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Next generation microbiological risk assessment meta-omics: The next need for integration

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ABSTRACT

The development of a multi-omics approach has provided a new approach to the investigation of microbial communities allowing an integration of data, which can be used to better understand the behaviour of and interactions between community members. Metagenomics, metatranscriptomics, metaproteomics and metabolomics have the potential of producing a large amount of data in a very short time, however an important challenge is how to exploit and interpret these data to assist risk managers in food safety and quality decisions. This can be achieved by integrating multi-omics data in microbiological risk assessment.

In this paper we identify limitations and challenges of the multi-omics approach, underlining promising potentials, but also identifying gaps, which should be addressed for its full exploitation. A view on how this new way of investigation will impact the traditional microbiology schemes in the food industry is also presented.

1. Introduction

The last decades have been characterized by exciting technological advancements in the field of analytical methods. In food microbiology this has allowed the introduction of alternative methods to traditional microbiology, the majority of them being based on molecular biology. A "cultural" evolution took place from the late '90, when microbes started to be detected in the food matrix without the need of cultivation on synthetic microbiological media (Cocolin and Ercolini, 2015). If foodborne pathogens are specifically taken into account, this approach dates back to the late '80, when polymerase chain reaction (PCR) was used to detect microorganisms from food without isolation.

Next Generation Sequencing (NGS) techniques undoubtedly represent a step change in the way microbiologists address ecology and diversity in foods. While with the traditional Sanger approach sequencing could be performed on a unique DNA molecule (Sanger and Coulson, 1975), with NGS it is possible to extract the nucleic acids from a complex ecosystem and profile in detail the microbial populations (identified as Operational Taxonomic Units, OTU) present. Moreover, NGS allows for a gene library creation, which can be used to understand the functions that are mostly present in a specific ecosystem. In metataxonomics (or metagenetics, rRNA metagenomics or more generally amplicon sequencing), the extracted nucleic acids are subjected to an amplification step, thereby becoming semi-quantitative due to the potential biases introduced by PCR. In metagenomics, DNA is subjected to direct sequencing, via the creation of shotgun libraries (Ercolini, 2013) (Fig. 1). It is clear that metagenetics is an approach that is "taxonomy" oriented, and for this the new term metataxonomics can be coined, while metagenomics is "function" oriented, although taxonomic composition of communities can also be inferred as well from a sample's metagenome (Bokulich et al., 2016; Franzosa et al., 2015). In fact, microbial diversity in food ecosystems can be described by extracting and elaborating separately the rRNA sequences from the rest of the data. In this case one essential point that should be taken into consideration is the number of sequences obtained for the rRNA genes with respect to the total (depth of the analysis). These approaches are valuable, without impacting the ecological description of the sample (coverage), when a high number of reads is obtained, which is depending on the complexity of the ecosystem. Inadequate sequencing depth and coverage result in an underestimation of the diversity

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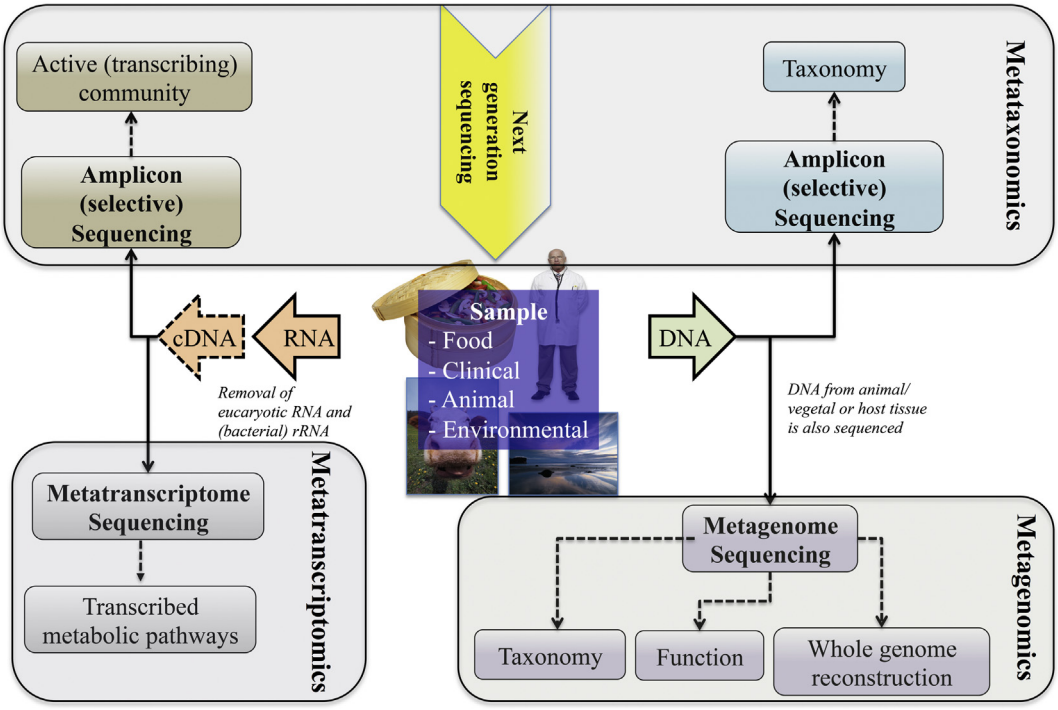

