FoodMicrobionet v4: a large, integrated, open and transparent database for food bacterial communities.

Eugenio Parente, Teresa Zotta, Annamaria Ricciardi

Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Potenza, Italy

Corresponding author

Prof. Eugenio Parente

Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali,
Università degli Studi della Basilicata,
Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy

Email: eugenio.parente@unibas.it
Tel.: +390971205561

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Abstract

With the availability of high-throughput sequencing techniques our knowledge of the structure and dynamics of food microbial communities has made a quantum leap. This knowledge is however dispersed in a large number of papers and hard data are only partly available through powerful on-line databases and tools such as QIITA, MGnify and the Integrated Microbial Next Generation Sequencing platform, whose annotation is, however, not optimized for foods.

Here, we present the 4th iteration of FoodMicrobionet, a database of the composition of bacterial microbial communities of foods and food environments. With 180 studies and 10,151 samples belonging to 8 major food groups FoodMicrobionet 4.1.2 is arguably the largest and best annotated database on food bacterial communities. This version includes 1,684 environmental samples and 8,467 food samples, belonging to 16 L1 categories and 196 L6 categories of the EFSA FoodEx2 classification and is approximately 4 times larger than previous version (3.1, https://doi.org/10.1016/j.ijfoodmicro.2019.108249). Both FoodMicrobionet and related data and software conform to FAIR (Findable, Accessible, Interoperable, Reusable/Reproducible) criteria for scientific data and software and are freely available on public repositories (GitHub, Mendeley data).

Even if FoodMicrobionet does not have the sophistication of QIITA, IMNGS and MGnify, we feel that this iteration, due to its size and diversity, provides a valuable asset for both the scientific community and industrial and regulatory stakeholders.

Key words: amplicon targeted high-throughput sequencing; 16S metagenomics; bacterial microbiota; database

1. Introduction.

In less than 15 years, next generation sequencing has added several layers of complexity to the study of food microbiomes. At the time of writing this article (January 2022) searches with the key words “microbiome” OR “microbiota” in Scopus and in Web of Science retrieved 118,416 and 127,424 hits, respectively. The number of papers using amplicon targeted next generation sequencing to study the structure of food bacterial communities has been rising exponentially since the first pioneering publications (Humblot and Guyot, 2009; Roh et al., 2010) (see Figure 1).

The evolution of -omic approaches to the study of the food microbiome and the methodological challenges in wet laboratory methods, sequencing and bioinformatic and statistical approaches have been reviewed multiple times (Bokulich et al., 2020; De Filippis et al., 2018; Pollock et al., 2018). While amplicon targeted approaches are still prevalent, with the decreasing cost of sequencing and the increase in power of sequencing platforms and of computational tools the number of studies using metagenomic or multi-omics approaches is rapidly increasing (Yap et al., 2021).

