FOOD MICROBIOLOGY AND MULTI-OMICS APPROACHES

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Abstract - Microbial functions in food have got more and more attention from the researchers so that the investigation in food microbiology has drastically changed. It is well established that traditional culture methods for analyzing food microbiota are very limited. High-throughput sequencing (HTS) and omic technologies allow to deep understand the behaviour of microorganisms in foods. We discuss different sequencing approaches and several HTS investigations of food microbiota. Furthermore, we focus on the four major types of omics approaches, genomics, transcriptomics, proteomics, and metabolomics and on their impact on food microbiology. However, most of the studies based on next generation sequencing just give a partial representation of the food-based ecosystem, and it is emerging the need to adopt omic combination approaches, referred to as multi-omic approach, which show more evidence for biological mechanisms. At the moment, only a few examples that combine different omic approaches have been found in food microbiology study. The current scenario and examples of recent works are discussed, showing the usefulness to adopt this integrated approach. Certain limitations are also outlined.

Index Terms - Food Microbiology, High-Throughput Sequencing, Omics Technologies, Multi-Omics Approach

I. INTRODUCTION

The traditional way for analyzing food microbiota has been through culturing methods. However, it has been shown that culturing doesn't allow the complete microbial characterization of many microbial communities, including those of foods. The isolation of microorganisms may require unknown factors and conditions present in natural habitats that are not reproduced in biological laboratories. Such limitations lead these techniques to underrate microbial diversity and fail to detect some microorganisms.

Over the last decades, a large number of culture-independent methods that overcome these problems have been developed; most of which have been used in food microbiology [1], [2]. These techniques are mainly based on the analysis of microbial nucleic acid sequences (DNA or RNA). In amplicon sequencing, marker genes are PCR amplified from DNA extracted from a mixed microbial community, sequenced and aligned against a reference database to determine the taxonomic composition of a sample. The most commonly used amplicon sequencing methods are the 16S rRNA gene sequencing and the 26S

rRNA gene sequencing (or Internal Transcribed Spacer, ITS), which are used to profile bacterial and fungal communities, respectively [3].

Subsequently, according to recent trends in environmental microbiology, food microbiology has taken advantage of the advances in molecular biology and adopted innovative strategies to detect, identify, and supervise microorganisms. Particularly, high-throughput sequencing (HTS), which identify the modern Next Generation Sequencing (NGS) techniques, has yielded unprecedented insights into wider microbial populations within different microbial environments [4], including food microbiology and its target microbes, that can be pathogens, spoilage associated, or starters and advantageous microorganisms [5]. HTS have promoted the emergence of new technologies, such as genomics, metagenomics, transcriptomics and metatranscriptomics, metaproteomics and metabolomics. Compared to previous culture-independent methods, the number of nucleic acid sequences analyzed by NGS techniques is much higher, allowing a deeper description of the microbial constituents of the ecosystems [6]. This new technologies, called omics technologies, focusing on advances in DNA sequencing methods, have so dramatically changed biological research [7], and allow us to supervise changes in microbiome, providing useful information regarding the dynamics of food fermentation, the monitoring of the growth of starter cultures, of pathogen and spoiling microorganisms.

Genomics can be defined as the analysis of whole-genome sequences of DNA extracted from an organism. In comparative genomics, bioinformatic platforms are used to individuate differences between the whole-genome sequences of different organisms [8].

Metagenomics is an extremely powerful tool that can be used to describe the genetic potential of several kinds of microbiome. In fact, it has been used in different environments, including the soil, the sea, the human gut microbiome and the food microbial community. Technological progresses, that have made possible cost-efficient and high-throughput microbial analysis, have allowed significant improvements in this omics technologies. Current metagenomics studies are based on shotgun techniques, that can produce reads from DNA, aligned to reference genomes to identify variants and community population. Targeted sequencing such as 16S rRNA gene sequencing can be also used to take a microbial community census. Typically, amplicon sequencing was limited to genus-level identification. In contrast, in

whole-metagenome shotgun (WMS), total genomic DNA extracted from a mixed microbial community is fragmented and sequenced to determine the gene content of a sample [9]. However, despite technological progresses, in order to improve the research results based on metagenomics analysis, it is important to create a unified microbial DNA extraction method, improve computational algorithms, and complete the reference databases.