Fig. 1. The use of NGS in applied microbiology can be used to better understand ecology and interactions of microorganism in specific complex ecosystems. Not only diversity, but also behaviour can be investigating targeting DNA and RNA, respectively.

regarding microbial populations and genes. Also, metagenomics offers the opportunity to look beyond the presence/absence of taxonomically defined entities (i.e. specific organisms) and instead to understand the relationships between microorganisms as well as their activities and functionalities in a particular niche. In this context, the focus on the presence of genes, and their transcripts, rather than the identification of a specific organism, may be a preferred approach (Brul et al., 2012). Moreover, the combination of data on the presence/absence of specific pathogenic or spoilage organisms and total metagenomics community fingerprints of specific niches may lead to the development of benchmarked risk profiles based on only the community fingerprint. Lastly, it becomes a very attractive possibility to sequence messenger RNA (mRNA), through the application of metatranscriptomics, in order to understand the behaviour of the microbial population in a defined ecosystem.

NGS approaches have attracted much attention in the last few years, due to their power in data generation, epidemiology, and microbiological risk assessment. Genomes can be generated with a speed that was not possible to imagine in the past (within 24 h) and they can be even reconstructed from metagenomic libraries, allowing for an acceleration in the process of pathogen-source attribution. Moreover, meta-omics data (especially metatranscriptomics) can be interpreted to better understand how a microorganism interacts with the surrounding environment (including other microorganisms) and as a consequence design strategies to improve safety and quality of foods. It must be underlined that the introduction of NGS in food microbiology will require new infrastructures and knowledge. Due to the high amount of information produced, data storage, processing and interpretation will have to be carried out properly following a trans disciplinary approach.

The greatest challenge that microbiologist are facing at the moment is how to integrate omics data in microbiological risk assessment (MRA). MRA is a qualitative, semi-quantitative or quantitative method, which provides a structured framework for identifying and evaluating various microbiological risks through the completion of four main steps the: a) hazard identification, b) hazard characterization, c) exposure assessment and d) risk characterization (CAC, 2014). Although methods and interpretation of omics data are currently too complex to be readily implemented into the current risk assessment paradigm, we envision a framework by which risk assessment moves beyond taxonomic and genotypic identification to a more functional approach based on the study of microbial behaviour (i.e. expression of genes). To live up to expectations, much will depend on whether the tools can provide the required resolution, e.g. detect microbes or traits present in low cell numbers and assess gene expression of such low-abundance species (Brul et al., 2008).

The aim of this paper is to evaluate the potential of meta-omics approaches and the obstacles, which should be overcome in order to foresee their full exploitation in MRA, including views not only from academia, but also from food producing companies.

2. Meta-omics approaches and their exploitation for food quality and safety

Meta-omics approaches have explored the complex microbiota of several environments i.e. water, soil, plants as well as human microbiota. One exciting and expanding area of investigation is the use of metagenome sequencing for diagnosing infection, as the potential to detect and identify causative organisms that are difficult to find by conventional methods (Thoendel et al., 2016). Phylogenetic analyses of pathogenic microbes using NGS (i.e. Whole Genome Sequencing, WGS) are powerful epidemiological tools for examining disease origins, and evolutionary relationships to identify transmission pathways and mitigate the factors affecting disease transmission (Khaledi et al., 2016; Rantsiou et al., this issue; Stumpf et al., 2016) leading to preventing future outbreaks and diminishing their effects.