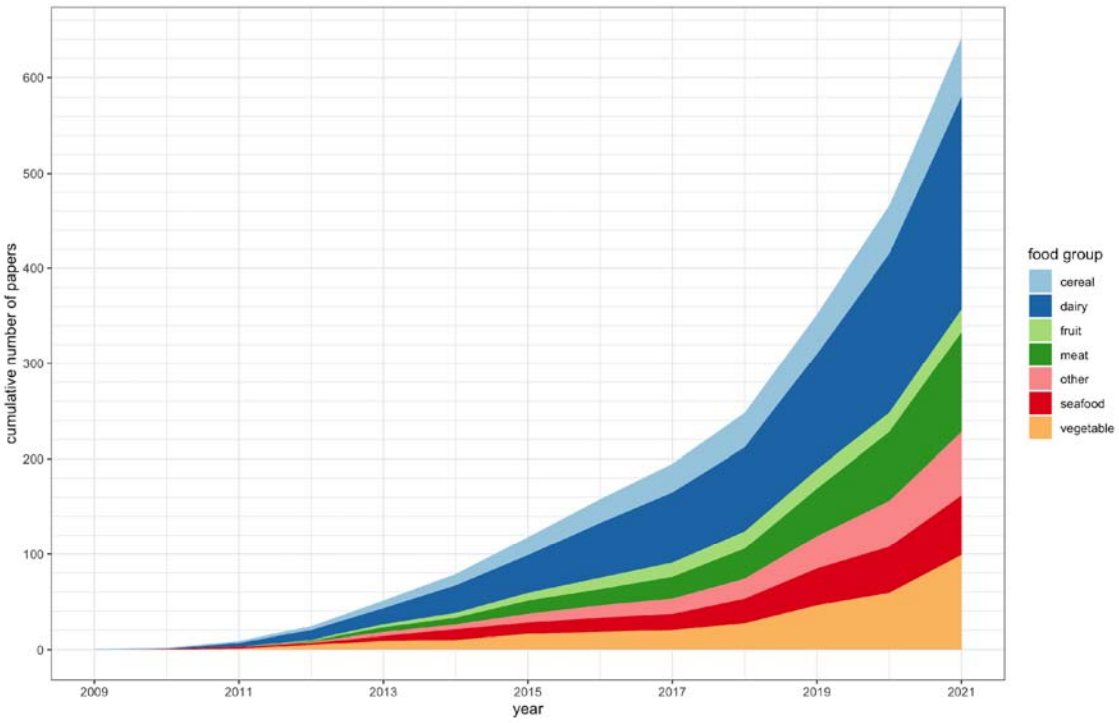

Figure 1. Cumulative number of papers using metataxonomic or metagenomic approaches for the study of food microbial communities. The data derive from a manually curated search on Web of Science™.

Shotgun metagenomics offers a significantly higher resolution (down to the strain and perhaps to single nucleotide polymorphism variants (Hildebrand, 2021) and paves the way to accurate source tracking for contamination (De Filippis et al., 2020) and to a new paradigm in food safety (Kovac, 2019). However, amplicon targeted approaches still have the power to describe, in a sensitive and cost-effective way, the structure of food microbial communities down to the genus level or below (Callahan et al., 2016a).

More than 640 papers describing the food microbiota have been published since 2009. Exploitation of this wealth of information for metastudies or for the development of descriptive or predictive tools requires FAIR (findable, accessible, interoperable,

reusable/reproducible: Lamprecht et al., 2020; Wilkinson et al., 2016) data and software.

Three large on line repositories on microbiome data have appeared in recent years: IMNGS (Lagkouvardos et al., 2016), QIITA (Gonzalez et al., 2018) and MGnify (Mitchell et al., 2019).

The Integrated Microbial Next Generation Sequencing platform directly accesses 16S rRNA gene targeted next generation sequencing data on NCBI Short Read Archive and provides powerful tools for searching for taxa or sequences or for performing analysis of user deposited 16S sequences (Lagkouvardos et al., 2016).

MGnify hosts metagenomic, metatranscriptomics and metataxonomic datasets which are either contributed by users or obtained from public repositories and offers powerful platforms for data analysis but does not allow the integration of data from different studies (Mitchell et al., 2019).

QIITA offers a powerful suite of tools for the analysis of sequences of public and private datasets, and allows the search and integration of data from different studies (Gonzalez et al., 2018).

At the time of writing of this paper (January 2022) MGnify included 3,696 public studies, and 325,323 public samples, but only 83 studies/2,805 samples on food biomes (https://www.ebi.ac.uk/metagenomics/) while QIITA included 620 public studies and 303,313 public samples but only a very limited number of public studies on food biomes (https://qiita.ucsd.edu/stats/). The data and interfaces for all these tools respond well to FAIR principles for scientific data (Wilkinson et al., 2016) but, unfortunately, the structure of the metadata for studies and samples is not optimized for foods.

Some years ago, we created a database for metataxonomic studies on food bacterial communities, FoodMicrobionet (Parente et al., 2019, 2016), whose main strength is the annotation system for studies and samples, based on the hierarchical classification of foods

developed by the European Food Safety Authority, FoodEx 2(EFSA, 2015). Here we describe the structure and new features of the latest version of the database.

2. Materials and methods.

The procedure used to add data to FoodMicrobionet has not changed significantly since the last version (Parente et al., 2019) and it is represented schematically in Figure 2.

