is rare for microbial communities in the human body to be dominated by a few species, and relative abundance data are easy for microbial ecologists to incorporate and analyse. Crucially, the problematic properties of relative abundance data (heteroscedasticity and compositionality) do not violate the assumptions of RST, which is our motivation for using this type of normalisation.

The only assumption required in RST is that each object has an associated set of attributes used to describe the object, and that the data are a true and accurate reflection of reality [21]. Thus, the application of RST resolves the problematic aspects of microbiome census data described above. Extensive steps were taken to denoise the microbiome census data, described
further in Section III, to ensure that the accuracy assumption is not violated. Additionally, RST makes redundant the requirement for more complex normalisation algorithms; the semantics of easily intuited relative abundance microbiome census data are maintained — aiding interpretation by domain experts and providing a possible solution to an open question in the microbiome research community regarding choice of an optimal normalisation algorithm (which can differ depending on data and analysis task [7]).

As far as can be ascertained, there have been no previous attempts to perform data analysis on microbiome census data using RST described in the literature. However, aspects of RST have been implemented for bioinformatics applications in the wider field of metagenomics. The metagenome is defined as the collection of genomes and genes from the members of a microbiota [1]. RST has been applied to remove superfluous $K$-mers and to improve DNA fragment classification compared with standard bioinformatics tools [22]. A rough reduction method based on Particle Swarm Optimisation has also been applied to the same problem [23]. RST has also been used to predict the presence of operons in metagenomic data. A decision tree classifier based on the Variable Precision Rough Set Model (VPRSM) was applied to genomic data from Escherichia coli to identify if a gene belonged to an operon [24]. The VPRSM had an accuracy of 89.4% using five features: maximum distance, minimum distance, direction, cluster of orthologous groups, and gene order conservation. The use of a decision tree meant that the decisions of the classifier were easy to interpret and could be validated by domain experts.

B. The microbiome and depression

Depression is a mental disorder that causes a persistent low mood, low self-esteem, and chronic anhedonia. Depression is currently the leading cause of global disease and affects over 300 million people worldwide [25]. A growing body of evidence supports the hypothesis that the gut microbiota, the complex community of microorganisms that inhabit the human gastrointestinal tract, play a key role in the aetiology of depression via regulation of the central nervous system in a complex network known as the microbiome-gut-brain axis; an in-depth explanation of this phenomena is outside the scope of this paper, but comprehensive reviews are available on the subject [26, 27]. Despite work in animal models that links the gut microbiota and depression [28], limited work has been done using a human cohort: faecal samples isolated from Norwegian [29] and Chinese [30] cohorts have identified some alterations in a depressed cohort. Our rationale for applying RST to a publicly available depression microbiome dataset, described further in Section III, is to enable the extraction of new knowledge from the data as previous work has relied on standard analysis techniques.

C. Core RST concepts

A microbiota profile can be represented by a $M \times N$ decision table. The rows of a decision table correspond to the universe of discourse, $X$ [21]:

$$X = \{x_1, x_2, \ldots, x_N\} \quad (1)$$

The columns of a decision table correspond to the set of features $A$ (the set of microbes) [21]:

$$A = \{a_1, a_2, \ldots, a_M\} \quad (2)$$

Decision table $DT$ consists of a subset of condition attributes (input features, different microbial species) and decision attributes (class labels e.g. disease or healthy; $DT = C \cup D$). Each attribute has an associated value set, which represents the abundance of the microbial species [10]:

$$V_a = \{v_1^a, v_2^a, \ldots, v_p^a\} \quad (3)$$

where $a \in A$. The value set must be discrete (continuous variables must be discretised). Although microbiome census data are discrete counts of sequences, they are typically converted into continuous variables by a normalisation process to mitigate uneven library size bias. A thorough discussion of bias in microbiome census data and normalisation approaches to counteract this is available [7]. Therefore relative abundance microbiome census data, which is used as input data throughout this chapter, must first be discretised. The maximal discernibility heuristic was used to discretise the microbiome census data throughout this paper [21]. Any condition or decision attribute subset $P \subseteq C$ or $D$ can induce a partition in $X$ [10]:

$$X \xrightarrow{P} X(P) = \{X_1^P, \ldots, X_N^P\} \quad (4)$$

where $X_i^P$ is the partition of $X$ induced by $P$. The subsets [10]:

$$X = X_1^P \cup \ldots \cup X_Q^P \quad (5)$$

correspond to the set of equivalence classes, called indiscernibility classes in RST. Discernibility is the core concept of RST: if $(x, y) \in \text{IND}(P)$ (where $\text{IND}(P)$ is the indiscernibility relation induced by attribute subset $P$) then $x$ and $y$ are indiscernible by attributes from $P$. For example, if two bacterial species have the same abundance in both healthy and sick subjects, then using only the abundance of the bacterial species it is impossible to discern between the two subjects. In RST, a set is approximated by two sets known as the lower and upper approximations [21]:

