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1 **Microbiome and -omics application in food industry**

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8 **ABSTRACT**

9 The enormous potential of multi-omics approaches to unravel microbiome-related links between food  
10 quality, sustainability and safety still requires experimental work and extensive data integration to  
11 increase knowledge and understand the biological and ecological processes involved in the assembly  
12 and dynamics of microbial communities along the production chains. Data spanning from DNA  
13 sequences to transcripts and metabolites need to be integrated in order to be translated at industrial  
14 level and literature showed several successful examples. The application of microbiome studies in  
15 food systems has shown the potential to improve food quality. Nevertheless, classical microbiological  
16 methods are still highly relevant even if isolation and characterization of strains in pure culture is  
17 often laborious and time-consuming and requires the use of several specific growth media that take  
18 into the account microbial growth characteristics as well as food characteristics. Studies on  
19 microbiomes has become a popular topic in the food industry since it can be used as a tool to improve  
20 quality and safety in the food chain.

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## 27        **1. Introduction**

28        As recently re-defined, the term microbiome refers to “*the community of microorganisms and their*  
29        *“theatre of activity” (structural elements, metabolites/signal molecules, and the surrounding*  
30        *environmental conditions) in a defined habitat”*(Berg et al., 2020).

31        Microbiome theme in food systems has been identified as a key priority research area due to its  
32        potential to improve safety, sustainability, production yield or to discover new strains, probiotics or  
33        mobile genetic elements. A better knowledge of the microbiome resource is helping in precision food  
34        system management not only at research level but especially at industrial level. Several European  
35        projects are currently active in microbiome research along the food chain: CIRCLES  
36        (<https://circlesproject.eu/>), HoloFood (<https://www.holofood.eu/>), MASTER (<https://www.master->  
37        [h2020.eu/](https://www.master-h2020.eu/)), SIMBA (<https://simbaproject.eu/>) as well as MicrobiomeSupport  
38        (<https://www.microbiomesupport.eu/>). The latter, supports the set-up of an internationally agreed  
39        microbiome definition (Berg et al., 2020), best practices and standards (Ryan et al., 2021) as well as  
40        tutoring public and stakeholders about microbiomes and microbiome applications (Schelkle and  
41        Galland, 2020). As currently reviewed, microbiome-based applications are expected to be important  
42        contributors to the global economy in the coming years, however an effort is needed in food science  
43        to transit from observational to mechanistic studies (Meisner et al., 2022). The rapid development of  
44        high throughput techniques in the last 20 years has improved the ability to characterize microbiomes  
45        from complex food matrices. It is now common to apply two or more omics techniques in parallel,  
46        referred to as multi-omics analysis (Dugourd et al., 2021) to decipher in depth the biological features  
47        of the microbiome systems. In the last decades several authors successfully applied multi-omics  
48        analysis in food microbiology. The application of two or even more omics techniques is needed to  
49        move from theoretical conclusions to reliable and valuable results (Zapalska-Sozoniuk et al., 2019).  
50        For example, the application of genomics and transcriptomics alone cannot fully depict the events  
51        taking place within a cell; even when the information from DNA is transcribed to mRNA, proteins  
52        may not be biologically active. In this light an appropriate study design plays a central role. Most of

53 the times, monetary resources are one of the determining factors that have an incidence on a  
54 successful experiment. As a consequence, study design suffers from low number of samples collected  
55 or biological replicates in favor of depth of information pursued (in terms of number of  
56 sequences/metabolites detected). However, it has been recently reported that collecting more samples  
57 with less depth (number of information obtained from each sample) enriches the value of a study  
58 (Tripathi et al., 2018). Sampling depth and collection procedure are critical points when an -omics  
59 platform is chosen since specific standard requirements characterize the different platforms. In this  
60 light comparing targeted and untargeted techniques generates different considerations. Targeted  
61 analysis includes the detection or quantification pre-defined analytical target that can be chemicals or  
62 biologicals while the application of the untargeted is referred to a detection of several unspecified  
63 analytes (Ballin and Laursen, 2019).

64 It is obvious that an untargeted technique (like DNAseq, shotgun proteomics or GC-MS-based  
65 metabonomics approach) requires a lower number of samples but higher sampling depth (Pinu et al.,  
66 2019). The development of several platforms for data integration is helping researchers to move from  
67 a single -omics approach to applying different tools, since the basic requirements essential for  
68 genomics are fully compatible with metabolomics, transcriptomics and proteomics (Pinu et al., 2019).  
69 Simultaneous analysis of all aspects of a microbiome dataset must be generally considered a hopeless  
70 task. So far, multi-omics studies in food science have been primarily applied to study fermented dairy  
71 products followed by meat/meat products and vegetable-based foods.