Conversely, metatranscriptomics has focused on the genes activity and its approach is based on the retrieval and sequencing of environmental mRNAs from a microbial ecosystem. Metatranscriptomics researches have been applied initially to samples from water and soil environments [10]. These studies demonstrated the feasibility of RNA-based profiling of microbial consortium and also produced large amounts of novel sequence information. Investigations based on this approach have subsequently been applied to food and human gut microbiome, showing, in the latter case, strong inter-subject in microbial gene expression [11] [12]. However, it is very difficult to obtain high-quality and sufficient amounts of RNA from environmental samples. Furthermore, it is a challenge to separate the mRNA of interest from the more abundant types of RNA such as rRNA [13]. In addition, it requires high-depth sequencing to detect differentially expressed transcripts present in low abundances [14]. Consequently, we may consider that metatranscriptomics is the most expensive of the omics approaches [15].

Metaproteomics allows the analysis of protein, which is important to understand the functions of the microbial community. Several investigations have been concerned the human gut microbiome [16] and have showed as metaproteomics is able to clarify functional aspects related to physiological states and to provide detailed insights about the link between microbial diversity and the impact on the host biology. However, technical limitations are even now present and standardized procedures are still to be established.

Metabolomics allow to define the metabolic different microbiome profile, including food microbiome [17], and to individuate and quantify classes and compounds of interest. Biological systems based on this omics approach have opened a new scenario in the comprehension of different microbiome by supporting the understanding of its state, modulation and interaction with microorganisms. However, some limitations restrict even now the development of metabolomics in microbiology, in fact databases are incomplete and there are metabolites not included.

II. OMICS APPLICATIONS IN FOOD MICROBIOLOGY

Understanding the microbiota evolution over time is a priority in studying the microbial composition of foods. Changes in microbiota provide useful information to follow natural dynamics processes as fermentation, to monitor the effect produced by starter cultures, to observe the food spoilage process or to supervise changes during fresh food storage.

The first and most frequent technique aimed to analyze the food microbiota evolution has been the amplicon sequencing, which has been applied to the microbial composition of a food matrix for studying the microbiome or the mycobiome. Amplicon sequencing has been also used to characterize fermentation processes in numerous foods, including cheeses and meats [18], [19]. Several researches has been aimed at estimating the microbial diversity in different dairy microbial community using 16S rRNA as the target and many of them shown the presence of several contaminant taxa, likely coming from the environment, which can lead to the decay of food quality [20]. Similarly, ITS gene sequencing have been also used in many investigations to individuate fungal diversity [21].

Using these approaches, it was shown that the initial microbiota of beef changes dramatically upon storage of the same beef for 40 days in completely different packaging conditions [22].

The most recent shotgun metagenomics approach has been applied largely to environmental microbiology, and in the last few years also in food microbiology. The use of the shotgun DNA sequence approach in food microbiology is very relevant, in fact it provides higher resolution taxonomic information than 16S rRNA sequencing and can profile many uncharacterized species, especially those present in low abundances, as well as provide information about the microorganisms gene content. One of the application in food concerns the possibility of understanding the change in the gene content during different food process, regarding meat, kefir, cheese or traditional Korean fermented food [23]-[26].

Methods based on genomics analysis have also been used for classifying and detecting foodborne viruses [27]. A main application based on shotgun metagenomic approach concerns the ability to detect foodborne pathogens [28] [29]. Other applications of metagenomics in food safety include the identification of novel and non-culturable agents that cause foodborne disease [30], as well as the characterization of microbial communities in foods and the characterization of human intestinal microbiome to individuate microorganisms that may protect against infection caused by foodborne pathogens. For example, a study [31] used metagenomics approaches to identify Campylobacter jejuni as the reason for a foodborne illness case that was not diagnosable by conventional microbiological culture.

Generally, we can affirm that the use of whole genome sequencing (WGS) as a tool for subtyping of foodborne pathogen isolates allows us to improve our ability to rapidly detect foodborne disease outbreaks [32].