$$\text{PS} = \{x : [x]_P \subseteq S\} \quad (6)$$

$$\text{PS} = \{x : [x]_P \cap S \neq \emptyset\} \quad (7)$$

where $S \subseteq X$ and $[x]_P$ are the equivalence classes of the $P$-indiscernibility relation. The tuple $(\text{PS}, \text{PS})$ is known as a rough set. $P$ and $Q$ are sets of attributes inducing equivalence relations over $U$. The region between the upper and lower approximation sets is called the boundary region. The boundary region represents the set of objects that can possibly be predicted to be from a specific decision class (non-deterministic; see Figure [1] [21].
RST to these data. The human body site dataset was analysed using the RoughSets package \cite{33} implemented in R.

The public gut dataset consists of a cohort containing 59 faecal samples (30 control, 29 depressed). Briefly, the bacterial DNA present in the samples was extracted and sequenced with a 16S marker gene survey. This process generated millions of DNA sequences, 200–400 nucleotides in length. Before this raw sequence data can be input to the rough set microbiome characterisation process, it must be first processed with bioinformatics algorithms to generate an accurate survey of bacterial species. The gut data were denoised according to standard operating protocols using an R bioinformatics pipeline \cite{18} to ensure that the truth assumption of RST was met. The human body site dataset was input to the rough set microbiome characterisation process in its preprocessed form, which is often used to simplify analysis. The data were discretised with the maximum discernibility discretisation algorithm implemented in the Java rseslib library \cite{33}. Due to the scale of the depression datasets (both contained 3000–4000 features) the rough set microbiome characterisation was implemented with rseslib, as R (the language in which the RoughSets package was implemented) suffers from poor computational performance compared with other languages such as Java \cite{35}. To aid analysis, discrete data in the depression datasets were labelled low and high if the bacterial species abundance had two cuts, and low, medium, and high if the bacterial species abundance had three cuts. We evaluated the ability of RST to model microbiota profiles by testing classification performance on the following tasks:

1) Classify microbial communities from different areas of the human body (tongue, skin, or faeces);
2) Classify depression status from the gut microbiome.

We began by generating a single reduct for the first decision table (the demonstrative dataset; see Figure 2). The rationale for generating a single reduct is that the first decision table serves as a demonstration of RST applied to a simple problem, and a single reduct can be used to provide a simple description of microbial communities across the human body. For the depression datasets, all local reducts were computed for each decision table using the rseslib Java library. To determine the classification performance of the partition in \( X \) induced by the set of reduct attributes \( A_k \) two measures were used \cite{10}:

\[
\text{Accuracy} = \frac{\sum_{L=1}^{Q} \text{Card}(A_k X^L_{A_k})}{\sum_{L=1}^{Q} \text{Card}(A_k X^L_{A_k})}
\]

\[
\text{Quality} = \frac{\sum_{L=1}^{Q} \text{Card}(A_k X^L_{A_k})}{\text{Card}(X)}
\]

where Card is cardinality, which represents the number of elements in a set, and \( L \) is the total number of upper \( (A_k X^L_{A_k}) \) and lower-approximation \( (A_k X^L_{A_k}) \) set tuples. Accuracy represents the ratio of the size of all lower-approximation sets to the size of all upper-approximation sets \( (0 \leq \text{Accuracy} \leq 1) \). If the family of lower approximation sets is an empty set (i.e. no objects can be said to be certainly predicted) then accuracy is zero. Quality represents the ratio of all objects in the family of lower approximation sets to the

### III. Rough Microbiome Analysis

This work uses two datasets:

1) a publicly available human body site dataset \cite{32}, which contains environmental samples gathered from across the human body (tongue, skin, or faeces);
2) a publicly available gut microbiome depression dataset \cite{30}, which contains faecal samples gathered from depressed and control subjects.

The human body site dataset serves to demonstrate the RST approach before it is applied to more complex data for knowledge discovery. The human body site dataset contains 3 samples for each body site: human skin, human tongue, and human faeces (9 samples total). This dataset forms part of the larger “Global Patterns” dataset — in microbiome research the Global Patterns dataset is widely used to benchmark new algorithms or tools \cite{6, 7}, which is the rationale for applying

\[
\text{BND}_P(Q) = \bigcup_{X \in U/Q} P \bar{S} - \bigcup_{X \in U/Q} PS
\]

The positive region, in which objects can be predicted to belong to a decision class with certainty, is given by:

\[
\text{POS}_P(Q) = \bigcup_{X \in U/Q} PY
\]

The negative region represents the set of objects that cannot be predicted to a decision class:

\[
\text{NEG}_P(Q) = X - \bigcup_{Y \in X/Q} \bar{P}Y
\]

Attributes that cannot be removed without changing the partitioning of objects amongst the indiscernibility relations are indispensable. A minimal set of indispensable condition attributes is known as a reduct.

