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Short communication

Advancing integration of data on food microbiome studies: FoodMicrobionet 3.1, a major upgrade of the FoodMicrobionet database

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ABSTRACT

We present a new version of FoodMicrobionet, a database for the exploration of food bacterial communities. The database, available as an app built with the Shiny package of R, includes data from 44 studies and 2234 samples (food or food environment), covering dairy, meat, fruit and vegetables, cereal based and ready-to-eat foods. The interactive interface allows exploration of data, access to external resources (on line versions of the studies, sequence data on SRA, taxonomic databases), filtering samples on the basis of a number of criteria, aggregation of samples and bacterial taxa and export of data in a variety of formats. FoodMicrobionet is the largest collection of data on food bacterial communities and, due to the structure of sample metadata, largely derived from the European Food Safety Agency FoodEx2 classification, makes comparison and re-analysis of data from published and unpublished studies easy. Data exported from FoodMicrobionet can be readily used for graphical and statistical meta-analyses using open-source software (Gephi, Cytoscape, CoNet, and R packages and apps, such as phyloseq and Shiny-Phyloseq) thus providing scientists, risk assessors and industry with a wealth of information on the structure of food biomes.

1. Introduction

The availability and the steadily decreasing costs of high throughput sequencing (HTS) technologies have undoubtedly revolutionized the study of microbial ecology, by providing methods that increase the resolution and capabilities of our experimental approaches directed to understanding structure and functions of microbial communities (De Filippis et al., 2018a). Large national and international coordinated efforts have been directed to the study of the human (NIH human microbiome https://hmpdacc.org/hmp/, https://hmpdacc.org/ihmp/), urban (http://metasub.org) and environmental microbiome (http://www.earthmicrobiome.org; Thompson et al., 2017), with well-standardized protocols (Anonymous, 2016; http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/; http://www.microbiome-standards.org/#SOPS). The number of studies using either amplicon-targeted or metagenomic/metatranscriptomic approaches for the characterization of food microbial communities has also been rising steadily in recent years (De Filippis et al., 2018a). Besides differences in wet-lab procedures and protocols, the lack of standardized procedures for data analysis can make comparisons between the results of different studies difficult (Clooney et al., 2016; De Filippis et al., 2018b).

Co-ordinated efforts have resulted in the creation of databases (such as QIITA https://qiita.ucsd.edu/, Gonzalez et al., 2018; and MGnify https://www.ebi.ac.uk/metagenomics/, Mitchell et al., 2018) which integrate deposit/retrieval of sequence data with analysis tools for both amplicon-targeted and shotgun studies. The European Bioinformatics Institute (EBI) metagenomics platform MGnify includes, at the time of writing of this article (April 2019), 3155 studies and 171,886 samples, which were analysed using a standardized pipeline (Mitchell et al., 2018). However, only 59 studies and 1650 samples of food biomes are included in MGnify, metadata and information for sample classification are limited and comparisons/analyses can only be performed within a given study. Although QIITA includes an even larger number of studies and samples (both private and public), only very few public studies are related to food biomes.

The difficulties in retrieving and combining information on food bacterial communities from different studies motivated us to release in

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2016 the first public version of FoodMicrobionet (v1.0.3; Parente et al., 2016), which included 17 studies, with data mostly contributed by partners in the form of OTU and metadata tables. The database was based on a hierarchical classification of foods developed by EFSA, the FoodEx2 classification (EFSA, 2015), and emphasis in the first version was on exploration of the bipartite network of taxa and samples (Parente et al., 2016), using an interactive visualization developed with the Gephi graph visualization tool (Bastian et al., 2009). Subsequent additions (versions 1.1 and 2) brought the number of studies to 33, and improved the possibilities of extracting and processing data through R scripts (De Filippis et al., 2018b; Parente et al., 2018). Here we describe the third release of the database, which, with 44 studies and 2234 samples, is the largest and most structured collection of data on food bacterial microbiomes, and the Shiny-FoodMicrobionet app, an interactive app designed to facilitate access, filtering and export of data.

