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Eugenio Parente[1], Luca Cocolin[2], Francesca De Filippis[3], Teresa Zotta[4], Ilario Ferrocino[2], Orla O'Sullivan[5], Erasmo Neviani[6], Maria De Angelis[7], Paul D. Cotter[5], Danilo Ercolini[3]

Affiliations

1 Dipartimento di Scienze, Università degli Studi della Basilicata, Potenza, Italy

2 Department of Agricultural, Forest and Food Science, University of Torino, Grugliasco, Italy

3 Department of Agricultural Sciences, Division of Microbiology, University of Naples Federico II, Portici, Italy

4 Istituto di Scienze dell'Alimentazione, CNR, Avellino, Italia

5 Teagasc Food Research Centre, Moorepark, Fermoy and APC Microbiome Institute, Cork, Ireland

6 Department of Food Science, Parma University, Parco Area delle Scienze 48/A, Parma, Italy

7 Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Bari, Italy

Corresponding author

Prof. Eugenio Parente

Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano, 10, 85100 Potenza, Italy

E-mail eugenio.parente@unibas.it

tel +390971205561

Abstract

Amplicon targeted high-throughput sequencing has become a popular tool for the culture-independent analysis of microbial communities. Although the data obtained with this approach are portable and the number of sequences available in public databases is increasing, no tool has been developed yet for the analysis and presentation of data obtained in different studies. This work describes an approach for the development of a database for the rapid exploration and analysis of data on food microbial communities. Data from seventeen studies investigating the structure of bacterial communities in dairy, meat, sourdough and fermented vegetable products, obtained by 16S rRNA gene targeted high-throughput sequencing, were collated and analysed using Gephi, a network analysis software. The resulting database, which we named FoodMicrobionet, was used to analyse nodes and network properties and to build an interactive web-based visualisation. The latter allows the visual exploration of the relationships between Operational Taxonomic Units (OTU) and samples and the identification of core- and sample- specific bacterial communities. It also provides additional search tools and hyperlinks for the rapid selection of food groups and OTUs and for rapid access to external resources (NCBI taxonomy, digital versions of the original articles). Microbial interaction network analysis was carried out using CoNet on datasets extracted from FoodMicrobionet: the complexity of interaction networks was much lower than that found for other bacterial communities (human microbiome, soil and other environments). This may reflect both a bias in the dataset (which was dominated by fermented foods and starter cultures) and the lower complexity of food bacterial communities.

Although some technical challenges exist, and are discussed here, the net result is a valuable tool for the exploration of food bacterial communities by the scientific community and food industry.

Key words Food bacterial communities; Network analysis; 16S rRNA amplicon-based high-throughput sequencing.

1. Introduction

The degree of complexity of the microbiota that potentially impacts on food quality and safety is extremely variable. In both fermented and non-fermented foods, the type of contaminating microbiota can initially be rather diverse, reflecting mainly the original microbiota of the raw material, processing, handling and storage conditions and the level of good manufacture practice (Bokulich et al., 2012; Bokulich and Mills, 2013; Chaillou et al., 2015, Cocolin and Ercolini, 2015; De Filippis et al., 2013). However, depending on the storage conditions and other extrinsic factors, only a few species and strains will be able to develop sufficiently in the food matrix to significantly affect the food quality (by spoilage or fermentation) or safety. The methods employed to study microbes and microbial diversity in foods have evolved, and in turn, have also revolutionized our overall understanding of the microbial ecology of foods (Cocolin and Ercolini, 2015). The culture-independent evaluation of food microbial diversity by high-throughput rRNA gene sequencing has become an increasingly popular approach to food microbiology. After microbial nucleic acid extraction from the food, the DNA (or cDNA in cases where RNA was targeted) is used as a template to amplify variable regions within or across the rRNA genes of bacteria (16S) or fungi (internal transcribed spacer [ITS] or other target) and an amplicon library is then sequenced using high-throughput sequencing (HTS; Ercolini, 2013) platforms. The result is a food (sample)-specific profile of the microbiota where all the microbial entities are identified at variable taxonomic depth. Based on the number of sequence reads assigned to a given taxon, the relative abundance of each identified operational taxonomic unit (OTU) can be determined. Therefore, for each food sample