72 Most of the studies (targeted or untargeted) can be applied for different purpose in food, such as:

- 73 i) Map the microbiome along the food chain
- 74 ii) Discover low abundance taxa or new taxa and microbial adaptation strategies
- 75 iii) Connect specific microbiome assets with the final food quality and safety
- 76 iv) Extend microbiome applications to the industry for actionable results
- 77 v) Microbial Risk assessment

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## 79        2. Microbiome mapping

80 From a biological point of view, food microbiome can be considered part of the extended hologenome  
81 of a human individual (Dunn et al., 2021) and needs to be deeply studied and characterized.  
82 Metagenomics studies already confirmed the transmission of microbes from food products to gut  
83 environments (Pasolli et al., 2020) and also showed their persistence in the human gut (Milani et al.,  
84 2019) (**Figure 1**). Since thousands of microbes, microbes' metabolites and mobile genetic elements  
85 (MGEs) are daily ingested with foods it is important to deeply characterize them and discover how a  
86 particular biogeography can modify the autochthonous microbiome (**Figure 1**). Mapping the complex  
87 microbial communities *in situ* with high taxonomic and spatial resolution is a main challenge due to  
88 the high density and rich diversity of taxa (also at strain level) (Shi et al., 2020). Origins,  
89 diversification, biodiversity and biogeography of foods' microbiome are becoming very relevant in  
90 recent years. With high throughput techniques (metataxonomic amplicon sequencing as the most  
91 applied) researchers have discovered vast and previously unrecognized ecological niches.  
92 Environmental factors (microclimatic conditions, pH,  $a_w$ , availability of nutrients) determine what  
93 kind of microbes can succeed in a particular place. This detailed analysis can also reveal how those  
94 microbes can interact and work together (Woo, 2018). Mapping exercises are still a new research area  
95 widely explored in humans (Huttenhower et al., 2012; Nash et al., 2017), soils (Thompson et al.,  
96 2017) and environment (Danko et al., 2021), however few studies reported an in-depth mapping or  
97 meta-analysis of foods and foodstuff. Food microbiota can originate from raw materials,  
98 environment, from the exposure to human manipulation and is influenced from the geographical area  
99 of cultivation/production (**Figure 1**). The mapping exercise to deeply characterize the food  
100 microbiome is one of the key fundamental actions that needs to be performed. Mapping can offer  
101 different possibilities in food microbiology, helps in microbe characterization and is essential in study  
102 design. Maps of molecules, MGEs and microbes across different food ecosystems will fundamentally  
103 transform the types of questions that can be asked of microbiome and metabolomics data. In this light  
104 the application of several biostatistics tools can help identify dynamic networks of species

105 interactions as well as relevant functions. Among them, ordination methods (principal coordinates  
106 analysis (PCoA)), gradient analysis (non-metric multidimensional scaling (NMDS)), dimensionality  
107 reduction, co-occurrence and network diagrams (Tripathi et al., 2018) are valuable tools to be used  
108 to resolve the degree of complexity of the microbiota. In food microbiology examples of extensive  
109 mapping and data integration methods are currently available.

110 The mapping exercise approach has been recently applied to dairy products, where 184 cheese  
111 samples were analyzed in depth coupling DNA-seq and metabolome analysis (Walsh et al., 2020).  
112 By this mapping exercise the authors discovered new putative genomes (belonging to genera  
113 associated with the rind) that display highest correlation with unpleasant molecules. Findings of this  
114 type may help in designing strategies to control the microbiome during cheese production and obtain  
115 desired final products. However, it should be point out that culture independent high throughput  
116 techniques must be used with culture dependent in order to provided complementary information.  
117 Sequencing technique may lead to possible biases deriving from DNA extraction, RNA quality, PCR  
118 amplification steps, as well as the failure in discriminating between live or death cell. The presence  
119 of all this unmeasured confounding factors cannot be excluded but can be solved by culturomics.