The use of NGS approaches to investigate the microbial ecosystem of food has dramatically increased in the last five years and has been reviewed recently (Ercolini, 2013, Kergourlay et al., 2015). In the case of microbial spoilage, high-throughput sequencing (HTS) has opened up new perspectives of characterization and control options. For most food products, spoilage microbiota had remained poorly characterized due to their diversity and the lack of selective culture media for their isolation. With the overview of the whole ecosystem given by metagenomic or metagenetic approaches, a precise description of the bacterial

species that are present at the time of spoilage becomes available. Pothakos et al. (2014) used HTS of 16S rRNA genes to describe the microbial communities involved in several food spoilage cases before the end of shelf-life to identify some spoilage-specific microorganisms. These methods have also been used to investigate environmental contamination (De Filippis et al., 2013) or effect of the process on the microbiota (Nieminen et al., 2012). Metagenetics has also revealed the existence of core communities sharing similarities between meat and seafood products and evidenced that unculturable bacteria were dominant in spoiled cod (Chaillou et al., 2015). Notably, beyond the powerful nature of HTS, in several studies, the taxonomic identification is limited to the genus level and no further advanced information is provided (Kergourlay et al., 2015).

These studies have laid the basis for ecosystem characterization, but other steps are required. The first one is to change focus from presence to function (roles). As far as spoilage is concerned, the traditional culture-based methods or the first non-cultural methods (Denaturing Gradient Gel Electrophoresis -DGGE, Temperature Gradient Gel Electrophoresis -TGGE) have shown in some cases that dominant species on microbiological plates were not the main spoilers, e.g., responsible for odors or texture degradation (Jaffrès et al., 2011; Macé et al., 2013). Metagenetics targeted on specific metabolic activities (e.g. acidification, protein degradation) would have thus to be considered to better evaluate food decay. This approach has been used in the fermented product Kimchi, where a specific group of genes was monitored: those involved in carbohydrate fermentation, which may better describe the bacterial communities that are linked to the fermentation characteristics (Jung et al., 2011). This group of genes was also reported to be involved during ripening of a Dutch-type cheese (Porcellato and Skeie, 2016). Using functional annotation, Escobar-Zepeda et al. (2016) have also evidenced the active role of the microbial communities in the production of flavour compounds in a ripened Mexican cheese. In the same way, Bokulich et al., (2015), have combined microbial ecosystem studies with the detection of spoilage genes to predict contamination routes in breweries.

Regarding food safety risk, the presence of foodborne pathogens may not be evidenced using HTS technologies, due to their low number compared to the dominant microbiota. Indeed *Listeria monocytogenes*, *Salmonella* spp., *Yersinia* spp. and *Brucella* spp. were not detected by metagenomics in ripened cheese (Escobar-Zepeda et al., 2016). Using 16S amplicon sequencing, low read numbers corresponding to L. *monocytogenes* were detected in some fresh meat or seafood samples but none was recorded at the spoilage time (Chaillou et al., 2015). In both cases, the presence of these foodborne pathogens was not investigated using traditional methods, and only global indicators such as total biota or *Enterobacteriaceae* were enumerated. To improve pathogen detection with NGS, the identification of virulence among the sequences in metagenomic studies, or their use as targets for amplicon sequencing, may be investigated. This approach was used in the metagenomic study of Escobar-Zepeda et al. (2016) where the presence of virulence genes of pathogenic *Escherichia coli* was check after amplification due to the low bacterial number of *Enterobacteriaceae* family, but not detected by metagenomics, and by Yang et al. (2016) who investigated, using shotgun metagenomics, *E. coli* virulence genes throughout the beef production chain. The evaluation antibiotic resistance of bacterial communities using HTS has not been reported yet in food. However, in other ecosystems like wastewater, the effect of genes *bacA* carried by *Bacillus* and RND-related ARGs (antibiotic resistance genes) carried by *Pseudomonas* was highlighted and mainly contributed to enhance the ARGs abundance in UV treated water (Hu et al., 2016).

Another field of investigation for safety risk assessment concerns bacterial interactions and their impact on safety. In the research studies mentioned above (Escobar-Zepeda et al., 2016; Yang et al., 2016), high numbers of different lactic acid bacteria (LAB) species or other main spoilage bacteria were detected suggesting that they affected the survival and/or growth of enteric and pathogenic bacteria. The possible mechanisms responsible for this inhibition include; organic acids, such as the lactic acid produced by LAB, pH decrease and the presence of bacteriocins. In metagenomic studies, the analysis of genes involved in such inhibition could be a first assessment of the ability of a microbial community to have an effect on the inhibition of foodborne pathogens (Escobar-Zepeda et al., 2016). With the increase of metagenomic data for microbial food ecosystems, the use of a novel ecological approach, called network inference, which investigates the co-occurrence and mutual exclusion of species inside communities and predicts microbial interactions (Faust and Raes, 2012) becomes possible. Moreover, co-occurrence/co-exclusion analysis was recently applied in a study of the microbial ecology of an Italian hard type cheese, in which it was demonstrated that specific LAB could exclude spoilage species, responsible for significant changes in the microbial ecology during the fermentation and ripening process (Alessandria et al., 2016).