77 analysed, a clear understanding of the composition and relative abundances of the
78 microorganisms populating the food at that time can be provided. The advantages and
79 disadvantages of this methodology have been discussed elsewhere (Bokulich and Mills, 2013;
80 Ercolini, 2013).
81 Many different sequencing technologies are available for the generation of sequence data
82 (Glenn, 2011. http://www.molecularecologist.com/next-gen-fieldguide-2014). Regardless of
83 how the data is generated, accurate data analysis tools are pivotal in any study of microbial
84 ecology; from quality filtering to graphical representations, the software and the algorithms
85 selected can greatly impact on the results and interpretation. Essential steps in any analysis
86 pipeline include post-sequencing quality checking (based on both length and quality scores),
87 clustering into OTUs, chimera removal, alignment, taxonomical assignment and diversity
88 analysis. The choice of the pipeline has been proven to significantly affect the results in terms
89 of estimated diversity and microbial community structure (May et al., 2014). Diversity can be
90 calculated from both a within sample (alpha diversity) and between sample perspective (beta
91 diversity). Numerous packages have been developed for rRNA gene amplicon data analysis,
92 primarily designed for UNIX based operating systems. The most widely used packages are
93 QIIME (Caporaso et al, 2010) and MOTHUR (Schloss et al., 2009). They have become popular
94 because they provide a pre-compiled and user-friendly analysis pipeline, but also due to their
95 constant maintenance and updates.
96 Beyond the power linked to the sensitivity and the throughput of sequencing-based
97 microbiota analysis, a fundamental advantage is the possibility of using the raw sequence data
98 in meta-studies. In fact, in contrast to previous culture-independent approaches, the
99 sequencing-based tools offer the unprecedented advantage of making the results readily
100 available for the scientific community through the deposit of the sequences in public
101 databases (e.g. the Sequence Read Archive (SRA) of the National Center for Biotechnology

102 Information (http://www.ncbi.nlm.nih.gov/Traces/sra) or the European Nucleotide Archive
103 of the European Bioinformatics Institute (http://www.ebi.ac.uk/ena). This allows researchers
104 to easily access datasets corresponding to diverse food samples generated by different
105 laboratories and with different scopes.
106 Network analysis (Newman et al., 2006) tools have recently been used to provide effective
107 and information dense displays of microbial communities for several environments (de
108 Menezes et al., 2014; Deng et al., 2012; Muegge et al., 2011; Zhou et al., 2011a), including
109 foods (Chaillou et al., 2015; De Filippis et al., 2013; De Filippis et al., 2014; Dolci et al., 2014;
110 Ercolini et al., 2013; Oakley et al., 2013). In a network representation, objects (OTUs and/or
111 samples) represent the nodes (or vertices), and are connected by links (edges). The edges can
112 be directed (i.e. when the direction of the connection is of importance) or undirected and are
113 usually associated with a weight. The latter can store information on the abundance of an OTU
114 in a sample or the probability of a significant co-occurrence/co-exclusion relationship.
115 Two types of displays have been used in microbial ecology. In OTU - sample nets, the network
116 is bipartite i.e. two types of nodes exist, sample and OTU nodes, and connections occur only
117 between samples and associated OTUs. Conversely, co-occurrence/co-exclusion networks,
118 which show significant positive or negative interactions among members of microbial
119 communities have rarely (Chaillou et al., 2015; Mounier et al., 2008; Oakley et al., 2013) been
120 used in food microbial ecology, but have been successfully applied to the study of the
121 microbial communities of a variety of environments (miscellaneous environments: Deng et al.,
122 2012; water: Liu et al., 2014; soil: Zhou et al., 2011a) and for the human microbiome (Faust et
123 al., 2012). The methods and models used to derive interaction networks have been reviewed
124 by Faust and Raes (2012) and, although they are riddled by pitfalls related to the structure of
125 the data and to the sensitivity to the methods and parameters selected in the analysis (Faust
126 and Raes, 2012; Kuczynski et al., 2010), they offer significant advantages with respect to