120 Several available online repository platforms offer the opportunity to collect high throughput  
121 information on microbiome in different research areas. The Earth Microbiome Project (Thompson et  
122 al., 2017) (available via QIITA website) contains a collection of more than 20.000 samples where  
123 microbial genomes as well as global metabolic models can be extracted and then re-analyzed and  
124 used in a comparative study or for meta-analysis purposes. In food microbiology, FoodMicrobionet  
125 (Parente et al., 2019, 2016) and its extension DairyFoodMicrobionet  
126 (<https://data.mendeley.com/datasets/3cwf729p34/4>) is one of the main examples of public repository.  
127 It includes 180 studies and 10,155 samples belonging to 8 major food groups and can be considered  
128 the largest database on bacteria communities based on amplicon sequencing dataset. The database  
129 contains also information including pH,  $a_w$ , presence and/or concentration of preservatives and redox  
130 potential value (Eh). Collectively these databases have enormous potential and allow microbial

131 information from a particular food/condition to be extracted and used for comparative, statistical and  
132 graphical analysis. An example of the potential of this information is the analysis of spoilage-  
133 associated core microbiota in meats, seafoods and their production environment that highlights a  
134 common core shared between different food types and their environment in relation to the degree of  
135 spoilage (De Filippis et al., 2018). It should be highlighted that researchers need to understand that  
136 sharing datasets as well as associated metadata is fundamental for the progress of food microbiology  
137 in the era of big data. By the power size effect, all this information collectively can help in discovering  
138 new potential ecological niches or uncommon microbial associations that are often disregarded when  
139 using only few samples. Importantly, efforts are needed in updating information and that those  
140 databases are constantly updated in terms of taxonomy and nomenclature (Zheng et al., 2020).  
141 However, many of the largest microbiome mapping studies have been performed with the cost-  
142 effective 16S rRNA gene amplicon sequencing that provides genus-level assignments as highest level  
143 of taxonomic resolution.

144 The global sourdough project (<http://robddunlab.com/projects/sourdough/>) is a multi-omics,  
145 intercontinental scale study with the aim to collect metadata, taxonomic and metabolomic information  
146 over 500 bread bakers in North America, Europe and Australasia. By using such extensive sampling  
147 procedure coupled with integration tools it was possible to demonstrate that geographic location does  
148 not determine changes in structural sourdough microbial composition, even if previous studies on  
149 few samples revealed the opposite and indicated that variations in acetic acid bacteria (AAB)  
150 abundance are the key driver during fermentation and boost the development of volatile compounds  
151 (Landis et al., 2021). This finding clearly implies that a large number of samples is needed for a better  
152 overview of the structure-function linkages. Co-occurrence/co-exclusion network analysis reveals the  
153 complementarity or competitiveness of inter-species interactions and for example allowed to observe  
154 that *Levilactobacillus brevis* is able to persist in a community while *Fructilactobacillus*  
155 *sanfranciscensis* displays the ability to persist only when grown with the yeast *Kazachstania humilis*  
156 (Landis et al., 2021). The ability to predict from a set of known species what community will be

157 formed is crucial in designing, predicting and controlling new microbial communities for food  
158 fermentations, probiotic therapeutic developments, bioremediation or biomanufacturing and offers  
159 valuable insight into biotechnologically important processes (Friedman et al., 2017).

160

### 161 **3. Discovering low abundance taxa or new taxa and microbial adaptation strategies**

162 Currently, there is no real consensus regarding which next-generation sequencing platforms or  
163 techniques are most suitable for low-complexity microbial communities, such as those in foods.  
164 Metagenomic shotgun sequencing or amplicon-based ones are widely used by researchers in food  
165 microbial ecology. 16S amplicon sequencing is still preferred due to the apparently lower cost per  
166 sample but is biased by the number of 16S rRNA encoding genes per genome and by the lower power  
167 in discriminating at species level. On the opposite, deep shotgun sequencing allows to reach the  
168 species level since most of the tools are based on the alignments with species-specific marker gene  
169 sequences like MetaPhlAn (Beghini et al., 2021) however suffers from the size of the reference  
170 genome. Despite this bias, the advantages of DNaseq is the ability to detect also genes and mobile  
171 genetic elements important in food microbiology (Walsh et al., 2018). Recently, it was proposed to  
172 use shallow shotgun sequencing as an alternative to 16S amplicon based sequencing at the same  
173 cost/sample of the 16S, with the advantages of retrieving also microbial functional profiles, more  
174 precise taxonomic resolution than 16S (Hillmann et al., 2018) and obtain informations on novel  
175 putative bacterial taxa (Lugli et al., 2022). Rare or low abundance taxa often play an important role  
176 in the overall metabolic flux and the differential functions of the rare species remain poorly  
177 understood (Ranjan et al., 2016). Individual samples may harbor thousands of rare taxa that are often  
178 discarded from the analysis but can have a high transcription/abundance ratio. The functions of rare  
179 microbes are still unknown; they may however be relevant in total microbial community stability if  
180 rapidly respond to environmental changes (Shade et al., 2014). In foods several examples showed  
181 that rare members of a microbiome, especially those that are not expected to be present in the food,  
182 have a possible role in ripening and determining final product characteristics. Processing environment