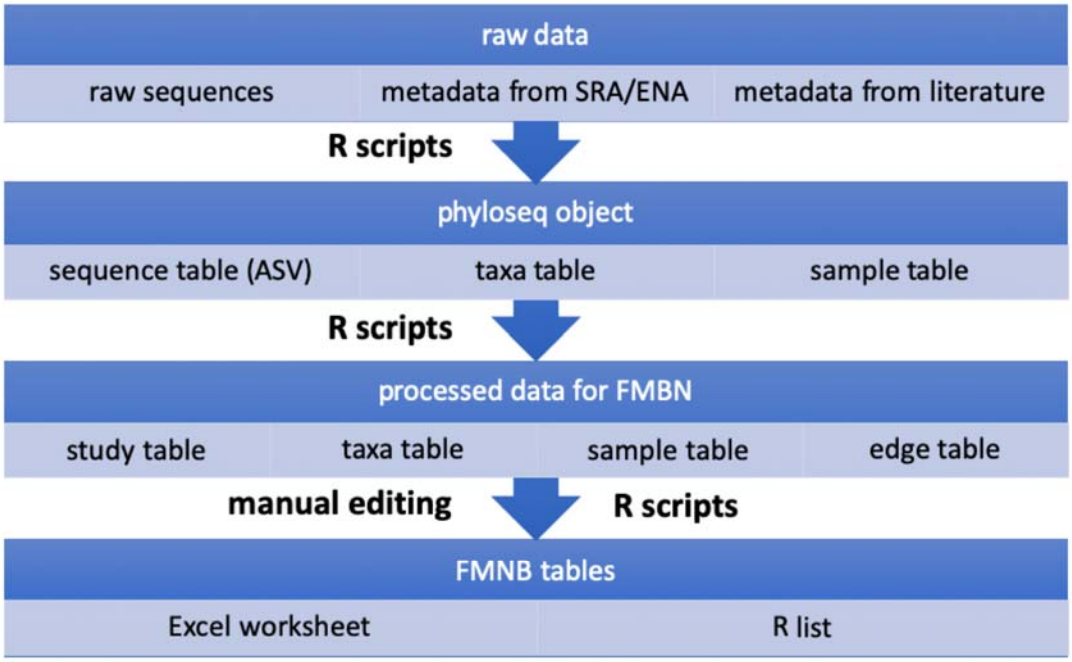

Figure 2. Schematic workflow showing how raw sequences and their metadata and additional information from the scientific literature are used to assemble data for FoodMicrobionet.

With the exception of 33 studies (out of 180), which were originally obtained by research partners as abundance tables for taxa and sample metadata tables (Parente et al., 2016), all

studies in FoodMicrobionet are obtained by reprocessing raw sequence data from NCBI Short Read Archive (SRA), and by using metadata from SRA and from the papers. Processing of sequences is carried out in R (R Core Team, 2021) using a modified version of the Bioconductor pipeline for amplicon targeted sequence analysis, using DADA2 for Amplicon Sequence Variant (ASV) inference and SILVA v138.1 for taxonomic assignment (Callahan et al., 2016a; Callahan et al., 2016b). The analysis results in the production of phyloseq (McMurdie and Holmes, 2013) objects which are further processed by R scripts and imported in Microsoft Excel for further manual editing of study and sample metadata. Finally, a R script is used to process Excel tables and for quality control checks. All scripts used for processing are publicly available in the FoodMicrobionet GitHub repository (https://github.com/ep142/FoodMicrobionet).

The structure of the database is schematically shown in Figure 3.

Studies, samples and taxa have all a rich metadata structure. The structure of the tables is described in detail on GitHub (https://github.com/ep142/FoodMicrobionet/blob/master/FoodMicrobionet_tablespecs.md), on Mendeley data (https://data.mendeley.com/datasets/8fwwjpm79y/5) and in supplementary material.

Recent additions compared to version 3 (Parente et al., 2019) include four fields which complete information for bioinformatic processing and one field for geolocation for studies, geolocation information for samples, and two further reference tables (primers and the FoodEx2 Exposure Hierarchy classification (EFSA, 2015).