127 detecting biologically and ecologically relevant relationships among members of microbial
128 communities.
129 The number of HTS studies of food microbial communities has been increasing steadily in
130 recent years (Mayo et al., 2014), but the information is dispersed in a large number of papers,
131 each analysing a single or a limited range of foods. It is therefore tempting and timely to
132 collect and integrate data from several studies in such a way that the results can be readily
133 searched and visualized, even by relatively inexperienced users. With the aim of providing
134 flexible means for meta-studies in food microbial ecology, here we present FoodMicrobionet,
135 a database and visualisation tool based on network analysis, and some examples of the
136 potential of the tool in terms of data display and analysis.
137

138 **2. Material and methods**

139 *2.1. Data sources*

140 FoodMicrobionet 1.0 includes data from 17 studies, on dairy products, dairy starter cultures,
141 raw and fermented meat, doughs and sourdoughs, or fermented vegetables. The list of studies,
142 with information on the sequencing platforms, software employed for bioinformatics analysis,
143 and the databases used for OTU assignment is shown in Table 1.

144 *2.2 Data tables*

145 Abundance tables, including taxonomic lineages for each OTU, were obtained from each
146 contributor and transformed in tab-delimited nodes and edges tables, which were collated
147 and curated to remove duplicates. The node and edge tables and their specifications are
148 provided in section 1 of Supplementary Material.

149 *2.3. Network analysis*

150 2.3.1. OTU - Food network

151 The edges tables were imported in Gephi 0.8.2-beta (http://gephi.github.io/; Bastian and
152 Jacomy, 2009) using the "Import spreadsheet" feature. Nodes tables were then imported to
153 retrieve the metadata for each node. Statistics (degree and weighted degree, centrality
154 statistics, network diameter, graph density, average path length) were then calculated for
155 each node and for the network using the statistical module of Gephi. A glossary of terms for
156 node and network statistics is provided in Table 2. Styles were then applied to the nodes to
157 enhance the display: the colour of the node was attributed on the basis of a custom field
158 containing families for OTUs and Food subgroup for samples; the size of the nodes was made
159 proportional to the weighted degree of the node; edge thickness was made proportional to the
160 weight of the connection. A Yfan Hu force based layout algorithm was finally applied (Hu,
161 2006). Simplified versions of the networks were obtained by filtering. The whole network was
162 then exported for web visualisation using the Sigmajs exporter plugin of Gephi.
163 2.3.2. Microbial interaction networks
164 Microbial interaction networks were generated for selected groups of samples extracted from
165 FoodMicrobionet using the CoNet app (Faust et al., 2012) of Cytoscape 3.2.1. OTU abundance
166 (as number of sequences per sample) tables were then imported using CoNet, and five
167 methods (Pearson, Spearman, Mutual information, Bray Curtis, Kullback-Leibler) were used
168 to mine for significant co-occurrence/co-exclusion relationships. Null distributions were
169 generated using the edge-scores routine and random distributions using the bootstrap
170 routine. Brown's method was used to merge method specific p-values and the Benjamini
171 Hochberg method was used to adjust the p-values for multiple testing. Interactions were
172 evaluated for both low level taxa and high level taxa, but a parent child exclusion filter was
173 used to avoid interactions between a high level taxon and its members (e.g.. between
174 *Lactobacillaceae* and members of the genus *Lactobacillus*). To simplify network visualisations,
175 interactions with high level taxa were included only if they did not duplicate interaction due

176 to a lower level taxon (i.e. if *Lactobacillales* and *Lactobacillus delbrueckii* shared the same
177 interactions, the former node was removed). Topological properties of the interaction
178 networks were evaluated using the NetworkAnalyzer tool of Cytoscape.
179
180 **3. Results and discussion**
181 *3.1. Building FoodMicrobionet: a bipartite OTU-sample network for food bacterial communities.*
182 The main purpose of FoodMicrobionet is to provide a user-friendly tool to explore multiple
183 datasets generated by 16S rRNA gene amplicon HTS studies of food bacterial communities.
184 The flowchart for the development of FoodMicrobionet and of its products (visualisations,
185 tables, graphs) is shown in Fig. 1. Data from seventeen published and unpublished studies
186 (Table 1) on dairy and meat products, starter cultures, sourdoughs or fermented vegetable
187 products (olives) were assembled in a database and a network was generated using Gephi
188 0.8.2-beta. The network has 964 OTU nodes and 552 sample nodes, with 18,115 edges
189 (sample-OTU relationships), and is by far the largest such collection of data of food bacterial
190 microbiota.
191 Network analysis software packages such as Gephi or Cytoscape (edges and nodes files
192 provided in supplementary material can be easily imported in Cytoscape) offer a wide range
193 of filtering, statistical analysis and graphical representation options but require some
194 informatics skills. Therefore an interactive network visualisation
195 (http://www.foodmicrobionet.org/fmbn1_0_3web/) was created using a publicly available
196 plugin. This visualisation allows even inexperienced users to explore FoodMicrobionet, to
197 select individual sample or OTU nodes, or to carry out group selections for sample and OTU
198 nodes. Relevant properties for both OTU and food sample nodes can be visualised by either
199 clicking on nodes or by selecting them using a search field. A user manual for the web
200 visualisation is provided in section 2 of Supplementary material.

