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Metagenomic insights into the dynamics of microbial communities in food

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ABSTRACT

Metagenomics has proven to be a powerful tool in exploring a large diversity of natural environments such as air, soil, water, and plants, as well as various human microbiota (e.g. digestive tract, lungs, skin). DNA sequencing techniques are becoming increasingly popular and less and less expensive. Given that high-throughput DNA sequencing approaches have only recently started to be used to decipher food microbial ecosystems, there is a significant growth potential for such technologies in the field of food microbiology. The aim of this review is to present a survey of recent food investigations via metagenomics and to illustrate how this approach can be a valuable tool in the better characterization of foods and their transformation, storage and safety. Traditional food in particular has been thoroughly explored by global approaches in order to provide information on multi-species and multi-organism communities.

1. Introduction

Metagenomics has become ubiquitous in the field of ecosystem exploration. Natural environments as diverse as air, soil, water, plants, as well as various human microbiota (e.g. digestive tract, lungs, skin) have been thoroughly explored by this approach, but food microbiota have until recently been less reported in the literature, perhaps because microbial communities of food are generally considered to have a low richness in terms of diversity. A long and well established tradition of determination of the main food contaminating species via cultural methods exists and has proven its efficiency for proposing and determining criteria and regulations in the field of food safety. However, this cultural approach has the drawback of detecting only cultivable bacteria, potentially only a small portion of the true microbial population (Giraffa and Neviani, 2001). At the beginning of 1990s, new approaches in the description of bacterial communities appeared through the development of culture-independent methods such as denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993), temperature gradient gel electrophoresis (TGGE) (Felske et al., 1998), terminal restriction fragment length polymorphism (T-RFLP) (Marsh, 1999), and several other automated PCR-based techniques still widely used today, such as temporal temperature gradient gel electrophoresis (*TTGE*) (Mace et al., 2012). Most of these methods allow accurate identification of part of the microbial community through the sequencing of ribosomal 16*S* rDNA targeted gene. Then, in the mid to late 1990s, two new methods for DNA sequencing were developed by Ronaghi et al. (1996) and Mayer et al. (1997), the pyrosequencing and the parallelized ligation-mediated and bead-based sequencing, respectively. Together, these two methods were considered as the "Next-Generation Sequencing" techniques (NGS). In the mid-2000s, commercially available sequencers based on these methods appeared (454 Life Science) leading to a revolution in the study of microbial ecosystems with the possibility of high-throughput sequencing of genes (HTS).

The development of these NGS technologies and their application in the field of food ecosystems revealed that these communities were perhaps more rich than expected and that some of them might play a yet unsuspected role. Ercolini (2013) recently reviewed high throughput workflow for food analysis by HTS. The use of these sequencing technologies to study food microbial communities is still relatively new, but its popularity is currently booming and its use has become affordable not only for researchers but also for the food industry as several companies now provide these services. The aim of this paper is to have an overview of information gained by this NGS approach to further our

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understanding of food ecosystems. We will focus in particular on the bacterial aspect of microbiota since many publications, using NGS for microbial food description, target the 16S rDNA gene. Yeast and filamentous fungi play a key role in food just as bacteria does, but the use of NGS to decipher an eukaryote ecosystem requires a different approach, targeting the internal transcribed spacer (ITS) region, a non-coding DNA sequence situated between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA genes in the chromosome. The ITS database is somewhat less advanced than for the 16S rDNA gene, but will gradually improve over the next few years (Santamaria et al., 2012). Nevertheless, some interesting reviews have already addressed this topic (Wolfe and Dutton, 2015). Specific challenges for food will be addressed and illustrated in the remainder of this paper.

2. Revisiting our vision of known foods

2.1. Metagenomics and metagenetics: a matter of scale and target

Metagenomics, based on gene fragment DNA sequencing, refers to the analysis of genetic material coming directly from the environment. In fact, in most cases the so called "metagenomic studies" are based on the analysis of a single type of gene: the 16S rRNA encoding gene, which is the most powerful marker for the identification of bacterial species and phylogenetic studies. Sequencing randomly amplified DNA fragments as per the metagenomic approach is less often reported, especially in food. It has been proposed that the term metagenetics may be restricted for studies dealing with 16S (Esposito and Kirschberg, 2014). In the field of food, mainly 16S-based studies have been performed.

The availability of new and affordable molecular techniques to characterize microbial flora has aroused interest regarding the potential to overcome classical microbiology limitations, or more accurately, to complement traditional microbiology with culture-independent strategies. Thereby, several kinds of food products, both fermented and unfermented, have been investigated using this duel-approach. In this way, the field of cheese making can be used to illustrate and summarize the various goals and concerns, which can be addressed by metagenetics and metagenomics. We will refer to two recent reviews for further readings (Irlinger et al., 2015; O'Sullivan et al., 2013).