The major barrier of those approaches is the overwhelming high proportion of human, environmental or other microorganisms to pathogen DNA in samples with low pathogen abundance. Microbial DNA enrichment methods offer the potential to relieve this limitation by increasing its quantity, while bioinformatic tools exist to help identify and remove reads from other sources (Ames et al., 2013; Scholz et al., 2016; Thoendel et al., 2016; Zhang et al., 2015). An additional challenge in such studies is to identify from which microorganisms and genes the DNA originated, thus the choice of the tools and databases which are available for annotating DNA sequences and can have a significant impact on the false representation of community composition and function is crucial (Randle-Boggis et al., 2016). The effect of DNA extraction method could not be underestimated too. In a recent study, the authors conclude that different DNA extraction methods lead to different results in downstream data analysis (Gerasimidis et al., 2016; Knudsen et al., 2016).

Advanced DNA sequencing techniques are becoming increasingly popular and economically viable, and there is a significant growth potential for such technologies in the field of food microbiology (Gill et al., 2006; Kergourlay et al., 2015). By enabling rapid identification of microbial communities someone can examine and compare microbiomes across a vast number of hosts, habitats, and species (Stumpf et al., 2016). In particular, the use of multi-omics approaches allows a combinational analysis of the microbiota and metabolites, which enables the better understanding of the interactions between environment, food product and microbiota (De Filippis et al., 2016). Also, in a situation where a potential pathogen is detected at very low levels, attention should be given to its presence due to potential implications for consumer's health. Besides these, interaction of the pathogens with the gut microbes during an infection process can affect the disease outcome, which constitutes the hazard characterization part of MRA. The relevance of multi-omics tools for hazard characterization is addressed in the paper by Nabida et al. (this issue).

3. From presence to behaviour

To fully exploit the potential of NGS in MRA it is essential that the results obtained are correctly interpreted, taking into consideration the biological meaning of the targeted DNA and/or RNA. Most often the approaches used to date in food microbiology are either based on the analysis of DNA or RNA and the outcomes obtained have to be interpreted knowing the physiological meaning of these two nucleic acids. While DNA is a chemically stable molecule, which can be found a long time after the death of a cell, RNA is more sensitive to degradation, especially in environments, like foods, in which enzymes, such as hydrolases, are present. While DNA can give a good overview of what microorganisms are or were present in a given ecosystem, it cannot provide any information on what microbes are doing regarding metabolic and spoilage activities and virulence factors expression. For this reason, if the goal of the investigation is to get an insight on how the microorganism is behaving, the RNA is better option (Cocolin et al.,