2. Materials and methods

2.1. The structure of the database

The structure of the FoodMicrobionet is graphically described in Fig. 1. The database includes five tables: a. the study table, with information on studies included in FoodMicrobionet; b. the samples table (linked to the study table via the studyId field), with information on individual samples; c. the taxa table, with taxa labels and lineages; d. the edges table, which stores the relationships between samples (via sampeId) and taxa (via taxonId) and provides the abundance of each taxa in a given sample; e. the FoodEx2 table, a service table with the EFSA FoodEx2 classification, linked to the sample table. A detailed description of tables and fields is presented in Supplementary Material.

2.2. New accessions and sequence processing

Eleven new studies were added to FoodMicrobionet 2.0 (Parente et al., 2018) by downloading raw sequences/metadata from NCBI Sequence Read Archive (SRA) and processing the sequences using R (R Core Team, 2018) and a bioinformatic pipeline based on the Bioconductor workflow for microbiome data analysis (Callahan et al., 2016a, 2016b). Briefly, functions from the dada2 package (Callahan et al., 2016b; https://benjjneb.github.io/dada2/) were used for visualization of quality profiles (plotQualityProfile()), quality filtering and trimming (filterAndTrim()), learning error rates (learnErrors()), dereplication (derepFastq()), inference of Amplicon Sequence Variants (dada()), merging or concatenation of paired end sequences (mergePairs()), construction of sequence table (makeSequenceTable()), chimera removal (removeBimeraDenovo()), and taxonomic assignment using the SILVA v132 database (Quast et al., 2013; assignTaxonomy(); species were added with addSpecies() whenever possible). Minor adaptations to the pipeline were used for 454 data or whenever paired-end sequences were deposited as merged sequences (https://benjjneb.github.io/dada2/faq.html). A phyloseq class object (McMurdie and Holmes, 2013) was then built using sample data (obtained from NCBI SRA as run info tables), sequence tables and taxa tables. Further processing in R included taxonomic aggregation of ASVs at the lowest taxonomic level available (species or genus), and formatting tables for inclusion in FoodMicrobionet. Study, sample and taxa information and edge tables were then converted to Excel tables, imported in FoodMicrobionet and further editing of study, sample and taxa information was carried out manually.

A list of studies in FoodMicrobionet 3.1 is available in Supplementary Material.

2.3. ShinyFMBN: a shiny app for accessing FoodMicrobionet

To facilitate access to FoodMicrobionet tables, we developed a Shiny (Chang et al., 2018) app, which allows to visualize, explore, filter and extract data from FoodMicrobionet. The app and a user guide are available from Mendeley data (https://data.mendeley.com/datasets/8fwwjpm79y/2). The manual for the app is also available as Supplementary Material.

3. Results and discussion

3.1. Changes in the database structure

The FodMicrobionet database has been modified compared to previous versions (De Filippis et al., 2018b; Parente et al., 2016; Parente et al., 2018) to provide better integration with external resources (i.e. by facilitating access to taxonomy databases, to bibliographic resources and to raw sequence data on the NCBI SRA) and to improve the classification of samples.

In the study table, bibliographic information can now be retrieved easily and exported as a table during extraction of data (see below), and the link to NCBI SRA now points to the run selector, which facilitates the selection of sequence runs for download.

The samples table stores information on individual samples. In addition to sample labels used in the original studies and a short description, classification keys including sample type (either food or food environment) and four fields with information derived from the FoodEx2 rev2 classification (EFSA, 2015) providing a hierarchical classification of samples, which greatly facilitates searches of the database and eliminates ambiguities. None of the studies included in FoodMicrobionet used the FoodEx2 classification either in the original publication or in the run info table deposited in NCBI SRA and therefore