201 Because of the high number of sample and OTU nodes, the information cannot be easily
202 presented into a readable graph. Therefore, simplified, filtered and node-labelled sub-
203 networks for meats, sourdoughs and dairy foods are shown in Fig. 2. The common features
204 found in OTU-sample networks previously published (De Filippis et al., 2013; De Filippis et al.,
205 2014; Dolci et al., 2014; Ercolini et al., 2013) are evident: sample nodes with similar
206 microbiota occupy defined areas of the graph and are close to the OTU nodes that dominate
207 their microbiota. This allows to identify easily the dominant, core and minor OTUs, that can be
208 clearly distinguished by their position and by their node size.
209 Taxon specific sub-networks can be easily extracted. Examples for members of the families
210 *Pseudomonadaceae* and *Enterobacteriaceae* are presented in Supplementary Fig. S1 and S2. In
211 this version of the display the size of sample nodes is related to the cumulative abundance of
212 the taxon and the size of the OTU nodes is related to the cumulative abundance of the OTU in
213 the sub network. Edge thickness gives an estimate of the abundance of a given OTU in each
214 sample, while colours can be used to estimate the relative abundance of food groups in which
215 the selected taxon is found.
216 The node degree distribution for OTU nodes is shown in Supplementary Fig. S3. The
217 distribution fits, albeit with a relatively low R^2 (0.832), a power law distribution with an
218 exponent (γ) of 1.12±0.04. Node degree power law distributions are indicative of a scale-free
219 network (Dunne et al., 2002; Newman et al., 2006). Such networks are widely distributed in
220 all fields (social networks, internet networks, power grids, bibliographic networks) and share
221 several properties. They are usually large and complex, highly connected (large average
222 degree), with a high number of nodes with low degree (in the case of FoodMicrobionet OTUs
223 which are found only in one or few food samples) but with a small numbers of OTUs
224 connected to a large number of samples (i.e. the 'signature' OTUs which make the core
225 microbiota of a given group of food samples). Because FoodMicrobionet 1.0 includes different

226 food groups, several signature OTUs with high degree are found, and this may affect the fit of
227 the power law distribution.
228 FoodMicrobionet can also be used to obtain further information on distribution of taxa in
229 different food groups by filtering and recalculation from nodes and edges tables. Information
230 on dominating OTUs can be gathered by plots showing the weighted degree distribution (i.e.
231 how abundant an OTU is in the whole dataset or in a subset) as a function of relative
232 occurrence (i.e. the fractions of samples in which an OTU is found). An example for raw meat
233 is shown in Fig. 3. Further examples for raw milk and mozzarella are shown in Supplementary
234 Fig. S4 and S5. More traditional plots for OTU distribution can also be obtained. An example of
235 the distribution of OTU belonging to different phyla in different food groups is shown in
236 Supplementary Fig. S6.
237
238 *3.2. Microbial interaction networks.*
239 Microbial interaction networks may help in formulating inferences on the phenomena
240 underlying the structure of food microbial communities, from co-occurrence or co-exclusion
241 patterns due to the occupation of different niches or to selective conditions allowing the
242 growth of a subset of taxa, to relationships such as amensalism, commensalism, symbiosis,
243 etc. The inference of microbial interactions is still affected by pitfalls: the results may be
244 strongly affected by the level of coverage of the microbial community, by the bioinformatics
245 pipelines used with specific options for clustering of the sequences and taxonomic
246 assignment, by the procedures used in normalization and by the methods used to estimate the
247 relationships, etc. However, robust methods have been developed to perform this analysis
248 (Faust et al., 2012). Since OTU abundance tables were available for all datasets included in
249 FoodMicrobionet, we explored co-occurrence/mutual exclusion patterns for all datasets for
250 which a high enough number of samples was available. Statistics for all interaction networks

