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# Zooming into food-associated microbial consortia: a 'cultural' evolution

Luca Cocolin[1] and Danilo Ercolini[2]

Foods are complex and dynamic microbial consortia where bacteria, yeasts and fungi can coexist. The advances in the culture-independent analysis of food microbiota have revolutionized the way we study these microbial ecosystems, leading to a 'cultural' evolution. This is not only because we have technically learned to avoid cultivation to study food microbes, but also because our mental approach to food microbiology has changed. We discuss the most recent achievements in the field of food microbial ecology and give examples of how current molecular biology tools can be used to study performance of microorganisms used for food fermentation, to explore the sources of technologically relevant and spoilage bacteria as well as to acquire knowledge on the behaviour of foodborne pathogens.

**Addresses**
[1] Department of Agricultural, Forest and Food Science, University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Italy
[2] Department of Agricultural Sciences, Division of Microbiology, University of Naples Federico II, Via Università 100, 80055 Portici, Italy

Corresponding author: Ercolini, Danilo (danilo.ercolini@unina.it)

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## Introduction

Regardless of their complexity, microbial populations in foods are the object of one of the widest attention from the scientific community because of the great influence they can have on quality and safety issues that are pivotal in food science. Foods are complex and dynamic microbial ecosystems in which bacteria, yeasts and filamentous fungi are cohabiting, interacting and communicating. Through these activities they contribute to the transformation of raw materials in final products in the case of food fermentations, but they can be also responsible for food spoilage. The advance in the technologies of analysis of food microbiota have completely revolutionized the way we study these microbial ecosystems and have allowed us to make progress in leaps and bounds in food microbial ecology. Food microbiologists often report in their scientific articles that the bias of cultivation can be overcome by the use of culture-independent approaches. At present, we are not yet fully sure that culturability is as much an issue in food studies as it is in other natural environments. Indeed, recent studies demonstrated the possibility of a full *in vitro* reproduction of complex microbial communities from cheese [1••]. Food microbial ecology has been based on the study of microbial isolates for decades. Still, culture-independent methods have allowed for the last 20 years a convenient and reliable analysis of food microbiota for the most different purposes. It all started with the translocation of the polymerase chain reaction — denaturing gradient gel electrophoresis (PCR-DGGE) approach from the field of environmental microbiology, where it was firstly used [2], to the analysis of food samples, where it was employed to monitor microbial populations during food production, storage and distribution to look at food fermentation and spoilage dynamics [3,4].

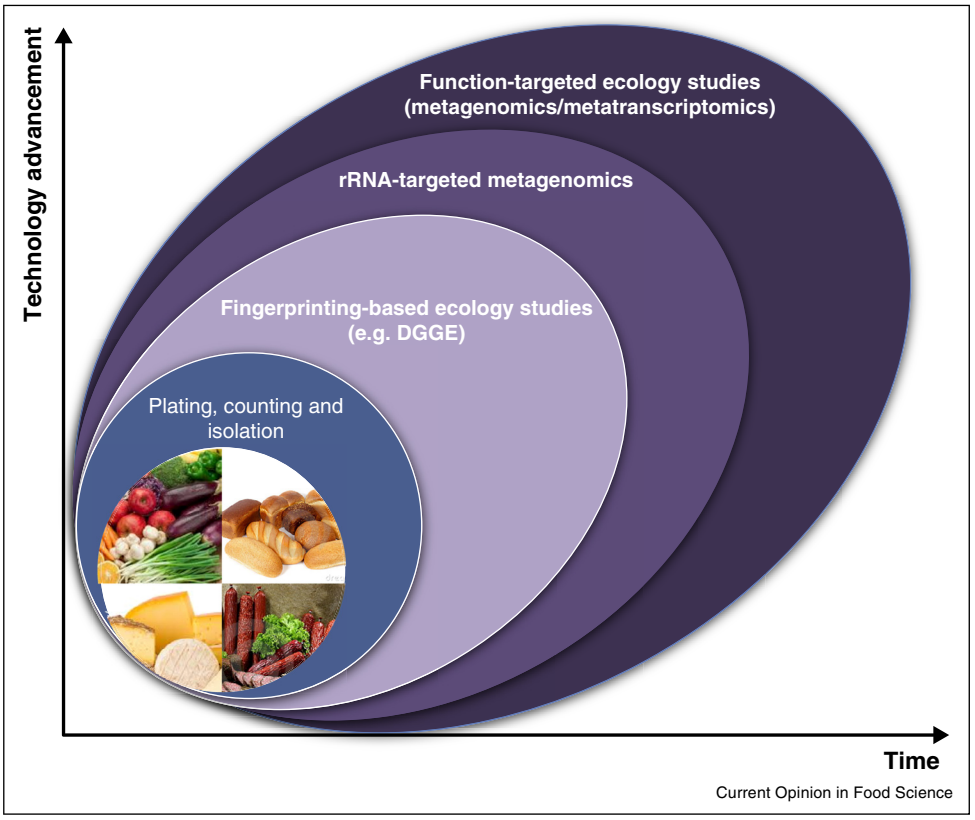
With the advent of the culture-independent analysis of food, it is a 'cultural' evolution we have been experiencing. This is not only because we have technically learned to avoid cultivation to study food microbes, but also because our mental approach to food microbiology issues has changed. We have evolved to think of food microbes as consortia and learned to monitor their occurrence, changes and activities as such.

