Applications of Metagenomics to Fermented Foods

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1. Introduction

Fermentation is a traditional way of food preservation and is of great importance for human food consumption as it enables the development of nutritional and organoleptic qualities of food. This key traditional process is used for the conservation and transformation of a wide variety of food products of different origins (animal or vegetal) and nature (liquid to solid). For instance, starchy cereal-based food, meat, fish and sea food, vegetables and fruits, dairy products, cocoa, coffee and many others are transformed by fermentation. Food fermentation is utilized in many different geographical areas and, most of the time, occurs spontaneously. In Africa and Asia, traditional fermented foods represent a large part of local population diet. They often require process optimization to extend their production and commercialization (Aidoo et al. 2006, Sanni 1993). Fermentation is either alcoholic, resulting in alcoholic beverages or bread, lactic as is usually seen for dairy foods and vegetables, or acetic (vinegar). But food products may also result from a combination of these. Moreover, frequently for traditional foods, fermentative agents are undetermined and may involve bacterial or mould species.

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Although empirically performed, many food fermentation processes gained scientific interest over the last decades. The aim of current research is to understand and control fermentation progress in order to ensure constant sensorial properties, to increase safety and to limit spoilage. Industrial microorganisms are usually very easy to analyse by traditional microbiological methods because their production is controlled and pure strains are usually used. On the contrary, spontaneous fermentations results from the activity of a complex microbial ecosystem whose diversity and level of organization remains largely underestimated (Botta and Cocolin 2012). This complex microbial ecosystem can be composed of microorganisms that are not cultivable or non-viable in laboratory conditions. In addition, similar physiological properties (leading to similar food characteristics), may result from the presence and/or activity of phylogenetically distant bacterial species. As a consequence, conventional microbiological methods are, by far, not the best tools to study fermentation in these cases (Cocolin et al. 2011).

Over the last decade, molecular methods were applied to environmental samples (including food) in a culture-independent manner to study microbial ecology as a complement to culturing studies. These first approaches of 'Metagenomics' greatly facilitated the description of fermentation ecosystems and the characterization of the microbial species involved in the process. Metagenomics, according to Chen and Patcher (2005), can be defined as "the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species". As compared to classical cultural-based methods, metagenomics approaches are meant to give a quicker and more comprehensive as well as a complementary description of the microbial diversity associated with the fermentation process.

Metagenomics relies on nucleic acid (DNA/RNA) analyses and appears frequently associated to massive sequencing tools and bioinformatics. However, other molecular methods used to investigate microbial diversity fall into this field. Target DNA (or RNA) regions are often analysed by fingerprinting methods coupling PCR amplification, enzymatic restriction and electrophoretic techniques (such as T-RFLP—Terminal Restriction Fragment Length Polymorphism, DGGE—Denaturing Gel Gradient Electrophoresis, TGGE—Temperature Gradient Gel Electrophoresis, SSCP—Single-Strand Conformation Polymorphism, ARDRA—Amplified Ribosomal DNA Restriction Analysis, ARISA—Automated Ribosomal Intergenic Spacer Analysis) or cloning. (For a review, see Nocker et al. 2007.) When studying food microbial ecology, PCR amplification is performed with primers usually targeting rRNA coding regions (16S, 26S and 28S for bacteria, yeast and fungi respectively) for global analyses so-called community profiling. Such global approaches generally provide an overview of major taxons but poor resolution for the identification at the species level. On the other hand, targeted studies focus on specific microbial groups in order to get insight into their abundance and/or diversity; specific genes can also be targeted when searching for specific (enzymatic) functions. Functional metagenomics explores the diversity microbial enzymatic activities potentially or actively present in a given ecosystem at the gene (DNA) and gene expression (RNA) levels (metatranscriptomics). (For a review, see Bokulich and Mills 2012.)