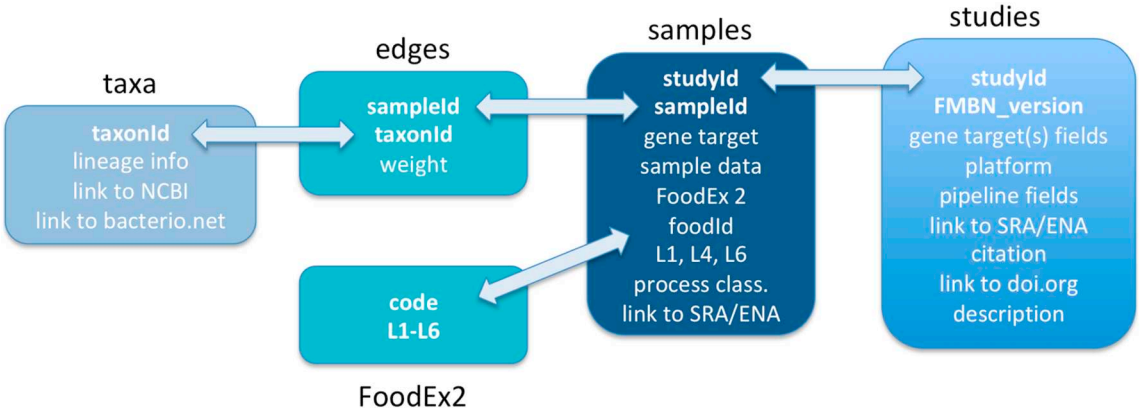

Fig. 1. Tables and relationships in FoodMicrobionet 3.1. The rounded rectangles represent the four main tables (studies, samples, taxa and edges and the "service" table with the FoodEx2 classification), while the double arrows represent the relationships among fields of different tables.

addition of this information required manual annotation during addition of new data to the database. Three further manually curated fields are used for sample classification: nature, process, fermentation/spoilage. This allows to add further information on individual samples (nature: raw, process intermediate, finished product; process: an indication of the application of a potentially lethal treatment; fermentation/spoilage: occurrence of fermentation, spoilage or both; see Supplementary Material for details). Although the FoodEx2 rev2 classification does provide state attributes and facets related to part-nature and process (EFSA, 2015), their implementation is complicated due to the lack of complete information for most samples.

3.2. New data

New studies were fed into the database starting from raw sequences obtained from SRA/ENA using the Bioconductor workflow for microbiome data analysis (Callahan et al., 2016a, 2016b), which was slightly adapted to make the output data (a phyloseq class object) compatible for addition to FoodMicrobionet. A flowchart showing the process of data addition and extraction in FoodMicrobionet 3.1 is shown in Fig. 2. The current version includes 44 studies and 2234 food biome samples, more than either QIITA and MGnify (as of April 2019). The variety of samples is also higher than in other databases: the samples belong to 9 major food groups and 76 different food products and there are 148 different combinations of food, nature, process, fermentation/spoilage (see Table specifications in Supplementary Material).

3.3. Improved accessibility

FoodMicrobionet makes data extraction significantly more flexible than existing databases and provides users with the choice of the comparison to be made (within or across studies).

To facilitate exploration of the database and data extraction, the current version is available as a Shiny app, which can be downloaded and used offline.

The app was built with several users (researchers, risk assessors, R&D in food industry) in mind but the most common use case scenario is that of researchers wishing to perform a meta study or to compare their own data with published data. A use case diagram for this scenario is provided in Supplementary Material.

The app (which requires R and RStudio to run), once launched opens in a browser window. The flow of tasks is shown schematically in Fig. 3, while a few examples of the workflow for data extraction and analysis is included in Fig. 2 and described in more detail in the examples provided in Supplementary Material.

Ideally, a hypothetical user of the app would start with exploration of data in the Explore pane, where data on studies and summary data on samples can be visualized. This pane can also be used to seamlessly reach external resources such as the journal articles for each study (via DOI) or sequence data on NCBI SRA archive. Once the user has chosen the criteria to use for selecting a subset of samples, filtering can be carried out in the Filter pane, which offers a flexibility in the choice of options (based on the study, the food group and food code, but also on number of available sequences, nucleic acid target, etc.) notably higher