183 is one of the main sources of rare microbes or uncommon ones that can easily affect the final structure  
184 of the microbiota in foods and are responsible for microbial food spoilage. Dairy (Sun and D'Amico,  
185 2021), raw meat processing environments (Stellato et al., 2016) or facilities for ready-to-eat meal  
186 (Pothakos et al., 2015) including fish and fruit preparations (Bokulich et al., 2015; Einson et al., 2018)  
187 are currently the main sources of uncommon food microbes. In the dairy production chain, brine tanks  
188 and ripening rooms are the main microbial sources and their distribution is strictly connected with  
189 cheese variety and layer (crust or core) (Calasso et al., 2016; Montel et al., 2014). Not only equipment  
190 but also human, extrinsic factors [air flow, temperature and humidity] and antagonistic microbial  
191 adaptations take part in the distribution of microbes in the environment (Doyle et al., 2017).  
192 Microbiomes distribution in processing plants could increase food safety through improved hygiene  
193 related SOPs. Novel disinfection interventions can be selected based on the occurrence in a particular  
194 environmental niches from which they were disseminated (Botta et al., 2020; Zwirzitz et al., 2020).  
195 Network analysis based on correlation methods is often used to identify significantly concomitant or  
196 co-exclusion relationships. Spearman's or Pearson's correlation are the most straightforward  
197 approaches for multi-omics data integration (Zhang et al., 2019) and for detecting interactions in  
198 meta-communities. However, the relative frequency or abundance (from OTUs or ASVs) used in the  
199 metataxonomic datasets instead of the absolute abundance can reduce the sensitivity of the methods.  
200 As suggested, including as many samples as possible (Berry and Widder, 2014) needs to be taken in  
201 consideration when assessing effectiveness and reliability. Studying and understanding structure,  
202 interaction and function of environmental microbes is helping increase food safety since bacteria from  
203 the environment may also harbor antimicrobial resistance genes (ARGs). Monitoring resistomes in  
204 the environment can provide essential information to better understand whether ARGs transfer  
205 actually occurs (Lopez et al., 2020) (**Figure 1**).

206 DNA extracted from environmental samples can be directly sequenced without any prior PCR steps.  
207 In this way the global microbial community are sequenced. Data processing then helps obtain the  
208 structure of the microbial ecosystem, including detection of mobile genetic elements but also

209 information about all the microbial categories including fungi, yeast and viruses/phages. Obtaining  
210 information about virus is crucially important especially in a dairy environment since the viral  
211 communities especially phages can likely act as vectors for horizontal gene transfer (Somerville et  
212 al., 2019) and are involved in the mobilization of antimicrobial resistance genes or CRISPR defense  
213 mechanisms among bacterial populations (Colombo et al., 2018).

214 In order to characterize microbial transmission along the process chain, several integration tools are  
215 adopted. This is crucial in order to obtain precise information about microbial structure. Among the  
216 statistical tools, the source attribution analyses are able to qualitatively determine possible microbial  
217 sources but also quantitatively estimate the proportion of source contributions to a sample  
218 community. SourceTracker utilizes a Bayesian classification model to map not only microbial  
219 populations but also gene flows in a variety of ecosystems (Bokulich et al., 2015; Zwirzitz et al.,  
220 2020) and also for monitoring microbial transmission and gene dispersal (**Figure 1**). However, to  
221 reduce the effect of false predictions, a high number of samples should be analyzed (Chen et al.,  
222 2019).

223

#### 224 **4. Connecting specific microbiome asset with the final food quality and safety**

225 Microbes present in viable but not cultivable (VBNC) state or microbes not expected to be present in  
226 the food are then difficult to be detected by classical methods and this leads to losing several important  
227 pieces of information about the whole microbiome. A meta-omics approach can offer the possibility  
228 to deeply study the composition of the food microbiome by detecting also few cells in a sample  
229 brought the food industry closer to the theme of microbiome. An 'omic-based analysis can include  
230 metagenomics (all the genetic repertoire in a community), meta-transcriptomics (the expressed  
231 genes), metabolomics, proteomics or lipidomics. In every biological system phenotype informations  
232 are transferred from nucleic acids to proteins and metabolites (Santiago-Rodriguez and Hollister,  
233 2021). From a quantitative point of view, proteins and metabolites abundances are the result of gene  
234 abundance and transcriptional activity. However, the use of a single omics technology cannot