251 are shown in Table 3 but a detailed discussion of microbial interactions is beyond the scope of
252 this paper and only two examples are discussed below.
253 A microbial interaction network for the kefir dataset (Marsh et al., 2013) is shown in Fig. 4.
254 The dataset included milk kefir and grains from different sources. Due to the very simple
255 structure of bacterial communities in kefir and kefir grains only a few interactions were
256 significant. The network has a very low complexity (7 nodes, average degree 3.41 and average
257 path length 2.19), with a clustering coefficient of 0.714, and no fit of the power law for the
258 node degree distribution. The occurrence of *Acetobacter* was negatively related with the
259 occurrence of *Lactobacillales* and that of *Lactobacillus* with *Leuconostoc*, *Lactococcus* and
260 *Streptococcus*. In fact, while *Lactobacillus* dominated the kefir grain microbiota, the latter
261 genera showed a better ability to grow in milk kefir. Members of the family *Lachnospiraceae*, a
262 minor group in the kefir microbiota, also systematically occurred in kefir grains, while they
263 were almost always absent in milk kefir. On the other hand, the co-exclusion relationship with
264 *Acetobacter* was observed in both grains and milk and may reflect conditions for storage and
265 production of kefir.
266 A very complex interaction network was obtained for the beef dataset (De Filippis et al.,
267 2013). The dataset included swabs from different points of bovine carcasses cuts and
268 beefsteaks obtained thereof, sampled at 0 days and after 7 days of aerobic storage at 4°C, for
269 two different samplings. The full network is shown in Supplementary Fig. S7, while a
270 simplified version, including only the most abundant taxa, is shown in Fig. 5A, together with
271 the interaction network inferred for spoiled beef steak samples (Fig. 5B). The complexity of
272 co-occurrences and mutual exclusions in fresh raw meat mainly reflects the high diversity of
273 bacterial communities (Fig. S7, Fig. 5A). Significant interactions among the most abundant
274 taxa (*Moraxellaceae, Pseudomonadaceae*, *Aerococcaceae, Staphylococcaceae,*
275 *Flavobacteriaceae, Rhodobacteriaceae* and *Corynebacteriaceae* on carcass swabs and freshly

276 cut beefsteaks; *Pseudomonaceae*, *Listeriaceae*, *Moraxellaceae* and *Enterobacteriaceae* on
277 spoiled steaks) confirm the co-occurrence and mutual exclusion patterns due to different
278 samplings, different cuts, and spoilage described by De Filippis et al. (2013). Spoilage
279 dramatically reduced diversity (De Filippis et al., 2013) and simplified the microbial
280 interaction network (Fig. 5B). The co-occurrence relationship between *Acinetobacter*
281 *guillouiae* (a species occurring at low abundance) and *Enterobacteriaceae* is independent of
282 the sampling and of the cut. On the other hand the mutual exclusion relationship between
283 *Staphylococcus equorum* and *Serratia* is clearly related to the contamination patterns of the
284 beef cuts, with the former species occurring systematically in thick flank cuts and members of
285 the genus *Serratia* occurring in brisket and chuck cuts. The last set of interactions reflects
286 different spoilage environments. In fact, the dominating spoilage organism was *Pseudomonas*
287 in sampling 1 and *Brochothrix* in sampling 2 (De Filippis et al., 2013). *Carnobacterium*,
288 *Acinetobacter johnsonii*, *Chryseobacterium* and members of the class *Actinobacteria* also
289 occurred more frequently in beef steaks from sampling 1.
290 The interaction networks inferred in our study (Table 3) are less complex (sometimes
291 dramatically) than those inferred for environmental bacterial communities (Deng et al., 2012)
292 or for the human microbiome (Faust et al., 2012). In addition, they do not show any fit of the
293 power law for either the node degree distribution or the clustering coefficient/degree
294 relationship, showing that they are neither scale-free nor show a hierarchical structure.
295 However, the average clustering coefficient is often higher than that of random networks with
296 the same size and average degree. In general, interaction networks for fermented or spoiled
297 foods show the lowest complexity, and a high correlation (r=0.92) was found between the
298 number of OTUs detected in the dataset and the number of nodes in the interaction network.
299 More complex networks, with >20 nodes were obtained when raw foods (milk, meat) were
300 included in the dataset or when the dataset reflected different environments (milk and