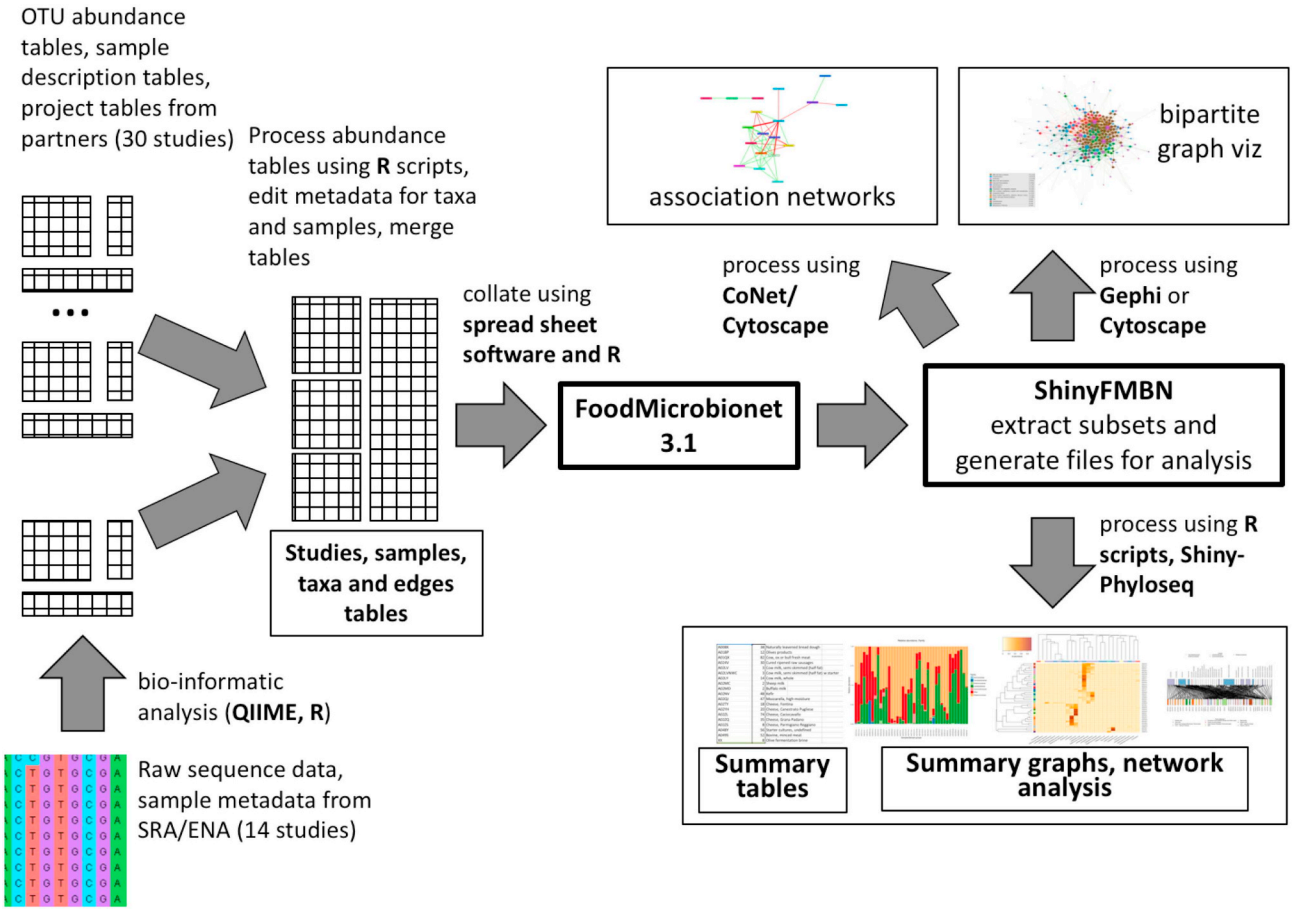

Fig. 2. A flow chart of the process of data addition to FoodMicrobionet 3.1 and data extraction and processing using the ShinyFMBN app, Gephi, Cytoscape, Conet, and R.