Concerning metatranscriptomics, gene-expression microarray and RNA-sequencing studies have been

recently applied in several food-microbiology research fields [33]. Particularly, the importance of shotgun RNA sequence approach is growing because it is able to discover the functions of food microorganisms. This technique has recently been applied to select biological markers in order to improve cheese quality assessment [34], or just to assess the microbial physiology during cheese manufacturing [35]. Flexible transcriptomics platforms has been applied in food quality and safety and can involve oligonucleotide microarrays, focused on PCR arrays and, more recently, on high-throughput "next generation" sequencing technologies [36]. In other studies [37] a complete genome sequence has been used to investigate metatranscriptomic gene-expression profiles during kimchi fermentation.

Metaproteomics is employed for the identification and quantification of microbial proteins, within a food matrix [38]. During the last decade, proteomics has been applied to different research areas within food technology. Many food applications deal with quality issues related, for instance, to the identification of bioactive peptides and proteins, which are very important from the nutritional point of view, or to study of post-mortem processes in animal foods. Furthermore, we now are susceptible to food safety issues, and proteomics can give assurance within different safety aspects, such as food authenticity, detection of animal species in the food, and identification of food allergens. Proteins can be also used as markers for many food properties, in fact they may provide insights about the processes to which the food has been subjected, and can provide further evidence of the foods labeling claim [39].

Metabolomics is employed for the identification and quantification of microbial metabolites in food [40]. Potential applications of metabolomics are related to different areas of food technology, as food component analysis, food quality/authenticity assessment and food consumption monitoring. A relevant study couples the amplicon approach with metabolomics to create a tool that can be used to identify the potential candidate metabolites related to specific taxa [41].

III. MULTI-OMIC APPROACHES IN FOOD MICROBIOLOGY

Many investigations described the usefulness of amplicon sequencing to study the microbiology of foods. However, there is a need to move beyond the identification of the microorganisms that are present and instead focus on their roles [42]. This aim can be achieved through other approaches, as WMS sequencing or metatranscriptomics (RNA-Seq).

However, single omics technologies provide a comprehensive food microbial community view, but it is generally limited only to single layers, like genomic, transcriptomics, proteomic, metabolomics layers. On the contrary, their integration generates a global view which provides more evidence for biological mechanisms [9]. This is very important, in fact, according to the investigation needs in food microbiology, recent studies concern the role of microbes within the complex food ecosystem and how the environmental conditions (e.g. changes in the fermentation process the addition of a starter culture and changes in ingredients) can affect the development and functioning of microbiota. In this context, a multi-omics approach can help researchers to obtain relevant insights into the mechanisms that can affect the final characteristics of products, in term of organoleptic proprieties, as well as safety. "Fig. 1" shows this combination, which is referred to as multi-omics approach.

Only a few examples that integrate different omics approaches have been found for food.

Interesting studies concern the food fermentation process. In some of them metagenomics has been combined with metaproteomics to investigate the microbiology of a fermented food [43], in particular, to characterize the bacterial and fungal composition of Pu-erh tea. These results allowed to identity the microorganisms and the associated proteins involved in the fermentation of this product.

A recent study focusing on cheese ripening process is an example in which data coming from metagenomic, metatranscriptomic and biochemical analyses have been combined to obtain an overall view of what happens during this process [44]. Another research showed that coupling metatranscriptomic and metabolome data is effective in discovering the functional diversity of cheese microbiota affected by different ripening conditions [45] .

Further investigations have used 16S rRNA and metabolomics to study the production of metabolites during the fermentation of several types of traditional Korean seafood [46] [47].

Recently, an integrated amplicon sequencing and metabolomics approach was employed to investigate the microbial evolution of Zhenjiang aromatic vinegar during fermentation [48]. A limitation of using this approach is that amplicon sequencing is unable to individuate variations in the food microbial composition. For this reason, this approach is not particularly suitable when it needs to ensure the food quality [49].