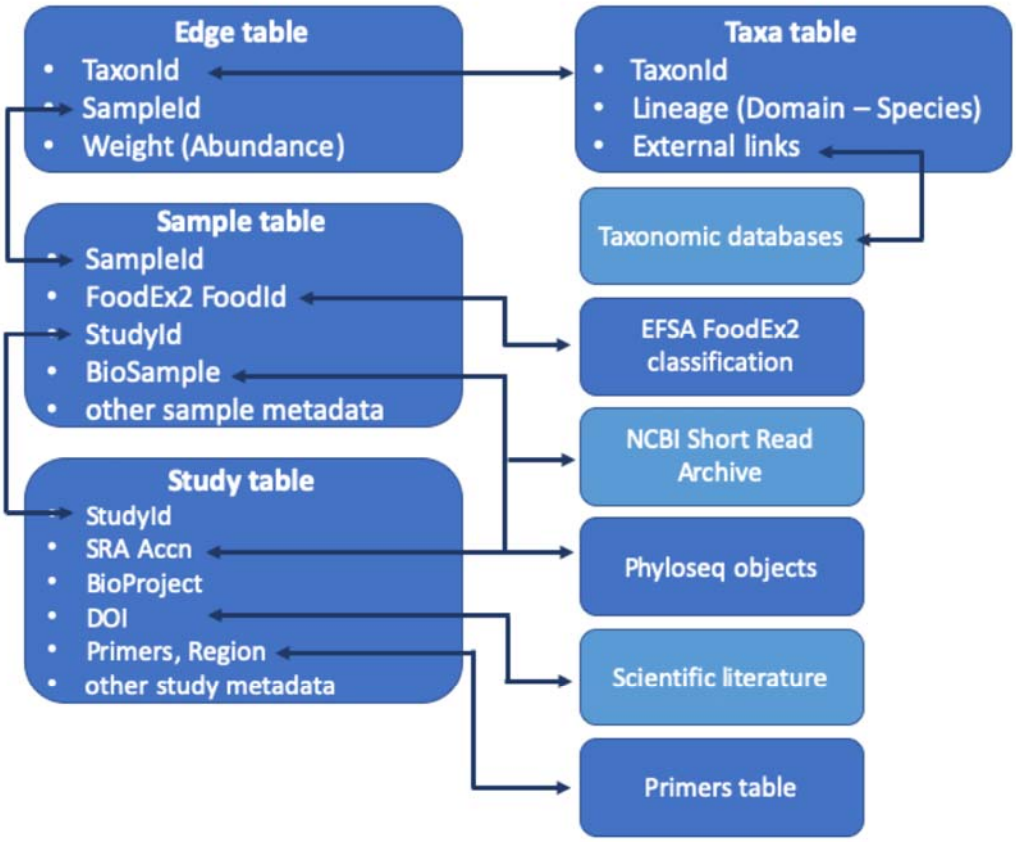

Figure 3. Schematic representation of the structure of FoodMicrobionet tables (Study, Primers, Samples, FoodEx2, Taxa, Edges) and their relationship with phyloseq (McMurdie and Holmes, 2013) objects and external databases.

FoodMicrobionet is available either as a R list, which allows experienced programmers to run their searches and analyses in the most flexible and sensitive way and as an interactive Shiny app (Parente et al., 2019) which requires minimum installation and configuration and allows users to perform searches using a large number of criteria, to perform aggregation of samples and taxa, to rapidly reach external resources using hyperlinks, to export data in a variety of formats, and to obtain and save graphs and tables (Parente et al., 2019).

Use cases and example workflows have been illustrated in a previous work (Parente et al., 2019) and we have tried to demonstrate this approach in a series of proof-of-concept metastudies (Parente et al., 2020, 2021; Zotta et al., 2021). The code for generating graphs and statistical analyses is fully reproducible and reusable (https://data.mendeley.com/datasets/8fwwjpm79y/4; https://github.com/ep142/MAN_in_cheese) and allows to reproduce the figures and tables when a new version of FoodMicrobionet is published.

3. Results and discussion.

3.1 FoodMicrobionet facts and figures.

FoodMicrobionet v 4.1.2 has grown significantly since its last iteration (Parente et al., 2019; Figure 4): the number of studies has grown from 44 to 180, and the number of samples from 2,237 to 10,151.

The growth of FoodMicrobionet reflects the growth of published studies and the distribution of target gene regions reflects the shift in technology in Next Generation Sequencing, from the defunct Roche 454 platform (with most studies targeting the V1-V3 region) to Illumina platforms, with V4 and V3-V4 regions of the 16S rRNA gene as the most frequent targets (Figure 4, Supplementary Table 1).

The number of reads for sample after processing is also saved in the database (Supplementary Figure 1): 70% samples have $>10^4$ reads after processing, but a significant number of samples in studies targeting the V4 region has $>5x10^4$ reads. This makes the detection of rare components of the microbiota possible.