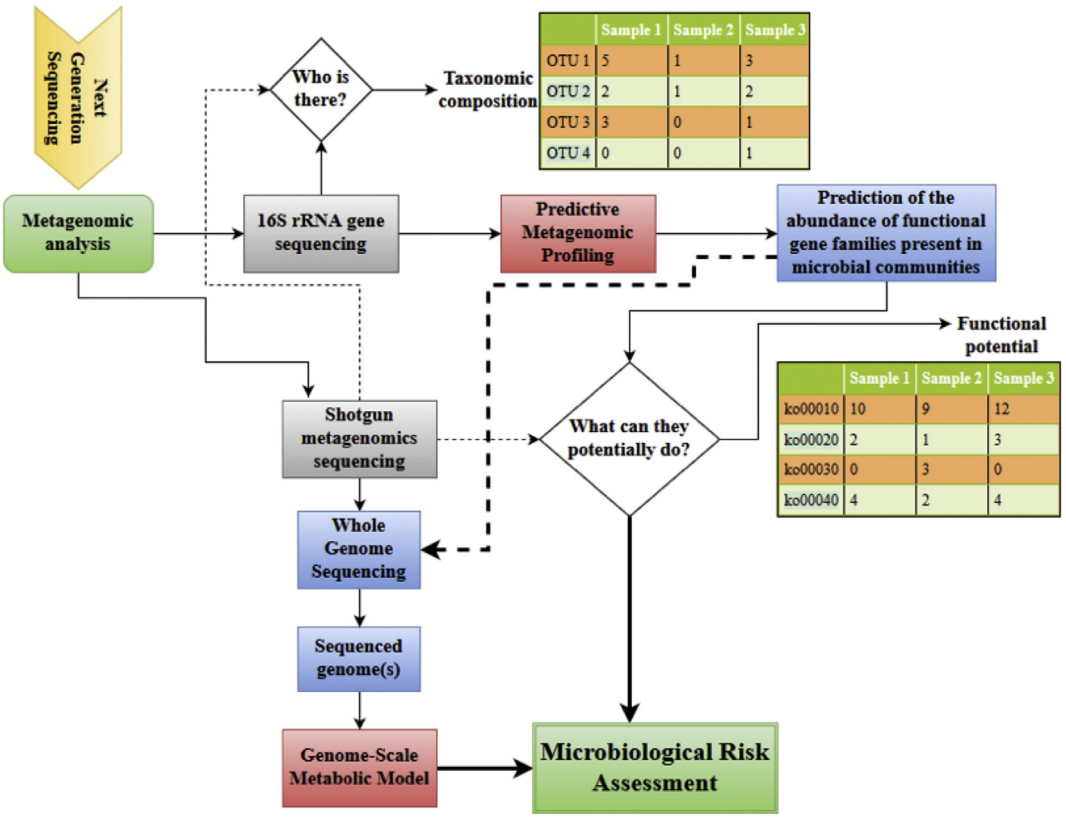

Fig. 2. The potential link between metagenomics and microbiological risk assessment.

2013). To get quantitative information on the real activity of microorganisms in their ecosystem, transcriptomic and metatranscriptomic approaches are necessary. Specific spoilage potential activities can be evaluated among communities such as biogenic amines production using conserved decarboxylase genes (Diaz et al., 2016) or microbial genes causing food discoloration (Andreani et al., 2015). Till now metatranscriptomic studies have been mainly applied in fermented products to investigate changes in the overall gene expression during fermentation of Kimchi (Jung et al., 2013) or to understand the impact of temperature and relative humidity on the ripening dynamics of an Italian cheese (De Filippis et al., 2016). Metatranscriptomic studies will probably rise with the availability of whole-genome sequences of the bacterial species identified in food ecosystems.

However, data on foodborne pathogen behaviuor are still lacking. At the moment of the writing of this paper, this is principally due to technical hurdles, which have to be solved to allow a better exploitation of such analysis. Because mRNA represents the target molecule to sequence, nucleic acid mixtures extracted from the complex ecosystems must be subjected to some purification steps, in which first the DNA is degraded, then the eukaryotic RNA is digested, and finally, the bacterial mRNA is purified. In all these steps particular attention should be given to the prevention of the RNA degradation and the preservation of a sufficient quantity to allow sequencing. Unfortunately the available possibilities (ready-to-use kits) present on the market, apart from being expensive, do not guarantee a successful purification of mRNA molecules, making the metatranscriptomic approach complicated. It is expected, in the near future, that better solutions for mRNA purification will be offered to the researchers to avoid these limitations. Moreover, the application of metatranscriptomics in the food safety area has to face additional challenges, such as the low number of pathogenic bacteria usually present in food and their physiological state. In fact, dormant foodborne pathogens still represent a risk for human health, however the level of expression of virulence genes is expected to be very limited.

One possible solution to circumvent the problems above is to look for biomarkers. Those are genes that can be correctly correlated to a specific physiological manifestation, such as virulence or stress response. By analyzing the trends in the expression of biomarkers, it would be possible to predict microbial behaviour. This strategy has been used for both spoilage and pathogenic bacteria (Desriac et al., 2012, 2013; Mataragas et al., 2015), however, for biomarker identification, it would be best to rely on metatranscriptomic data or large sets of relative expression data in which scan for potential biomarker genes (den Besten et al., this issue; Rantsiou et al., this issue).

Independently of the approach used (metatranscriptomics or biomarker identification), the simple analysis of mRNA is not sufficient to provide inputs to MRA because often the expression of a gene does not necessarily imply a physiological response. For this reason, transcriptomic data must be coupled with proteomics (metaproteomics) and metabolomics (metametabolomics). Therefore, the multi-omics approach is necessary to get the information needed to be considered in MRA. This aspect assumes even more importance when we analyze the intra-species diversity that foodborne pathogens and spoilers present in food matrices. By tackling the diversity in the microbial world by using these high-throughput methodologies it will be feasible to construct mechanistic models which will take into consideration this biodiversity and will improve the reliability of MRA schemes, even in the event of no growth but increased virulence in certain food matrices (den Besten et al., this issue).