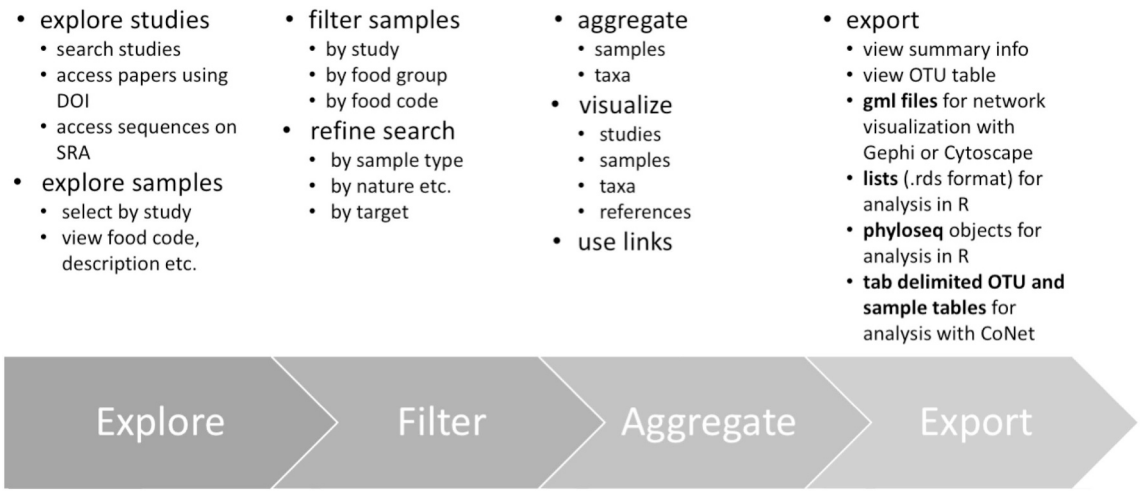

Fig. 3. A detailed flow chart of data extraction from FoodMicrobionet using the ShinyFMBN app.

than MGnify or QIITA. Once the filtering is completed, aggregation (of taxa and/or samples) can be performed in the Aggregate pane, which also allows to visualize summary information and access external resources (research articles, sequence data, information on taxa in taxonomic databases); a list of literature references for the subset of samples is provided for convenience.

We feel that one of the most useful features of the app is the possibility of exporting the data in a variety of formats, ready for statistical and graphical analysis. Use case scenarios for potential users with different objectives are presented in Supplementary Material using samples for fruit and vegetables extracted from the database. The data and R scripts used to analyze them are available on Mendeley data (https://data.mendeley.com/datasets/8fwwjpm79y/2).

Firstly, we have retained the possibility to produce graph files (in .gml format) which can be imported in free, open source graph visualization and analysis software, such as Gephi (https://gephi.org; Bastian et al., 2009) and Cytoscape (https://cytoscape.org; Shannon et al., 2003) to represent food microbiome data as a bipartite network. An example is provided in Fig. 4 (a high-resolution version is provided as Supplementary Material). This representation is a map of the microbiota of different food products, where the most abundant and prevalent taxa are close to the food groups where they are dominant, while minor taxa are pushed to the border of the graph. In addition, areas of the graph dominated by food groups of similar nature (fermented products, both of dairy and meat origin; unfermented raw meat products, fruits, vegetable products, composite dishes, etc.) are visible. In addition, Gephi can be used to interactively explore the data (see Supplementary Material and material available on Mendeley data, https://data.mendeley.com/datasets/8fwwjpm79y/2).

ShinyFMBN can be also used to extract the data in a file format which is suitable for use with CoNet, a Cytoscape app for the inference of microbial association networks (Faust and Raes, 2016). Examples are available in Parente et al. (2018).

In addition, data can be extracted in formats which can be readily used for statistical and graphical analysis. Two formats are provided: one that can be used in the R package phyloseq (McMurdie and Holmes, 2013, 2015), providing a suite of functions for the reproducible analysis of microbiome data, and another (in the form of a list including study information, references, taxa and sample metadata and abundance tables) which can be used in a workflow we designed to carry out both alpha and beta diversity analyses. Examples using data for vegetable and fruits extracted from FoodMicrobionet are shown in the supplementary material (Supplementary Table 2 and Supplementary Figs. 1 to 6).

Finally, OTU tables, taxa descriptions and sample metadata tables obtained from FoodMicrobionet can be easily modified and used in online analysis tools (such as MicrobiomeAnalyst, Dhariwal et al., 2017).

We feel that the range of output files provided by the app would satisfy the needs of most users and is significantly richer than that provided by other tools, such as MGnify. With little additional coding effort, interested users can quickly compare their own data with data extracted from FoodMicrobionet. A proof of concept is available as Supplementary Material and on Mendeley data (https://data.mendeley.com/datasets/8fwwjpm79y/2; a R script that use as an input sequence data from Pérez-Díaz et al., 2019; these data are not included in FoodMicrobionet but are available from NCBI SRA archive with accession number SRP132735). The procedure illustrates how a phyloseq object (McMurdie and Holmes, 2013) obtained with a pipeline based on DADA2 (Callahan et al., 2016b) can be merged with a phyloseq object extracted from FoodMicrobionet. The resulting object can be readily used for statistical and graphical analysis, allowing easy comparison of own samples with previous studies.