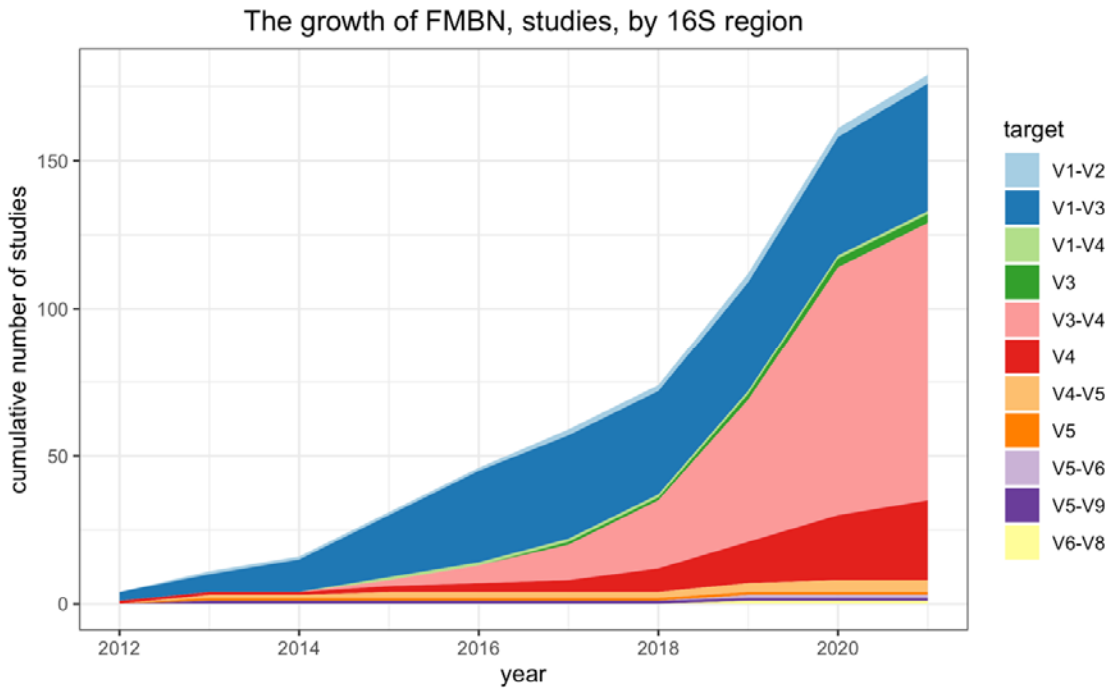

Figure 4. Cumulative distribution of studies in FoodMicrobionet 4.1.2, by year and by 16S rRNA gene target region.

The variety of food groups and food types in FoodMicrobionet is also very large: samples in FMBN belong to 16 major food groups (L1 level of FoodEx2 exposure classification; Supplementary Table 2). Approximately 80% of samples belong to the first three categories (Milk and dairy products, Meat and meat products, Vegetables and vegetable products). Samples in FMBN are further classified using levels L4 and L6 of the FoodEx2 exposure classification, and additional fields (which allow to identify raw products, intermediates or finished products, the level of thermal treatment and the occurrence of spoilage and/or fermentation) allow a finer classification. Samples in FMBN belong to 109 L4 food groups and 197 L6 food groups. There are 163 individual food types, and, combining further information on samples (nature, heat treatment, spoilage/fermentation), there are 316 combinations (Parente et al., 2019). This number and variety of samples makes

FoodMicrobionet the largest database of metataxonomic data on food bacterial communities, with significantly more samples compared to QIITA and MGnify. FoodMicrobionet stores samples with 51 countries, but the 90% of samples are from 14 countries only (Supplementary Figure 2). This does not reflect the distribution of samples and studies in published studies but rather, the distribution of those which are available in NCBI SRA.

There are currently 9,098 taxa in the taxa table of FoodMicrobionet. Taxa have a unique numeric and text identifier and may represent ASV identified at the species (4,497, 49.4% of taxa) or genus level (3,259, 35.8%) or above using the DADA2 assignTaxnomy() and assignSpecies() functions. The % of the taxa and sequences identified at the genus level or below varies by study, depending on the quality and length of sequences and on the gene target. Length of reads (in bp) in FoodMicrobionet studies varies between 150 and 610 bp (median 422). Although one may question the value of taxonomic assignment at the species level, especially for the shortest reads (Callahan et al., 2016a; Edgar, 2018; Johnson et al., 2019; Meola et al., 2019; Pollock et al., 2018), due to the detailed information provided for both studies and samples (gene target and region, the number of issues observed during processing of raw sequences) and to the possibility of accessing to the processed sequences (ASV) in phyloseq objects, users of FoodMicrobionet can make informed decision on how and when taxonomic units should be combined at a level higher than the species, an operation which is easily performed with the ShinyFMBN app (Parente et al., 2019).

FoodMicrobionet is fully connected to external databases: external links (as dynamically built Uniform Resource Locators) in the study, sample and taxa tables allow rapid access to external taxonomic databases (NCBI taxonomy, the List of Prokaryotic Names with Standing in Nomenclature and the Florilege database, Falentin et al., 2017

http://migale.jouy.inra.fr/Florilege/#&about) and to the scientific literature (via DOI). Using NCBI SRA Study accession number it is possible to access fine grained data on ASV sequence and abundance stored in the phyloseq objects obtained from processing the raw sequences (these are not public but available on request).