Fig. 1. Rough set example. The universe of discourse is partitioned into 9 indiscernibility classes by a set of attributes. The blue line represents the set being approximated (e.g. sick subjects). The green section is the lower approximation, and the red sections are the upper approximations of the rough set. In the complement of the upper approximation (grey) it is certain that no objects in the rough set will be present (e.g. a healthy subject could be in the grey section)
and analysing a set of IF-THEN Patterns and trends in data can be described by generating valuable, it forms only one aspect of a microbiome experiment. The power of them is not assessed: although prediction can be noted that the generated rules are descriptive and the predictive accuracy and quality are not tested on independent validation data due to insufficient data: these metrics are only capable of explaining how well a rough set is describing a microbiota profile. IF-THEN decision rules were generated from the indiscernibility classes defined by the reduct attributes using the rseslib library. The descriptive strength of the rules was evaluated by measuring the support each rule has. Once species of interest were identified by this procedure, a literature review was conducted to identify associations between the generated rules and biological phenomena.

IV. RESULTS

A. Human body site data

A decision table was created from the human body site data which contained 3878 conditional attributes and 9 samples (3 skin samples, 3 faecal samples, and 3 tongue samples). Each conditional attribute defines the abundance of a bacterial Operational Taxonomic Unit, which approximates a bacterial taxa (group e.g. species). A single reduct was generated for this first characterisation task as it serves as a demonstration before the RST approach is expanded to the depression data set for knowledge discovery. A single feature was present in the reduct: the bacterial species Propionibacterium acnes. The characterisation ability of the reduct rough set was tested using the accuracy and quality measures described in Equations 12 and 13 (see Table I). The lower approximation set contained all of the samples for each sample type so the accuracy and quality of classification was 1. This demonstrates that RST is capable of excellently discriminating between samples collected from different sites across the human body. The next step of characterisation is to describe the alterations identified by RST using IF-THEN rules and linguistic variables.

Rules were generated from the reduct. It is important to note that the generated rules are descriptive and the predictive power of them is not assessed: although prediction can be valuable, it forms only one aspect of a microbiome experiment. Patterns and trends in data can be described by generating and analysing a set of IF-THEN rules. However, the quality of characterisation can be measured by the strength of the generated rules, which is defined as the number of instances in the dataset that are concordant with each rule. The human body site characterisation task generated three rules (with 100% strength) for three classes regarding the bacterial species P. acnes:

IF P. acnes 0 THEN Faeces (Rule 1)
IF P. acnes [0, 4.91 × 10^-5] THEN Tongue (Rule 2)
IF P. acnes [4.91 × 10^-5, 1] THEN Skin (Rule 3)

The generated rules are supported by compelling biological evidence. Relating the output of the RST characterisation process to biological phenomena is simple because the semantics of the original data were not destroyed by complex normalisation approaches. Typically P. acnes is a commensal member of the skin microbiome, but it can act as a pro-inflammatory opportunistic pathogen, causing acne [36]. Its pattern of abundance matches descriptions in the literature: most prevalent on skin, but capable of colonising other areas of the body including the tongue and large intestine [36]. The absence of P. acnes in stool samples could be related to the sensitivity of the sequencing process or the low sample size of the cohort (P. acnes is not a major member of the gut microbiome, the most complex of all human microbiomes). Alternatively, as faeces are not a perfect proxy for the large intestine P. acnes may be present in the large intestine but be undetectable in stool samples. The RST characterisation of human body sites has therefore identified a biologically plausible process that represents a key change across habitats. We will now apply this approach to the more complex depression dataset to enable knowledge discovery.

B. Gut data

A decision table created from the gut dataset using the approach described in Section III. The gut decision table had 2900 conditional attributes, and 59 samples (30 control, 29 depressed). Each conditional attribute defines the abundance of a denoised amplicon sequence variant which approximates the true DNA sequence present in the samples. The denoising paradigm offers a range of benefits — including sampling accuracy — compared with the operational taxonomic unit approach, which is our motivation in using the approach. A thorough explanation of the benefits the denoising paradigm offers is outside the scope of this paper; reviews are available [13]; the denoising approach did not exist when the human body site data were first created. All local reducts were computed for the gut decision table. The reducts contained 12 features that covered the bacterial genera Bacteroides, Prevotella, Anaerostipes, Phascolarctobacterium and Odoribacter. One of the features could not be mapped to a specific

| TABLE I | CLASSIFICATION METRICS |
|----------------|-----------------------|
| Classification task | Accuracy | Quality |
| Human body site     | 1         | 1        |
| Gut microbiome      | 1         | 1        |

Fig. 2. Overview of rough microbiota profile analysis
The lower approximation set contained all of the samples for both classes (depression and control), so the accuracy and quality of characterisation was 1 (creating a crisp set), which demonstrates that the rough set microbiome characterisation is capable of excellently discerning between samples collected from depressed and control subjects.