3.4. Limitations

One of the criticisms which can be made to our approach is that it literally puts together apples and oranges (Clooney et al., 2016), i.e. studies in which different wet lab and dry lab procedures and bioinformatic pipelines, each with its own set of biases and problems, are used. Although this is true, we feel that the advantage of being able to access a large collection of data still offsets the potential disadvantages. In a previous study, we showed that when data are compared at a genus or higher level of aggregation (possibly grouping samples in food groups), the use of different bioinformatic pipelines gives comparable results (De Filippis et al., 2018b). However, comparisons between older studies, mostly carried out using the Roche 454 platform and targeting the V1-V3 region, and more recent studies, frequently using Illumina protocols targeting the V4 or the V3-V4 region of the 16S rRNA gene, may be more difficult. Different targets and sequencing platforms may indeed generate different results for the same samples (Clooney et al., 2016; Fouhy et al., 2016) and it is clear that the development of standard operating procedures (SOPs) shared by the community of food microbiologists and the use of internal controls (in the form of mock communities) would be invaluable in the future. As an example, SOPs for human microbiome data generation and analysis span from sample collection and handling, through DNA extraction and sequencing, till data analysis (http://www.microbiome-standards.org/), and the need for best practices has been recently reviewed (Pollock et al., 2018). Such level of shared standardization does not exist for food microbiome studies, and even QIITA and MGnify, whose analysis pipelines are