235 guarantee an overview of what really happens in food systems. Metagenomics (amplicons or shotgun  
236 sequencing) reflects only the global view since DNA molecules, as the most stable, can be originated  
237 from live as well as dead cells and the absolute abundance of a gene is not necessarily associated with  
238 molecules/proteins synthesis. mRNA sequencing (a less stable molecule), if used to confirm  
239 (meta)genomic-data, does not necessarily predict the translation of the genetic information into  
240 functional/active protein/metabolites and does not provide taxonomic information. Metabolites or  
241 proteins on the other hand can be of mixed nature since they can originate from host or food  
242 ingredients, are highly labile and need specific collection, handling or preservation methods to  
243 maintain integrity. All these considerations highlight that at least two -omics techniques are required  
244 to have a more comprehensive overview of what happens in food system. Based on the initial  
245 biological questions and taking into account sample issues (e.g. host molecules/sequences) an  
246 appropriate study design based on combinations of two or more omics tools should be chosen in order  
247 to overcome those limitations (Ferrocino et al., 2022). The microbiome asset shapes the final  
248 characteristics of the product and by coupling RNA-seq with metabolomics it is possible to see that  
249 perturbations during the food process chain modify the function of the microbiome. Examples of this  
250 multi-omics approach showed that ripening temperature during cheese (De Filippis et al., 2016), fruit  
251 (Li et al., 2021; Xu et al., 2019), plant based fermentation (An et al., 2021; Kim et al., 2020) and  
252 vinegar production (Wu et al., 2021) modifies the gene expression of the microbiome with important  
253 changes in volatilome profile of the final products.

254 Several examples of data integration between two or more omics in food-based systems are already  
255 available. DNA is most frequently the primary target molecule since it is easier to manipulate if  
256 compared to RNA and scientific literature in food-omics is mostly oriented to DNA based  
257 approaches. The advantages of using DNA are the simultaneous detection of bacteria, fungi, virus  
258 (Beghini et al., 2021; Manni et al., 2021), mobile genetic elements (ARGs, bacteriocins etc..)  
259 (Raymond et al., 2019) as well as the ability to reconstruct genome at strain level (De Filippis et al.,  
260 2019; Franciosa et al., 2021; Walsh et al., 2018). To decipher the interaction among microbes in order

261 to shape the final characteristic of the product, DNA-seq with metabolomics can be considered the  
262 optimal combination of omics techniques. The most common data integration step is based on  
263 correlation-based network analysis in order to generate and easily visualize metabolic microbiome  
264 networks/models. Microbiome-scale metabolic reconstruction is now the most straightforward  
265 approach in order to discover how microbes shape the final characteristic of the products. In food  
266 microbiology several examples showed how this tool can be applied to detail for examples how color  
267 modification, variation of pH and flavor development are associated with shifts in microbiome  
268 composition and function in cheese (Bertuzzi et al., 2018), soy sauce (Sulaiman et al., 2014),  
269 fermented meat (Ferrocino et al., 2018; Franciosa et al., 2021), fermented cocoa (Mota-Gutierrez et  
270 al., 2021), fermented fish (Zhao and Eun, 2020), *Daqu*, *Baijiu* and *Xiaoqu jiu* chinese liquor (Huang  
271 et al., 2020; Yang et al., 2021; Zhao et al., 2021) or kefir (Verge et al., 2019). Correlation analysis  
272 seems to be the most common tool to decipher microbial putative functions or new co-abundance and  
273 interaction strategies. In food microbiology this statistical tool was successfully applied to discover  
274 interactions at sub-species level in *Lactobacillus* populations highlighting that *L. helveticus* and *L.*  
275 *delbrueckii* specifically co-evolved and in the same way also *L. plantarum* and *L. paracasei* (Milani  
276 et al., 2020).

277 The correlation among bacteria and fungi is also of great interest because several bacteria are inhibited  
278 by the presence of certain fungi or are not able to grow without the synergic effect of fungi (Wolfe et  
279 al., 2014).

280 By using correlation analysis it was observed that *Geotrichum candidum* if present at high relative  
281 abundance can release growth factors that support bacterial growth, which in turn allows for the  
282 biosynthesis of some volatile compounds (Bertuzzi et al., 2018).