4. Next generation sequencing, predictive metagenomics profiling, genome-scale metabolic models and their potential link with microbiological risk assessment

MRA can benefit from the revolution that NGS has brought to the analysis of microbial ecology of foods. Metagenomic analysis usually takes two forms (Fig. 1). The first is the 16S rRNA gene sequencing, which answers the question '*Who is there?*' (taxonomic composition).

The 16S rRNA sequencing data can be analyzed with tools belonging to the field of Predictive Metagenomic Profiling (PMP). The objective of PMP is to predict the abundance of functional gene families present in microbial communities (Wood, 2016), answering the question '*What can they potentially do?*' (functional potential). The PMP analysis produces a matrix containing the predicted gene family counts as KEGG orthology identifiers (KO), which can be grouped into pathway level categories (KO modules). Furthermore, for each OTU, 16S rRNA copy number is used to measure the relative contribution of gene families to the functional potential of the microbial community (Wood, 2016). As such, the results from the PMP analysis can be used to support MRA studies regarding the prediction of a phenotypic behaviour (e.g. antimicrobial resistance, survival or increased virulence) of the identified OTU under concern. The results of PMP can be validated and more details regarding the phenotypic behaviour of the identified OTU can be acquired with the investigation of the functional potential of the microbial community using shotgun metagenomics sequencing (Fig. 2).

MRA is the process of building a risk assessment model for the estimation of the risk, i.e. the adverse effect to health due to the exposure to a specific hazard of microbiological nature. Although, Genome Scale Metabolic Models (GSMMs) can be applied to build dynamic network models for microbial communities (Steinway et al., 2015), an MRA refers to a single microorganism. From this point of view, GSMMs and constrained-based modelling approaches for single species populations, i.e. the microbial hazard under concern, seems more appropriate and may enhance specific parts of an MRA study. Some basic information on GSMMs is provided below and interested readers should consult excellent reviews on this topic for more details (Bordbar et al., 2014). Based on the WGS information of a given organism it is possible to reconstruct a genome-scale metabolic network of chemical reactions also known as GSMM. It contains information about the genes and proteins (enzymes) implicated in the metabolic reactions of a microorganism. A GSMM can be integrated and/or validated using genomic, transcriptomic, proteomic and metabolomic data (Patil and Nielsen, 2005). If validation takes place in situ (i.e. in a food or environmental sample), through meta-*omics* approaches, it may be possible to evidence the influence of the microbial community or other, abiotic factors, on the predictions made using a GSMM. From this point of view, it might enrich MRA studies through understanding and prediction of the relationships between genes, proteins (enzymes) and reactions. For example, it is possible to identify the active metabolic pathways, which may lead to increased resistance of a foodborne pathogen under a specific environmental condition, using a GSMM developed for this pathogen. In a given environmental condition, however, the identification of the presence of genes that encode enzymes for a particular metabolic pathway does not necessarily mean that there will be a direct effect on the flux output (Andersen et al., 2001; Koebmann et al., 2002). The kinetic of enzymes determines which flux will be ultimately affected (Teusink and Smid, 2006) and, in this context, the development of kinetic models for the specific metabolic pathways identified earlier by the GSMM as active will have a positive influence on an MRA study. Moreover, it will provide information on how this environmental condition affects the pathogen's metabolic network, i.e. predictions about its phenotype; resistant or sensitive.

For this purpose, there is an inventory of analytical tools that can be applied to GSMM to obtain additional information. For example, Flux Balance Analysis (FBA) can be implemented to a GSMM to get the optimal value for product yield and biomass formation or even to predict metabolic fluxes and specific growth rate. Teusink et al. (2009) used GSMM, FBA and physiological data to investigate the adaptive growth strategy of the microorganism *Lactobacillus plantarum* in an unusual and poor carbon source conditions, while Métris et al. (2011) have exploited FBA to determine essential genes for *Campylobacter jejuni*. Therefore, specific parts of an MRA study such as Hazard Identification and Exposure Assessment may benefit from the introduction and application of GSMMs. Nowadays, the determination of maximum growth rate (μ_{max}) for instance, used to assess exposure in MRA studies, is mainly descriptive and empirical, while changes in μ_{max} reflect metabolic adaptations that can be explained with such global approaches (Adadi et al., 2012; O'Brien et al., 2013). Finally, the Hazard Characterization component of an MRA study, and especially its sub-component of the host-pathogen interaction, a significant component of an MRA study, may take advantage of the GSMMs. When both kinds of models, human and microbial metabolic models, are available the interaction between host and microorganism can be established (Bordbar et al., 2010; Nielsen, 2015).