2.2. Cheese: a product of interest

Cheese processing starts with fermentation by lactic acid bacteria (LAB). During this process, cheese evolves into 2 distinct parts: the inner core and the external rind, in which the microbiota and their interactions are different. The microbial populations originate either from raw products or from starter cultures; then other populations progressively replace the dominant starters during ripening. These starters, mainly LAB, induce the early acidification process, while the non-starter microbiota (which include bacteria but are also comprised of yeasts, molds, and filamentous fungi (Fox et al., 2000)), is involved in flavor, ripening or smear cultures.

Although numerous studies using molecular techniques (e.g. qPCR, DGGE, TRFLP) had already been published, the first extensive description of cheese bacterial microbiota with 16S metagenetic analysis by Quigley et al. (2012) set a new milestone with the first detection of several bacterial genera in cheese, such as *Prevotella* or *Arthrobacter*. Since this first publication on Irish cheese, the majority of the descriptive reports target a specific cheese type: water buffalo mozzarella cheese (Ercolini et al., 2012), Latin-style cheese (Lusk et al., 2012), Croatian cheese (Fuka et al., 2013), Belgian Herve cheese (Delcenserie et al., 2014), and Mexican Poro cheese (Aldrete-Tapia et al., 2014). This overview of traditional cheese studies was completed by two transversal studies on several kind of cheese (Almeida et al., 2014; Wolfe et al., 2014). All these studies assessed the microbial diversity in complement with classic microbiological culture. They revealed that cheese rind possesses a dominant core of 14 bacterial and 10 fungal genera (Irlinger et al., 2015; Wolfe et al., 2014). This core is completed by more specific sub-dominant populations, which are thought to be active in the cheese ripening and aging. The analysis of the flora detected by HTS and by classic microbiology reveals significant differences. Metagenetics capture a broader range of bacterial population where bacterial cultivation and isolation can often be more thorough in terms of identification and characterization. The limits of metagenomics are well known: the length of sequencing fragments, the depth of sequencing effort, and bias on DNA extraction and amplification. However, it should be emphasized that microbiological media and culture conditions are biased too, since they often favor compatible and cultivable bacteria over more slow-growing and non-cultivable populations.

Beyond the ecology of cheese microflora, these HTS surveys were often performed regarding specific concerns. First, the characterization of a particular cheese with a protected specific appellation will be useful to underline and explain its typicality (Aldrete-Tapia et al., 2014; Delcenserie et al., 2014; Fuka et al., 2013). Second, the main goal is to develop a better understanding of the fabrication process (Bokulich and Mills, 2013; Ercolini et al., 2012; O'Sullivan et al., 2013, 2015). Metagenetics can be applied to various types of samples to identify spatial and temporal variations during cheese processing. In particular, O'Sullivan et al. (2015) have shown that during a day of production, late cheeses tend to present a higher bacterial diversity than early cheeses and that this diversity is persistent during the ripening process, which is a concern when traditional cheese makers want to scale up and standardize specific or raw milk cheeses. HTS analysis can be a powerful tool to measure the impact of process changes on the typical microbiota (Aldrete-Tapia et al., 2014; Fuka et al., 2013).

Finally, global metagenomics has been used in order to improve the functional knowledge of cheese products. In a first publication, Wolfe et al. (2014) described *in situ* and *in vitro* studies of rind formation in which they isolated bacterial and fungal populations and observed bacterial/fungal interactions. More precisely, using a metagenomic approach they studied the cheese rind microbial communities of 137 different cheeses across ten countries and revealed a widely distributed dominant community of 24 culturable genera of bacteria and fungi. The authors first investigated how taxonomic diversity varies within the three rind types studied: bloomy, natural, and washed. They then revealed putative functions of uninoculated organisms, such as the presence of methionine-gamma-lyase (MGL) (an enzyme responsible for the production of sulfur compounds in cheese), which has previously been reported only in *Brevibacterium linens* (Amarita et al., 2004) and was reported here in *Pseudoalteromonas*. Following this *in situ* study, authors proceeded to an *in vitro* experiment by culturing a representative cheese rind community composed of (at least) one isolate from the 24 dominant genera previously identified. By doing so they highlighted the importance of abiotic manipulations by cheese makers in the selection of specific microorganisms. This *in vitro* approach also allowed an easier way to describe interactions within the cheese rind communities, for example between bacteria and fungi.

A second paper by Almeida et al. (2014) performed massive genomic sequencing and functional metagenomic profiling of cheese samples. The authors selected 142 bacteria isolated from dairy products belonging to 137 different species and 67 genera. Via massive sequencing, they were able to reconstruct 117 genome drafts. Through their work, they actually doubled the number of sequenced genomes of known bacteria linked to cheese products with the ambition of building a functional genomic catalog dedicated to cheese bacteria. They also analyzed the microbial composition of communities present at the surface of different traditional cheeses and observed that a significant proportion of the species were present in the newly sequenced genomes part of their catalog. This revealed that some species not initially inoculated, named *Psychrobacter immobilis* and *Pseudomonas haloplanktis*, were in fact present as dominant species.