301 cheese, Dolci et al., 2014; Mozzarella produced with different acidification methods, Guidone
302 et al., 2015; raw and spoiled meat, different cuts and samplings, De Filippis et al., 2013). In
303 contrast, Deng et al. (2012) published figures on a wide range of complex bacterial interaction
304 networks from environmental or human sources: the network size ranged from 107 to 254
305 nodes, the node degree distribution showed a good fit of the power law for all networks and
306 the modularity (which measures the occurrence of modules which are strongly
307 interconnected) was significantly higher than that of random networks, while the occurrence
308 of a hierarchical structure was variable. Moreover, the bacterial interaction network of the
309 human microbiome (Faust et al., 2012) included 197 phylotypes with 3005 significant
310 interactions. The network showed a good fit of the power law model for the node degree
311 distribution but did not show a strong hierarchical structure, although the occurrence of body
312 site modules was found. Several factors may contribute to the lower complexity of microbial
313 interaction networks for food. Food microbial communities, and fermented food communities
314 in particular, are dramatically less complex than those found in environmental samples or in
315 the human or animal microbiome, and therefore a lower number of sequences is generally
316 sufficient to obtain a high coverage as it can be predicted by alpha rarefaction analyses
317 (Ercolini, 2013). This may prevent the detection of significant interactions for minor OTUs.
318 Finally, the interactions detected in this study mainly reflect co-occurrence and mutual
319 exclusion patterns in different food environments, and although they in some cases may
320 suggest true positive (commensalism, mutualism) or negative (competition, amensalism)
321 interactions (Gram et al., 2002; Ivey et al., 2013), these should be confirmed in independent
322 experiments.
323
324 *3.3. Future perspectives.*

325 Both the web visualisation and the full or filtered networks obtained from Gephi, although
326 visually pleasing and informative, are somewhat naïve and should be interpreted with
327 caution. The meta-analyses based on sequencing data published by different laboratories
328 carry some inevitable bias due to differences in data generation and processing. These include
329 possible differences from sample handling through nucleic acid extraction, variable 16S
330 region chosen as target, library purification and preparation, sequencing technology and
331 parameters, sequencing depth / sample coverage (Ercolini, 2013). Furthermore, it is
332 important to underline that the exact bioinformatics path chosen for the analysis can have a
333 strong impact too (May et al., 2014), and will have to be taken into account for a possible
334 standardization of the data handling and usage. In addition, detection of rare OTUs might be
335 affected by biases and reproducibility and repeatibility issues (Benson et al., 2014; Guidone et
336 al., 2015; Pinto and Raskin, 2012; Zhou et al., 2011b). To take this into account it may be
337 advisable to compare different studies at a lower taxonomic resolution and exclude rare OTU
338 from the comparisons. This can be easily done by processing data tables from
339 FoodMicrobionet. An example of a filtered network is shown in Supplementary Fig. S8. In this
340 case, OTUs belonging to the same genus were merged, and the interactive web visualisation is
341 available at http://www.foodmicrobionet.org/fmbn1_0_3gweb/.
342 Therefore, for a future larger scale meta-analysis it would be advisable to, as a minimum
343 requirement, process the sequences with the same standardized flow in order to limit at least
344 the post-sequencing bias of the analysis. Unfortunately an optimized bioinformatics pipeline
345 is still not available for food microbial communities. Most studies in FoodMicrobionet were
346 carried out using the same sequencing platform and similar or identical bioinformatics
347 pipelines (Table 1) and direct comparisons among studies can be carried out with a good
348 degree of confidence.