Is FoodMicrobionet FAIR?

FoodMicrobionet data and software are free (both are under MIT licence https://opensource.org/licenses/MIT), open and highly reusable and support analysis protocols which are reproducible. We have done our best (given the time and resource constraints) to conform as closely as possible to criteria for FAIR (Findable, Accessible, Interoperable, Reusable/Reproducible) data and software sharing (Lamprecht et al., 2020; Wilkinson et al., 2016).

Both the database and the software are Findable (using searches on Google, Mendeley data, or GitHub, for example) and deposited on permanent repositories (Mendeley data, GitHub) with unique identifiers. Even if data within FoodMicrobionet may not be directly findable by automated machine searches (and insofar they are not machine operable), since the database is not available on line except in the form of R lists or tab delimited text files, the richness of contextual metadata for all the main tables (studies, samples, taxa) makes it possible to devise precisely targeted searches.

FoodMicrobionet is Accessible through the above-mentioned repositories and through our website, and are confident that enough metadata are provided in these repositories to easily reach the resources. In terms of user accessibility (which is not a criterion in FAIR principles), we have done our best to make the resource accessible to both expert (the database in the form of a R list can be used for fine-tuned searches and analyses using R

scripts) and moderately expert users. The latter can, with a minimum effort, download and install the R Shiny app, ShinyFMBN (Parente et al., 2019), which, once launched in R, allows easy access via an interactive and intuitive interface. A detailed manual for the app is available on Mendeley data (https://data.mendeley.com/datasets/8fwwjpm79y/4). Although the app could be easily deployed on RStudio Shiny apps server (https://www.shinyapps.io), we feel that this would make the use unnecessarily slow.

FoodMicrobionet is fully Interoperable. The sample classification is based on a robust hierarchical classification, FoodEx2, rather than on arbitrary keywords and dynamic links are created in the studies, samples and taxa tables to reach a number of other databases. Conversely, other databases like Florilege might quite simply create new accessions using FoodMicrobionet and metadata in FMBN can, in principle, be used to populate QIITA and MGnify, by linking studies and samples via the SRA accession numbers.

Data and products of search results are highly Reusable, for the same reasons. In addition, the objects exported by the app are in formats which are compatible with metataxonomic analysis pipelines (like phyloseq and ShinyPhyloseq: (McMurdie and Holmes, 2015, 2013); MicrobiomeAnalyst: (Chong et al., 2020; Dhariwal et al., 2017); CoNet: (Faust and Raes, 2016); graph visualization and analysis software like Cytoscape and iGraph: (Csardi et al., 2006; Shannon et al., 2003), microbial association network inference tools: (Kurtz et al., 2015; Peschel et al., 2021).

5. Conclusions.

Even if FoodMicrobionet does not have the sophistication of QIITA, IMNGS and MGnify, we feel that this iteration, due to its size and diversity, provides a significant resource for both the scientific community and industrial and regulatory stakeholders. Scientists can access

and use a variety of stand-alone or online software tools and ShinyFMBN to compare their own results with literature results, carry out metastudies to answer a variety of scientific questions, build reproducible analysis workflows, get quantitative data on the ecology/distribution of bacteria of interest, use the database as an entry point for further searches in other databases. The size of this version, which includes >$9x10^5$ taxon/sample relationships, might even allow the machine learning approaches to predict contamination patterns of food. The ability to rapidly retrieve information on prevalence/abundance of taxon in different foods and on the structure of microbial communities in different food types may be useful to both the industry and regulatory agencies. Information on the distribution of beneficial genera and species may find use for regulatory purposes (for example to facilitate studies on the distribution of beneficial microorganisms to evaluate their inclusion in the Qualified Presumption of Safety). The fine-grained data on the structure of microbial communities for a large variety of raw materials, foods, food environments may be useful for both process and product development purposes to identify spoilage or contamination patterns, or for the design of microbiome-based starters. We are committed to keep adding data to FoodMicrobionet, but the openness and transparency of its software and documentation technically allows any interested party to create new versions of the database or to significantly improve its structure and functionality.

CRediT author statement

Eugenio Parente: Conceptualization, Methodology, Software, Writing- Original draft preparation. **Annamaria Ricciardi** Data curation, Writing - Reviewing and Editing. **Teresa Zotta**: Data curation, Writing - Reviewing and Editing.

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