283 Based on the correlation network analysis between microbes, metabolites and functional genes the  
284 role of several *Lactobacillus* during food fermentations was elucidated in different systems. For  
285 example the correlation between *Lactobacillus acetotolerans* and a high abundance of genes encoding  
286 alcohol dehydrogenases could explain why it was predominant at the late stage during grain

287 fermentation (Huang et al., 2020) or how *Pediococcus pentosaceus* contributes to flavor  
288 development in fermented meat by D-lactate dehydrogenase activity responsible for the formation of  
289 ethanol and ethyl lactate (Franciosa et al., 2021). In dairy industry it was observed that co-abundance  
290 and inter-species interactions are responsible for resilience toward colonization by spoilage or  
291 pathogenic microbes with detrimental effects on the final products for example in terms of safety,  
292 stability, organoleptic characteristics or colour (Milani et al., 2020). It was observed that the  
293 concomitance of *Streptococcus thermophilus* and *Lacticaseibacillus rhamnosus* determines an  
294 increase in the occurrence of *Clostridium tyrobutyricum* responsible for spoilage phenomena (Bassi  
295 et al., 2015), while with natural whey starter strains (formed by *Lactobacillus delbrueckii*,  
296 *Lactobacillus helveticus* and *Lacticaseibacillus casei*) the prevalence of spoilage microbial taxa is  
297 reduced (Alessandria et al., 2016). The univariate correlations used in those examples are relatively  
298 straightforward but lack context for interpretation in terms of biological plausibility and mechanistic  
299 insight (Chong and Xia, 2017).

300 Studying interactions, functions and diversity of each of the microbial species harbored in this  
301 complex system is a key factor towards effective monitoring and easy manipulation of a food system  
302 with the aim to increase quality and safety. However, the numerical relationships identified by  
303 Pearson or Spearman correlation may not reflect biological significance, nor do they specifically  
304 account for complex interactions (Santiago-Rodriguez and Hollister, 2021).

305 In the authors' point of view, a more complete study of the microbiome of food products requires  
306 sequencing coupled with an extensive culture-based approach, in order to confirm the presence of  
307 particular microbes/consortia. In this light the use of synthetic microbial communities (SynComs) is  
308 receiving great interest as a validation tool of the mapping exercise as well as to confirm the results  
309 of the mathematical models. Its principle is to design a small groups or consortia of microbes in order  
310 to mimic functions and structure of the natural microbiome. By using this scale reduction of the  
311 microbiome the role and the interactions among each microbes can be detailed investigated (De Souza  
312 et al., 2020; Karkaria et al., 2021). SynComs were successfully used in food fermentations in order

313 to modulate the production of organic acids and several microbial metabolites to increase yield and  
314 final taste of Kombucha and Baijiu (Wang et al., 2020; Du et al., 2021; Li et al., 2022).  
315 Applications of synthetic microbial communities can be expanded to food industry helping in design  
316 new microbiome community to confer specific characteristic to the products in term of quality and  
317 safety.

318

## 319 **5. Extending microbiome applications to the industry for actionable results**

320 Observation studies in food microbiology are currently widely used for artisanal niche products made  
321 without the use of commercial starter cultures (Belleggia et al., 2020; Maoloni et al., 2020; Mota-  
322 Gutierrez et al., 2021). All these studies highlight that natural and autochthonous microbes display a  
323 large interaction network that confers particular characteristics to the products if compared with  
324 samples obtained with selected starter cultures (Ferrocino et al., 2018). A single strain used as a starter  
325 culture is often not able to confer to the product all the desired characteristics, which are obtained by  
326 a mixture of different microbial genetic repertoires. For example, in the meat sector it is recognized  
327 that *L. sakei* has strain-dependent properties, distinct ecotypes but also intra-species, strain-level  
328 biodiversity and its large diversity represents a valuable and exploitable asset in the development of  
329 a variety of industrial applications (Chaillou et al., 2013; Franciosa et al., 2021). In fact one of the  
330 main challenges in improving and controlling industrial fermentation processes is the revealing  
331 microbial adaptation strategies also at strain level (Janßen et al., 2020). Multi omics network analysis  
332 clearly showed that autochthonous microbiome (AM) displays a higher number of genes involved in  
333 fatty acid biosynthesis and amino acid metabolism, that in turn boosts the formation of medium- and  
334 long-chain fatty esters enhancing the sensory profile of sausages. As a result, consumers preferred  
335 the spontaneously fermented sausages because of the flavour and aroma characteristics (Ferrocino et  
336 al., 2018). Selection of an autochthonous microbiome starter culture can be one of the new potential  
337 exploration areas of food microbiology. The use of an AM can not only guarantee quality but can  
338 also offer the possibility to cover safety issues. Selection of a correct AM can help control pathogens

