Microbiome and -omics application in food industry

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ABSTRACT

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

1. Introduction

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

Microbiome theme in food systems has been identified as a key priority research area due to its potential to improve safety, sustainability, production yield or to discover new strains, probiotics or mobile genetic elements. A better knowledge of the microbiome resource is helping in precision food system management not only at research level but especially at industrial level. Several European projects are currently active in microbiome research along the food chain: CIRCLES (https://circlesproject.eu/), HoloFood (https://www.holofood.eu/), MASTER (https://www.master-h2020.eu/), SIMBA (https://simbaproject.eu) as well as MicrobiomeSupport (https://www.microbiomesupport.eu/). The latter, supports the set-up of an internationally agreed microbiome definition (Berg et al., 2020), best practices and standards (Ryan et al., 2021) as well as tutoring public and stakeholders about microbiomes and microbiome applications (Schelkle and Galland, 2020). As currently reviewed, microbiome-based applications are expected to be important contributors to the global economy in the coming years, however an effort is needed in food science to transit from observational to mechanistic studies (Meisner et al., 2022). The rapid development of high throughput techniques in the last 20 years has improved the ability to characterize microbiomes from complex food matrices. It is now common to apply two or more omics techniques in parallel, referred to as multi-omics analysis (Dugourd et al., 2021) to decipher in depth the biological features of the microbiome systems. In the last decades several authors successfully applied multi-omics analysis in food microbiology. The application of two or even more omics techniques is needed to move from theoretical conclusions to reliable and valuable results (Zapalska-Sozoniuk et al., 2019). For example, the application of genomics and transcriptomics alone cannot fully depict the events taking place within a cell; even when the information from DNA is transcribed to mRNA, proteins 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
78

2. Microbiome mapping

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

287 fermentation (Huang et al., 2020) or how *Pediococcus pentosaceous* 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 Sperman 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 validations 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 β-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

assessing the presence of genetic elements involved in virulence. Next generation sequencing can help in this context to obtain a quite rapid overview of potential microbial hazards along the chain. Literature reports several examples of recent applications of these technologies to identify pathogenic strains especially in low abundance. Compared to classical methods, the sensitivity of these approaches can help in identifying the persistence of low abundance pathogens or spore-forming bacteria strains in processing facilities to mitigate the risks associated with the development of those microbial groups along the chain (McHugh et al., 2018). Risk assessment could benefit from a more precise characterization of the populations and their surroundings, as it can identify risk factors or even mitigation strategies. The analysis of the microbiome along the food production and processing environments can also play a role in pathogen persistence and survival since interspecies interactions can increase pathogens surviving and colonization. It was shown that *Pseudomonas* can help *Listeria monocytogenes* attach to stainless steel surfaces, while *Staphylococcus sciuri* reduces the ability of *L. monocytogenes* to form biofilm due to competition phenomena mediated by metabolites production (Tan et al., 2019). The synergistic interactions between foodborne pathogens with resident microbiota associated with food processing environments have also been demonstrated by several authors.

AM showed the potential to influence the growth survival and/or inactivation of pathogens It appears thus relevant to characterize the influence of the resident microbiome on both the pathogen survival and growth (Den Besten et al., 2018). Data analysis identify that *Veillonella* can be a possible indicator of the contamination of food processing surfaces by *Listeria monocytogenes* (Shedleur-Bourguignon et al., 2021), while the initial adhesion of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) was significantly enhanced in presence of *Bacillus paramycoides* (Xu et al., 2022). Longitudinal analysis in a meat processing revealed the co-occurence of *Listeria* spp. with biofilm producing microbes like *Pseudomonas*, *Acinetobacter*, and *Janthinobacterium* (Zwirzitz et al., 2022). The analysis of the huge amount of data obtained after sequencing requires significant time and computational power to perform genome assembly, however several tools have been developed in 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).

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

7. Conclusion

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

Figure Legends

FIGURE 1. Graphical representations of the food microbiome mapping analysis workflow. Processing environment, season, type of farm, temperature, operators and food chain parameters can be transmitted from production plant to food and then to human with possible implications on human health. Created with BioRender.com

FIGURE 2.

Graphical representations of the culture based and culture independent for strains characterization. Created with BioRender.com

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

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

Declaration of Competing Interest

The authors report no declarations of interest.

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