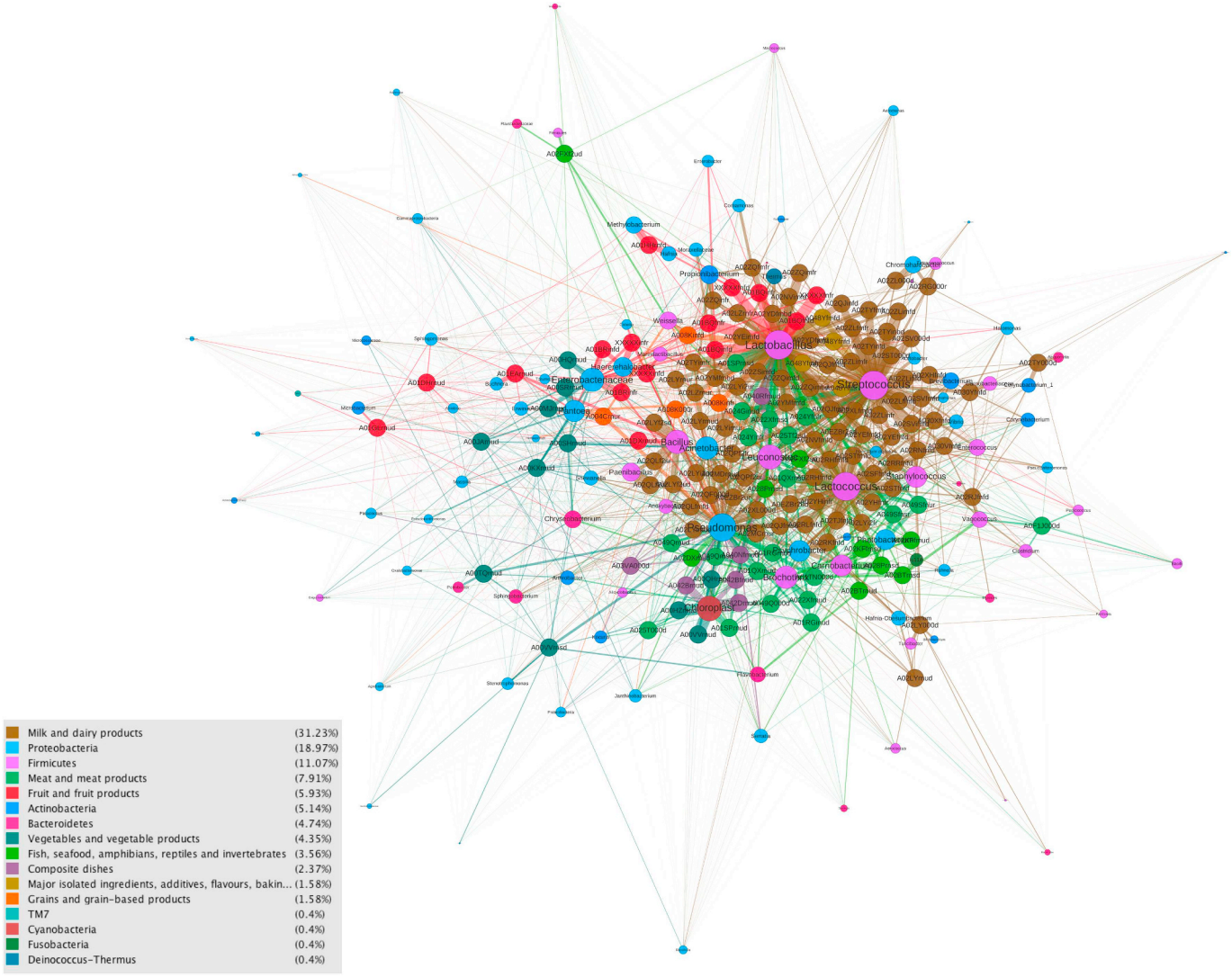

Fig. 4. A bird's view of FoodMicrobionet as a bipartite network, whose nodes are sample groups and taxa (aggregated at the genus level or above). Data were extracted from FoodMicrobionet by aggregating taxa at the genus level and samples at the expanded food label level. A .gml file was exported using the ShinyFMBN app, and imported in Gephi 0.9.2. After calculation of node statistics, including weighted degree, a filter was applied to retain only nodes with a weighted degree > 8 (i.e. for OTU nodes, only nodes with a sum of abundance in all connected food samples or food environments 8%; weighted degree for sample groups is 100 by default). After application of style elements (size of nodes was related to weighted degree, while the colour matched major food groups or phyla; size of edges was made proportional to weight), a combination of a Force Atlas 2 and no overlap layouts was applied. For a list of codes for food or environmental nodes see Supplementary Table 1. The codes include 5 characters codes from the FoodEx 2 classification and four further characters specifying nature (r raw material or ingredient; i intermediate; f finished, including the products during storage; 0 for environmental samples or when this information is not available), lethal process (n none; m mild; 2 type 2; 1 type 1; 0 for environmental samples or when this information is not available), fermentation/spoilage (u not spoiled nor fermented; f fermented; s spoiled; b spoiled and fermented; 0 for environmental samples or when this information is not available) and target (d DNA; r RNA). See supplementary material for details and for a .pdf version of this figure.

regularly updated, still cannot address problems in comparing studies related to the use of different sequencing platforms or nucleic acid target. With this in mind, we designed FoodMicrobionet in such a way that study metadata on gene targets, regions and bioinformatic pipeline used to process the data are easily accessible and can be used in searches to select studies/samples which have been processed with identical or similar approaches, thus providing the potential user with enough information to operate an informed choice.

Compared to online tools, such as QIITA or MGnify, access to FoodMicrobionet requires additional software installation (R and RStudio, both of which are frequently used by molecular microbial ecologists). Although this might be felt as a limitation, the Shiny-FMBN app, once installed, can be used off line, and operates in a browser window in a simple and user-friendly way. An Internet connection is only needed if the user plans to access external resources.

4. Conclusions

We believe that FoodMicrobionet 3.1 may be a valuable tool for food microbiologists wishing to carry out meta-studies on food microbial communities, and may facilitate the integration and accessibility of meta-omics data for food safety and quality studies (Cocolin et al., 2017). We plan to continue maintaining and expanding the database and to add new features. For example, further ecological data may be of interest for food microbiologists wishing to explore FoodMicrobionet: surprisingly, information on relevant chemical- or physico-chemical features of samples (pH, a_W, presence and/or concentration of preservatives, Eh) is often lacking or incomplete in published papers and in run info tables deposited in NCBI SRA. In the future, we plan to add these information at least in the form of categorical data by either retrieving it from the original paper or inferring it by analogy with similar

foods. We also plan to re-analyze older studies using the same pipeline used for most recents accessions. Although these may not necessarily make studies more comparable (older studies mostly target V1-V3 region using Roche 454 platforms), it may allow to include sequence tables with amplicon sequence variants (ASVs) in the database, thus allowing users to rerun taxonomic assignment whenever new releases of taxonomic assignment databases become available.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108249.

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