The next step of characterisation is to describe the alterations identified by RST using IF-THEN rules and linguistic variables. More complex rules were generated to characterise the gut microbiome characterisation task (see Table I). The abundance of bacterial taxa was defined as being low or high to aid comprehension. In the gut microbiome three rules were induced to characterise control samples, and four rules to characterise depressed samples. Control samples are characterised by low abundance of the bacterial genera, whilst depressed samples are characterised by a mixture of high and low abundant bacterial genera. There are significant underpinning biological justifications for the four rules that characterise the depressed gut microbiome. Phascolarctobacterium is a bacterial genus that is abundant in the human gut and produces short chain fatty acids, which are associated with modifying host metabolism and mood [26]. Additionally, Phascolarctobacterium has been previously positively correlated with positive mood in healthy adults [37]. The second and third rules for depression contain the multiple bacteria in the Bacteroides genus; Bacteroides are a major mutualistic member of the normal human intestinal microbiome, the described abundance patterns indicate a type of gut dysbiosis has occurred, which has been frequently associated with various diseases [38].

It is useful to compare our results for the gut microbiome with the original analysis that used a traditional (i.e. non-RST) methodology [30]. The low levels of Ruminococcaceae in rules 2 and 3 are concordant with the traditional analysis. The low abundance of Alstipes in rule 4 is not consistent with the traditional analysis. However, the low abundance is combined with a high abundance of Odoribacter, which was also not mentioned in the original analysis. Odoribacter are typically opportunistic pathogens, which can activate inflammatory pathways associated with the microbiome-gut-brain axis [39].

VI. Conclusion and Limitations

In this work we present the first application of RST to characterise microbiota profiles from a standard benchmark dataset. We then extend the application to a gut microbiome dataset to enable knowledge discovery concerning microbiomes in depressed subjects. We find that RST is capable of excellently characterising the gut microbiomes in depressed subjects and identifying previously undescribed alterations to the gut microbiome in depressed subjects. The minimal prior assumptions of RST also offer a potential solution to an unresolved question in the microbiome community regarding identifying which normalisation procedure is optimal for microbiota profiles. In addition, the application of RST tools such as reducts and rule induction allows domain experts to understand RST models without requiring an understanding of the mathematics involved and thus helps to shed light on the underlying biological processes present.

Rule-based systems suffer from the combinatorial rule explosion problem. As the number of features being considered increases, the number of rules increases exponentially [42]. This drastically reduces the performance and transparency of rule-based systems. The rough set theory applications to the microbiome census data in this paper have generated small reducts, with less than a dozen features, which avoids this problem. However, it is important to note some applications of the rough set characterisation approach may result in a rule explosion if many bacterial species are relevant to the characterisation. Rule optimisation would be an important method of tackling this problem while maintaining the transparency of the system.

The biggest limitation to the proposed approach is the small sample size of the analysed data. Microbial ecologists use a range of measures to ensure that sampling has been sufficient
TABLE II
RULES THAT CHARACTERISE THE GUT MICROBIOME. † INDICATES HIGH ABUNDANCE, ⇔ INDICATES MEDIUM ABUNDANCE, AND ‡ INDICATES LOW ABUNDANCE

| Rule | Antecedent | Consequent |
|------|------------|------------|
| 1    | IF Bacteroides (3) ‡ AND Bacteroides (6) ‡ AND Prevotella ‡ AND THEN Control |
| 2    | IF Bacteroides (3) ‡ AND Bacteroides (4) ‡ AND Bacteroides (6) AND THEN Control |
| 3    | IF Bacteroides ‡ AND Bacteroides (4) ‡ AND Bacteroides (6) ‡ AND THEN Control |

TABLE III
QUALITY OF MICROBIOME CHARACTERISATION.

| Decision | Rule # | Support |
|----------|--------|---------|
| Control  | 1      | 86.7%   |
|          | 2      | 83.3%   |
|          | 3      | 80.0%   |
| Depressed| 1      | 31.0%   |
|          | 2      | 27.5%   |
|          | 3      | 27.5%   |
|          | 4      | 27.5%   |

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to enable the accurate characterisation of an environment (e.g., taxon resampling curves). If our characterisation approach is expanded to include prediction of disease (biomarker analysis), hundreds of samples would be required to validate the predictions. Additionally, the application of fuzzy RST would be valuable for future work. Disease is a continuum and can rarely be described using a simple two-class paradigm. Fuzzy RST would also remove the need to discretise the data, preventing some information loss and resolving one of the largest drawbacks associated with rough set characterisation.
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