5. How new NGS approaches could impact traditional microbiology programs in the food industry?

Metagenomics is a powerful tool that allows both taxonomic and functional characterization of microbial communities, which would have been difficult if not otherwise impossible to determine using traditional microbiological techniques. Since the early 2000s, the impact of the microbial communities in the food-processing environment on the hygiene and food safety of the finished product, also known as environmental pressure, has been widely studied with the limited molecular tools available such as PCR and D/TGGE. While these tools created an improved picture of the microbial environment compared to traditional culture methods, a number of questions remain open: Why do some pathogens have higher prevalence (time, location) than others? How can one assess or identify the transfer of pathogens from the environment or raw materials to product or how do inadequate control measures lead to faults in finished products? As we move into a new paradigm using NGS in metagenomic studies the increased information provided will impact industry, whether it is greater insight into the composition of microbial communities in a factory, faster identification of specific strains, and their capabilities to cause disease or spoilage. Industry presently identifies ways to incorporate metagenomics into their Pasteurian based microbiological food safety and quality systems. This increased information will lead to a revolution similar to that which we are currently observing with the use of WGS strain typing in epidemiological investigations of foodborne illnesses (Rantsiou et al., this issue), as it is expected to move from culture to omics based indicators, not considering only presence, absence or enumeration of one type of organism or community, but also its potential impact on food quality and safety and public health (Tan et al., 2015).

Potential applications of NGS led metagenomics are far ranging and these community analyses could be used in a number of interesting ways. In the area of Food Authenticity the microbiota of a food product could be used as a fingerprint specific to that product and factory in order to define its point of origin (David et al., 2016). This will, however, require the preliminary knowledge of the microbiome of the specific raw materials and the effect of the location of origins. This DNA based fingerprint can be one additional tool, once defined, to establish identity of a food, water or beverage product. This same information, however, may prove useful to the food industry by enhancing or focusing the trace back of products and raw materials when spoilage or food safety issues occur as the microbial species and strains present in the microbiome could be specific to regions, countries or even factories and raw materials, in much the same way the Food and Drug Administration (FDA) has used WGS strain typing to identify the geographic origin of strains in specific outbreaks (Chen et al., 2016; Hoffmann et al., 2016; Wilson et al., 2016). These metagenomic studies may also be able to identify sentinel microbes (essentially indicator microbes linked to various pathogens), which could be incorporated into food safety plans.

While metagenomic studies may help to identify sentinel microbes and provide enhanced information for source tracking they cannot at least for now replace the classical microbial food factory control plans already in place. While the technology is becoming more accessible, it

still requires technical expertise that cannot be implemented in all food business manufacturing sites. However, well designed studies can challenge and improve factory control plans by identifying the most relevant target for routine testing, moving verification schemes from expert driven to data driven and most importantly providing insight for microbiological risk analysis across matrices.

The MRA framework can be applied both for food safety hazards and spoilage organisms. Codex Alimentarius Commission has provided a basis for performing MRA for food safety hazards. This systematic risk assessment for a particular hazard encompasses several sequential steps to enable the determination of a Food Safety Objective (FSO), which provides an appropriate level of protection for the public health against a particular hazard in a country. In the light of new insights brought by metagenomics, the outputs of the MRA framework are expected to provide new options for mitigation the hazard, such as adhesion capability, survival mechanism and ecological interaction. It is then up to the industry to derive applicable control measures to meet the FSO (CAC, 2014). With regard to spoilage, the task of establishing control measures can be more difficult; not only are there a large number of spoilage agents, but they also possess, quite different spoilage characteristics. Moreover there is also a lack of public data providing any clear qualitative links between processing line performance (specification met or not) and consumer satisfaction as there is with the GenomeTrakr network and foodborne illness (although much of this meta data is currently only available to FDA and CDC). Metagenomic tools, presently under development, are expected to contribute in providing food spoilage potential, helping food business operators to have a better rationale for product acceptance (Pujol et al., 2013; Cao et al., 2017).

Metagenomics in risk assessment can potentially bring value where (i) risk assessment requires evaluation of a microbial community and or (ii) to provide more details of a known hazard in the processing environment or in a food matrix (growth, survival, and ecological competition). A food safety risk assessment based on an entire microbial community present in a food matrix is not readily foreseeable, though applications such as meta-transcriptomics might help study the behaviour of a hazard in its natural environment in the presence of co-occurring microorganisms. The behaviour of a particular hazard and its virulence potential can be used in both hazard identification and characterization studies during risk assessment. Nevertheless, as highlighted by Franz et al. (2016), in their opinion on the applicability of whole genome sequencing of a single isolate in MRA, a paradigm shift will be required for the current risk assessment models to input genotypic information. Also, the predicted phenotypic characteristics have to be validated for biological relevance using experimental studies before its use in risk assessment models.