The rise of Next-Generation Sequencing (NGS) technologies (about a decade ago) revolutionized the field of microbial ecology. NGS analyses provide a comprehensive description of the microbial DNA content in a given sample by generating up to 10^9 sequence reads per run. These high-throughput approaches allow: (i) the sequencing of PCR amplicons from rRNA coding regions (16S for bacteria and ITS for fungi) or (ii) the direct sequencing of the whole-community DNA for a comprehensive exploration of the DNA diversity or 'metagenome' of the sample. Four types of NGS technologies (454 Pyrosequencing, Ion Torrent, Illumina and SOLiD systems) are available on the market and are able to generate per sample analysis from 500 million to 50 billion of short DNA sequence (100–400 pb) reads that are further aligned and either compared or assembled. The choice of technology and method depends on the type (or complexity) of fermented food and the aim of the study. The Pyrosequencing (and Ion Torrent) method generates about one million of 400–600 bp reads and provides a high taxonomic resolution (up to the species level) but a low coverage of the sample. At a lower cost, the Illumina (and SOLiD) technology provides a higher coverage by generating up to one billion short reads (100 pb) with low taxonomic resolution, and is more likely to detect rare or underrepresented sequences. NGS data are analysed using bioinformatics software (Mothur, Qiime, MEGAN, etc.) that allow the processing, alignment, assembly and statistical comparison of DNA/RNA sequences. Reference databases are then utilized for taxon assignment. The rapid, constant development of bioinformatics tools and software and the increasing amounts of data available contribute to enrich and increase the reliability of metagenomics-dedicated databases. (For a review, see van Hijum et al. 2013.)

This chapter reviews some of the most recent applications of molecular techniques in metagenomics approaches to understand fermentation microbial ecology dynamics and to improve the quality of fermented food.

2. Metagenomics as a Tool to Monitor Fermentation Process

Fermentation generally starts with a high microbial diversity which corresponds to the microbial contamination of raw materials, process equipment and human manipulation. Fermentation is characterized by modifications of the food composition: alcoholic fermentation results from the conversion of sugars into ethanol and carbon dioxide; lactic acid fermentation produces lactic acid and possibly, carbon dioxide, acetic acid and ethanol; acetic acid fermentation results mainly in acetic acid formation. Throughout the fermentation process, the interaction between the microflora and the fermenting food matrix creates a dynamic phenomenon, at both microbial and biochemical levels, characterized by changes in physicochemical conditions (pH, salt, temperature, etc.). All these biochemical modifications share the common feature of generating stress factors for microorganisms. The adverse effects of ethanol result from impairment of microbial cellular membrane properties (Aguilera et al. 2006, Li et al. 2012). An increase of organic acid levels generates a dissipation of the proton gradient across cellular membranes (Russell and Diez-Gonzalez 1997). The composition of the raw material may take part of the environmental stress: for example, high sugar levels result in an osmotic stress. Moreover, food preparation, such as salt addition

performed at the beginning of the fermentation process, may contribute to the stress effect. As a consequence, a decrease of microbial diversity is generally observed over fermentation progress, and a dominant microflora appears.

This is illustrated by many examples through different methodological approaches. For example, bread is traditionally made from sourdough which is a mix of flour and water containing yeasts and bacteria. The first studies that considered sourdough diversity were based on cultural approaches completed by molecular identification of dominant species. RAPD (Randomly Amplified Polymorphic DNA) and 16S ribosomal RNA coding region sequencing were used to determine the dominant species of lactic acid bacteria (de Vuyst et al. 2002). Afterwards, PCR-DGGE (Denaturant Gradient Gel Electrophoresis) was used in several studies (Meroth et al. 2004, Moroni et al. 2011). In rice sourdough, PCR-DGGE clearly showed a shift during process from mother sponge flora towards a dominating flora mainly composed of *Lactobacillus curvatus*. Over gluten-free sourdough elaboration process, a stable biota with a broad spectrum of autochthonous lactic acid bacteria and yeast species was established, as this biota was adapted and competitive in these conditions. Dominating species were commonly isolated from other types of sourdough (rye or wheat) or from other tropical product fermentations. For example, *Pediococcus pentosaceus*, *Leuconostoc holzapfelii*, *Lactobacillus gallinarum*, *Lactobacillus graminis*, *Lactobacillus vaginalis*, *Weissella cibaria* were detected though they were not considered as typical of sourdough fermentation. Other methods like PCR-TGGE (Thermal Gradient Gel Electrophoresis) and High Resolution Melting quantitative PCR (HRM-qPCR) were used in other studies (Ferchichi et al. 2007, Lin and Gänzle 2014). The use of PCR-TGGE was demonstrated as another efficient approach to monitor the main fermentative species in sourdough. The authors underlined the need of a careful choice of amplification regions and sampling, and of critical and reproducible DNA extraction, PCR amplification and analysis before drawing any conclusion. The advantages of HRM-qPCR rely on the simplicity of the method and its ability to detect single nucleotide differences in target sequences. However, this approach would not be appropriate for complex microbiota whereby multiple peaks are present, corresponding to multiple melting temperatures of target sequences.