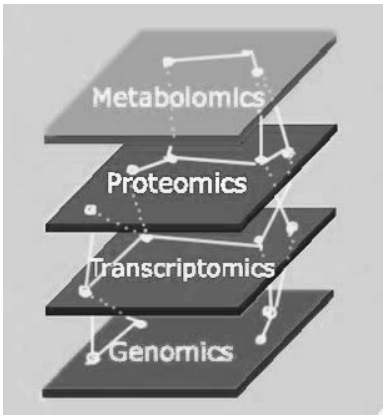


Fig 1 – multi omics approach

Concerning food quality field, the correlation of data from all omics techniques, in a multi-omics approach, would contribute to the overall quality of food products. Particularly, the integration of metabolomics technique with other technologies is important to allow the link among microbial metabolites and sensorial attributes [50]. Some investigations used multiple omics technologies combining metagenomics and metabolomics to find the microorganisms that are responsible for the production of metabolites, such as flavor compounds, in fermented foods. Particularly, WMS and metabolomics have been used to identify associations between different species and flavor compounds in kefir milk [51] [52]. The achieved results suggest that this combined approach may lead to the identification of strain starter cultures to obtain fermented foods with improved sensory qualities.

Even regarding food safety area, integrated omics approaches have been used. Particularly, integrated metagenomics and metabolomics approaches have also been adopted to enhance the safety of fermented foods by identifying the microorganisms which produce damaging metabolites. For this aim, 16S rRNA and metabolomics were used to identify the formation of biogenic amines in Chinese rice wine [53]. Similar approaches can be adopted to improve the safety of other fermented foods [54]. For example, during the fermentation process of kombucha tea, ITS and metabolomics were combined to demonstrate that fungal population evolutions was related to the improvement of the levels of antioxidants. This suggests that fungi contribute to the healthy characteristics of this beverage [55].

Finally, there is a great potential for transcriptomics and proteomics to be utilized in an integrated approach for rational development of new control strategies for foodborne pathogens [32]. A number of recent studies have assessed transcriptomes and/or proteomes of bacteria under particular conditions, simulating what a pathogen may experience on a food, such as the low water activity and low temperature that E. coli could encounter during beef carcass chilling [56].

CONCLUSIONS AND DISCUSSION

Omics and multi-omics techniques are revolutionising food microbiology. Pathogen detection, microbial profiling, genetic data mining, starters' fate determining, pathogens over food manufacturing and ripening, etc., are the objectives of these high-throughput technologies. In fact, food microbiology study is revisited with the contribute of these new techniques.

For this reason, it is important to make some considerations that may be useful for researchers aiming to design a study based on these approaches. These request on large numbers of statistical analyses, and a considerable investment of time and skilled human resources, thus, a careful activity planning is necessary [57].

Particularly, multi-omics approaches provide biological analysis coming from datasets that are usually large. The ability to individuate associations and to detect carefully the flow of information strongly depends on food sample size. An initial power calculation to ensure sufficient sample size is thus necessary in large-scale studies.

Another very important aspect when a food multi-omics approach is designed, is the data analysis requirements, that involves with multi-omics data integration. Therefore, developing a computational pipeline able to integrate data generated from different platforms it becomes necessary and this, often, requires a large storage space.

At the moment, several tools are available to really understand what happens in a food based system, but, in our opinion, a system level approach is still lacking. Below, we shortly discuss some studies that adopt such approach in multi-omics applications. A study concerns a research on the biological wastewater treatment [58], but the adopted approach may be used also in other areas, such food microbiology. Its aim is to develop a computational framework for the reconstruction of a large microbial community from multi-omics data. Particularly, the considered framework allows data integration within reconstructed community-level metabolic networks.

Generally, the reconstruction of metabolic networks based on genomic data represents a good alternative to metabolomics for obtaining the metabolic features of organisms, as discussed in a study which concerns human gut microbiome [59]. Ideally, top-down and bottom-up approaches should be combined to identify interplays between microbial community structure and functions, and to reduce the gap between the metabolic networks and the microbial community [60].

Finally, we may consider that multi-omics approaches suffer from certain limitations, due mainly to their relatively high cost and the need for specific bioinformatics and biostatistics skills for data analysis and for a computational system development. However, we are convinced that despite these difficulties multi-omics approaches will allow to study food microbiome more deeply and that will be powerful enough to elucidate its ecologic role.

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