Aside from its potential use in MRA, metagenomics is currently finding applications where traditional culture methods fail or are difficult to apply. Moreover, simulation of spoilage conditions can be tricky to replicate and study under laboratory conditions as the growth media used for the isolation may induce a selective bias. Amplicon sequencing or whole genome shotgun metagenomics sequencing can rapidly identify microbial population with a short turnaround time and also negates the influence of the selective enrichment bias thus providing immense value in spoilage studies. Additionally, metagenomic tools can help industries to anticipate this microbial spoilage by identifying genes coding for enzymes in the metabolic pathway leading to spoilage present at the beginning of a products shelf-life and using this data to create models that asses the risk of product spoilage (Kable et al., 2016). However, limitations in the method such as the quantification of organisms, live/ dead differentiation, biological relevance of functional prediction and sampling bias have to be overcome to successfully apply metagenomics studies to develop mitigation strategies to prevent product spoilage.

Metagenomics has also found utility in improving traditional pathogen detection methods. For example, The US FDA used metagenomics to study the enrichment step used for detecting *Salmonella* in the tomato phyllosphere (Ottesen et al., 2013). Similarly this can also be applied to optimize the sample preparation steps for the detection of pathogens in difficult matrices such as mineral mixes.

Metagenomics can also help to understand factory ecology and either validate or improve the current environment pathogen verification activities. For example, attempts have been made to devise biocontrol applications to manage L. *monocytogenes* in production facilities (Fox et al., 2014) by studying the microbial population. The findings of this study suggested the presence of sentinel organisms, which can signify the attachment and the potential for biofilm formation by L. *monocytogenes*, which contributes to the basic understanding of an industry-wide problem. Outcomes of such studies have to be validated but could significantly improve the current microbial hazard management practices.

Metagenomics can be of immense benefit to the food industry where the current microbial isolation and identification methods suffer serious limitations in addressing its concerns. This tool can also be used to gain new knowledge and information on known issues and can potentially result in bringing significant advantages to the current management practices. For example, metagenomics can be applied to either validate or significantly improve the applicability of *Enterobacteriaceae* family as a hygiene indicator in a dry manufacturing facility. In conclusion, metagenomics along with WGS of single microbial isolates will result in the generation of tremendous knowledge which has to be harnessed for the benefit of the food industry by defining relevant case studies and a paradigm shift will be required before these data can be used in food safety risk assessment models. Finally industry is still struggling to identify the best way to incorporate omics of all types into its food safety plans. While the previous sections provide many examples of how these technolgies may be used in industry and building upon these examples it is easy to envision a coordinated program during product development through scale-up and production in a factory where the manufacturers link metagenomics and metabolomics to better understand the microbiology of the product and the process to meet a FSO or Spoilage objective. As industry always desires faster more intigrated management of hazards it is easy to envision this future as similar to that proposed by the clinical microbiologist, were the ingredient and enviromental monitoring programs are metagenomic and metabolomics based with cultural conformation of the positive samples. This system would provide increased speed for industry. However a few challenges as discussed above remain. First the distribution and prevelence of many pathogens in ingredients such as dairy powders means we still have to find the needle in a hay stack. Therefore our reliance on classical enrichments will remain until this is solved. Additionally, industry needs to prepare for this radical change in how food microbiology is conducted; the skills at the bench and throughout the bioinformatic pipline are not common place in the food safety workforce and it will take time for these things to become common place across industry, which will make the interpretation of data and communication of its implications across all levels of a company from a risk manager to the Vice President of Food Safety seemless.

6. Conclusions

The application of multi-omics in food safety and quality has the potential to answer questions traditional microbiological methods could not address. Approaching the food ecosystem from different angles (metagenomics, metatranscriptomics, metaproteomics and meta-metabolomics) allows for a “holistic” representation of which microorganisms are present, how they behave, how they interact and which are the phenotypic manifestations in this complex arena. The expected outcome may have an invaluable impact in food safety, in order to reduce the risk associated to foodborne pathogens, but also to better control spoilage processes. However, before this becomes reality a number of obstacles and hurdles have to be overcome. More specifically we have to learn how to translate molecular events into practical

applications, which will give the food industries concrete solution on how to make food products more safe and stable.

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Appendix A. Supplementary data

The glossary and abbreviations list are presented in Supplementary Tables 1 and 2, respectively, and are reproduced in the four joint papers on “Next generation Microbiological Risk Assessment”. Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.ijfoodmicro.2017.11.008.

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