349 With these limitations in mind, the approach used here provides an appealing means for
350 microbiologists and food scientists dealing with food microbial community metadata analysis
351 by (a) providing access to a large set of curated data on the occurrence of different taxa in
352 foods most of which were obtained from studies published in peer reviewed journals, thus
353 facilitating the process of formulating and validating hypotheses on the structure and
354 dynamics of food bacterial communities and writing original articles and reviews; (b)
355 fostering open access to microbial ecology data by making curated nodes and edges tables
356 publicly available; (c) improving our understanding of the ecology of spoilage-associated and
357 beneficial microorganisms; and (d) providing information on the structure of bacterial
358 communities in raw materials, fermented and spoiled foods which can be used for food
359 process development.
360 While only part of this information is available through the online visualisation, the latter
361 provides a simple interactive interface to explore the microbial ecology of the food
362 environments included in FoodMicrobionet 1.0. Experienced users can import the nodes and
363 edges files provided as supplementary material in a variety of spreadsheet, statistical or
364 network analysis software packages to carry out graphical and statistical analyses or to
365 generate their own networks.
366 Future plans include (a) expanding the network to other food matrices and food
367 environments; (b) implementing an optimized data analysis pipeline to standardize the
368 treatment of the raw data; and (c) the addition of metadata describing food properties in
369 order to speculate on relevant ecological factors driving microbial interactions and to allow
370 the selection of FoodMicrobionet sub networks with defined range of specific ecological
371 factors. Contributions from other research groups will be welcome. Details on the submission
372 procedure are provided in section 3 of Supplementary Materials.

373 Ultimately, FoodMicrobionet will allow all researchers in the food microbiology to benefit
374 from the significant advances that HTS is providing in this key field of research.
375
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516

517 **Figure legends**

518

519 **Figure 1.** Flowchart of the development of FoodMicrobionet v 1.0. FoodMicrobionet is a
520 curated database of HTS studies on food bacterial communities which is implemented in
521 Gephi 0.8.2, a network analysis software. The network file can then be used to generate a
522 variety of products for visual and statistical analysis.

523 **Figure 2.** Filtered (only OTU nodes with a cumulative abundance >5% are shown) for raw
524 meat (A) sourdough samples (B) and dairy products and starters (C) extracted from
525 FoodMicrobionet 1.0. Node colour (grey scale for food subgroups for sample nodes or
526 bacterial family of OTU nodes) is used to highlight different sample and OTU nodes. Style
527 features are used to enhance the graph: node size is related to the weighted degree (i.e.
528 cumulative abundances for OTUs) while edge thickness is proportional to the abundance of an
529 OTU in a given sample. Areas of the graph in which samples belonging to a given group are
530 more abundant are enclosed by dashed lines.

531 **Figure 3.** Relative occurrence/ weighted degree scatterplot for OTU nodes in raw meat
532 samples (De Filippis et al., 2013). Only nodes with and weighted degree >1 are shown.
533 Different symbols are used for members of different phyla and the identity of nodes with a
534 weighted degree >5 is shown.

535 **Figure 4.** Microbial interaction network for the kefir dataset (Marsh et al., 2013). Each node
536 represents an OTU. Interactions were evaluated at different taxonomic levels. Only significant
537 interactions are shown ($p<0.0004$; $q<4x10^{-4}$). Edges showing negative interactions (co-
538 exclusion) are coloured red, those for positive interactions in green. The colour of nodes
539 corresponds to the class. The thickness of the edges reflect the level of significance of the
540 supporting evidence for the association (as q-values, $0\text{-}4x10^{-4}$), while the size of the nodes is
541 proportional to their degree.

542 **Figure 5.** Microbial interaction network for the beef dataset (De Filippis et al., 2013). A
543 simplified network for all samples (non-spoiled and spoiled, A) and the full interaction
544 network for spoiled beefsteaks (B) are shown. Each node represents an OTU (only low level
545 taxa are shown). Interactions were evaluated at different taxonomic levels. Only significant
546 interactions are shown ($p<0.0004$; $q<4x10^{-4}$). Edges showing negative interactions (co-
547 exclusion) are coloured red, those for positive interactions in green. The colour of nodes
548 corresponds to the class. The thickness of the edges reflect the level of significance of the
549 supporting evidence for the association (as q-values, $0\text{-}4x10^{-4}$), while the size of the nodes is
550 proportional to their degree. Actinobacteriac refers to the class *Actinobacteria*.
551