339 and reduce the use of nitrites/nitrates and reduce the prevalence of antimicrobial resistance genes  
340 (ARGs), mycotoxins or biogenic amines. The application of AM reveals also its importance in  
341 relation to the accumulation of mycotoxins especially in fermented meat due to the presence of  
342 indigenous fungi. For example Ochratoxin A (OTA) has negative effects including nephrotoxicity,  
343 immunotoxicity and neurotoxicity (Álvarez et al., 2020) and AM can be selected in order to obtain  
344 the same degree of protection as synthetic antifungal compounds. A mixture of autochthonous  
345 *Debaryomyces hansenii* and *Penicillium chrysogenum* was successfully used in dry cured meat in  
346 order to reduce the expression of genes involved in the production of OTA with a considerable  
347 reduction of contamination (Cebrián et al., 2019). Other example showed that AM strains possess the  
348 ability to reduce OTA accumulation by acting on the transcriptional level of the genes involved in  
349 OTA production (Peromingo et al., 2018).

350 AM can also be selected with respect to the presence of enzymes like  $\beta$ -1,3 glucanases, lytic proteases,  
351 and chitinases able to hydrolyze microorganisms cell wall components (Cence et al., 2019). A  
352 reduction of nitrites and nitrates can be obtained by using an AM since several *Debaryomyces*  
353 *hansenii* strains possess antioxidant and antimicrobial properties as well as positive effects on aroma  
354 (Perea-Sanz et al., 2020). In addition risks often linked with indigenous *Staphylococcus* or  
355 *Lactobacillus*, *Carnobacterium* and *Enterococcus* are due also to the production of decarboxylases  
356 that can cause biogenic amine production like tyramine, putrescine, cadaverine and histamine (Van  
357 der Veken et al., 2020). In this light an accurate and extensive use of the meta-omics approach is  
358 helping in studying the AM and can be considered as the first step in the selection of a microbiome  
359 starter culture able to maintain safety and the desired characteristics of products.

360

## 361 **6. Risk assessment**

362 Monitoring microbial hazards along the food process chain still requires extensive sampling  
363 procedures based on classical microbial methodology, several types of growth media, sample pre-  
364 treatments, specific incubation temperatures as well as several confirmation tests for pathogens or for

365 assessing the presence of genetic elements involved in virulence. Next generation sequencing can  
366 help in this context to obtain a quite rapid overview of potential microbial hazards along the chain.  
367 Literature reports several examples of recent applications of these technologies to identify pathogenic  
368 strains especially in low abundance. Compared to classical methods, the sensitivity of these  
369 approaches can help in identifying the persistence of low abundance pathogens or spore-forming  
370 bacteria strains in processing facilities to mitigate the risks associated with the development of those  
371 microbial groups along the chain (McHugh et al., 2018). Risk assessment could benefit from a more  
372 precise characterization of the populations and their surroundings, as it can identify risk factors or  
373 even mitigation strategies. The analysis of the microbiome along the food production and processing  
374 environments can also play a role in pathogen persistence and survival since interspecies interactions  
375 can increase pathogens surviving and colonization. It was shown that *Pseudomonas* can help *Listeria*  
376 *monocytogenes* attach to stainless steel surfaces, while *Staphylococcus sciuri* reduces the ability of  
377 *L. monocytogenes* to form biofilm due to competition phenomena mediated by metabolites production  
378 (Tan et al., 2019). The synergistic interactions between foodborne pathogens with resident microbiota  
379 associated with food processing environments have also been demonstrated by several authors.  
380 AM showed the potential to influence the growth survival and/or inactivation of pathogens It appears  
381 thus relevant to characterize the influence of the resident microbiome on both the pathogen survival  
382 and growth (Den Besten et al., 2018). Data analysis identify that *Veillonella* can be a possible  
383 indicator of the contamination of food processing surfaces by *Listeria monocytogenes* (Shedleur-  
384 Bourguignon et al., 2021), while the initial adhesion of *Salmonella enterica* serovar Enteritidis (*S.*  
385 Enteritidis) was significantly enhanced in presence of *Bacillus paramycoides* (Xu et al., 2022).  
386 Longitudinal analysis in a meat processing revealed the co-occurrence of *Listeria* spp. with biofilm  
387 producing microbes like *Pseudomonas*, *Acinetobacter*, and *Janthinobacterium* (Zwirzitz et al., 2022).  
388 The analysis of the huge amount of data obtained after sequencing requires significant time and  
389 computational power to perform genome assembly, however several tools have been developed in  
390 order to perform a comparison of single-nucleotide polymorphism (SNP) profiles without the need