## The structure of food microbiota described by a bunch of sequences

Recently we have switched from fingerprinting analysis in which food microbiota is visualized as band patterns, to sequencing where microbial consortia are described as nucleic acid sequences (Figure 1).

The current availability of the most powerful high-throughput sequencing (HTS) technologies have determined an almost complete replacement of the electrophoretic PCR-DGGE approach with direct sequencing of the rRNA genes of mixtures of barcoded food samples. At the moment, the novel sequencing-based tool is massively used in many research laboratories active in food sciences and it is pushing forward the culture-independent study of food microbial ecology [5]. The use of rRNA amplicon sequencing to study microbiota is the most common HTS application in food microbial ecology, although cost of analysis and need for bioinformatics skills are limiting industrial applications [5]. This entails the analysis of amplicons arising from a complex mix of

**Figure 1**




Evolution of the microbiological approaches used to study microbial diversity and ecology in food ecosystems.

microbial genomes directly extracted from a food sample. The target genes are those of taxonomic interest, with the 16S rRNA gene being the most widely used for bacteria, while ITS and 18S for fungi. rRNA amplicons, obtained from DNA/RNA extracted directly from foods, are sequenced and sequences are compared to reference databases to identify the operational taxonomic units (OTUs) through well established bioinformatics pipelines [6–8]. The approach, recently re-named metagenetics [9], is considered quantitative as the number of sequence reads identified with the same OTU allows for an estimation of the relative abundance of each microbial entity in the food sample analyzed. The methods require a constant and careful updating and maintenance of the sequence databases that are almost weekly enriched with new sequences. The unprecedented advantage of sequencing-based tools is having a quantitative monitoring of 'microbial species' in food ecosystems. The quantitative power of the PCR-based target amplicon sequencing is widely acknowledged by the scientific community. However, we would like to take this opportunity to remind that such tools bring all the possible bias linked to potential selective nucleic acids extraction and preferential PCR amplifications [5] that are currently perhaps too much overlooked. With sequencing-based detection systems, the achievement of species-level taxonomic identification can be very tricky and strongly depends on the sequences length. In several food products, varying from cheese to meat or fermented vegetables, many different species of the same genus can occur. In such cases, an HTS study at the genus level is not useful as being informed that '*Lactobacillus*' dominates in a cheese ripening is no news. For this purpose, long sequence reads including more variable regions of the 16S rRNA gene are required for accurate assignment.

Studying the changes in microbial populations can provide useful information to follow natural fermentation dynamics, monitor the fate of starter or adjunct cultures, or observe the shifts in spoilage-associated populations according to food storage conditions. Many different researches have been carried out unravelling the structure of the microbial consortia in dairy [10–14], meat [15] and vegetable foods [16•,17–19].

Food fermentations can be observed at community level with a glance at microbe-samples networks. In a case of sourdough fermentation, sourdough-microbe networks