391 of the assembly step, resulting in those methods being faster and less intensive computationally. Free  
392 software like MetaMLST (Zolfo et al., 2017), PanPhlAn and StrainPhlAn (Beghini et al., 2021) have  
393 the capability to perform SNP comparison of outbreak strain genomes versus non outbreak strains in  
394 a faster way (**Figure 2**). These approaches are more useful in the food industry that requires rapid  
395 testing (Martin et al., 2017). After this preliminary screening the application of more powerful  
396 computational tools can be used to reconstruct genomes directly from shotgun data. In particular SNP  
397 profiles have been used to obtain information about strains that can be transmitted from production  
398 plant to food and then to human with possible implications on human health (Milani et al., 2019).  
399 Several limitations of genome reconstruction should be highlighted since Metagenome-assembled  
400 genomes (MAGs) can be contaminated with sequences from phylogenetically close microbes or can  
401 share genes with prophages, plasmids or genomic islands. In this way, the determination of the  
402 pangenome may result in false genomes and data can be confirmed only by an extensive culture-  
403 based approach (Ferrocino et al., 2022) (**Figure 2**). Culturomics may take the advantage of the high  
404 throughput rapid identification of the colonies by Matrix Assisted Laser Desorption Ionization/Time  
405 Of Flight Mass Spectrometry (MALDI-TOF-MS) a promising tool that can open new horizons by  
406 speeding up the procedure replicating microbiome reconstruction *in vitro*. MALDI-TOF-MS coupled  
407 with metataxonomic analysis was used in order to provided complementary information by producing  
408 a more comprehensive view of the microbial ecology in food fermentations. Since culture-dependent  
409 method identify at species level and culture-independent identify non-lactic acid bacteria and yeasts  
410 (Kim et al., 2021). In addition, MALDI-TOF-MS is a promising screening tool for the rapid  
411 identification of foodborne pathogens like *Campylobacter jejuni* and *Listeria* spp. (Bowen et al.,  
412 2020, Campos Araújo et al., 2020).

413 In this context, combining -omics techniques to obtained information on the microbiome with data  
414 obtained by culture base approach on presence/absence of a pathogen can help to develop more  
415 realistic models for risk assessment (Cocolin et al., 2018).

416 The advent of software and sequencing platforms for on-site analysis (like MinION) can move  
417 forward the research in order to improve the industrial risk assessment and management procedure.

418

## 419 **7. Conclusion**

420 We are now able to collect Gbytes of data spanning from DNA sequences, transcripts and metabolites  
421 from a single sample and the integration of this information is helping in deciphering the composition  
422 and function of the microbiome. However, a lack in standardization of procedures and databases, or  
423 the absence of explicit legal requirements in food law regarding the concept of microbiome analysis,  
424 especially in the context of risk assessment (Merten et al., 2020), make it difficult to define standards  
425 in the analysis along the food chain. Food industry and related stakeholders have now grown closer  
426 to the microbiome theme and researchers need to push the use of multi-omics tools to improve product  
427 quality and safety. However, it is important to remember that all these powerful tools require also  
428 implementing culture-based approaches to help in data interpretation. Several researchers report the  
429 discovery of new putative genomes from sequencing data, however a lack of confirmation due to the  
430 absence of a cultivation step puts in doubt the newly discovered strains/function.

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442 **Figure Legends**

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444 **FIGURE 1.** Graphical representations of the food microbiome mapping analysis workflow.

445 Processing environment, season, type of farm, temperature, operators and food chain parameters can  
446 be transmitted from production plant to food and then to human with possible implications on human  
447 health. Created with BioRender.com

448

449 **FIGURE 2.**

450 Graphical representations of the culture based and culture independent for strains characterization.

451 Created with BioRender.com

452

453 **Conflict of interest statement**

454 Nothing declared.

455

456 **CRedit authorship contribution statement**

457 Ilario Ferrocino: Conceptualization, Writing - original draft. Kalliopi Rantsiou: Writing - review &  
458 editing. Luca Cocolin: Conceptualization, Supervision.

459

460 **Declaration of Competing Interest**

461 The authors report no declarations of interest.

462

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