Generally, the final fermented product results from a large number of chemical reactions caused by microorganisms. The example of cocoa bean fermentation is particularly convincing as alcoholic fermentation, lactic fermentation and finally acetic fermentation take place successively. The initial anaerobic conditions at low pH (3.6) and high sugar content of the pulp surrounding the cocoa beans (obtained after the breaking of the pod) promote yeast activity (naturally present or introduced by humans). In the pulp, sugar conversion into alcohol and carbon dioxide causes a rise in temperature and an increase in pH due to the consumption of the citric acid by yeasts. These changes promote the onset of lactic acid bacteria (LAB) that oxidize the alcohol to lactic acid. As conditions become more aerobic (due to aeration), the production of acetic acid by acetic acid bacteria (AAB) is favoured. The formation of acetic acid from alcohol is an exothermic reaction and the temperature reaches about 50°C; this temperature shift causes inactivation of acetic acid bacteria. And, due to the presence of acetic acid, the biochemical reactions which produce precursors of chocolate flavor compounds can occur in the seed. Towards the end of the fermentation, the strong

odour of acetic acid decreases progressively. Other aromatic molecules (alcohols, acids, ketones, sulfur compounds) are also produced by the microorganisms during fermentation, and confer organoleptic qualities characteristic of the product (Marilley and Casey 2004, Smit et al. 2005, Lacroix et al. 2010, Sicard and Legras 2011). Both yeasts and bacteria are involved in the production of compounds responsible for chocolate flavor (Shwan and Whaels 2004). If not performed well, fermentation can lead to serious defects, such as beans with slate colour without flavouring, bitter taste and astringent or purple beans, as well as poorly aromatic or rotten beans.

Although literature on a thorough description of the microbial species involved in cocoa fermentation is available, relatively little is known about their precise contribution to chocolate quality (for a review, see Saltini et al. 2013). Recent metagenomics approaches on cocoa fermentations allowed a re-exploration of the process and revealed new insight into microbial dynamics, diversity and interactions during the process (Camu et al. 2007, Hamdouche et al. 2014). Notably, rare taxons as well as viral communities were detected, providing a comprehensive view on the ecosystem (Illeghems et al. 2012). This shows that complex fermentative ecosystems, such as spontaneous coca bean fermentation, studied by metagenomics approaches allow new features to be discovered.

3. Metagenomics to Determine which Fermentative Agents are Really Involved

Targeting DNA in studies of fermented food provides a lot of information on its progress but is limiting. In fact, the DNA molecule has a variable half-life dependent on many factors including the biological activity of the matrix in which it is present. In addition, simple DNA analysis does not allow distinguishing living from dead microorganisms when they are present in a certain concentration.

The study of food fermentation focusing on RNA instead of DNA provides information on the function of the microorganisms and how they are influenced by different environments. Indeed, RNA analysis seems able to better highlight the microorganisms that contribute to the fermentation process (Cocolin et al. 2013). Messenger RNAs have a very short lifetime and their extraction from the food matrix remains technically difficult. Contrarily, the use of ribosomal RNA seems a good compromise. Indeed, its lifetime is greater than mRNA but less than that of DNA.

The literature on metatranscriptomics to monitor fermentation is scarce. Real-time PCR on cDNA, PCR-DGGE on cDNA and microarray targeting transcripts are the main techniques used in those studies. The amount of 16S rRNA in fermented rice has been monitored by RT-Q-PCR (Reverse Transcription-Quantitative-PCR) (Nakayama et al. 2007). In other studies, Tuf, GroL and 16S genes were targeted from Emmental cheese samples (Falentin et al. 2010) and RT-PCR-DGGE was applied on cheese samples (Dolci et al. 2013). Metatranscriptomic gene-expression profiles during kimchi fermentation (fermented vegetable product) were investigated (Jung et al. 2013). This work showed the prevalence of carbohydrate metabolism (transport and hydrolysis) and lactic acid fermentation. *Leuconostoc mesenteroides* was rather active at the beginning of fermentation and *Lactobacillus sakei* and *Weissella*

koreensis presented a higher activity during later stages. A DNA array was developed to study kimchi and it was concluded that a minority of species mainly contribute to the development of kimchi (Nam et al. 2009). LAB communities of sourdough fermentations were monitored using a DNA microarray constituted of functional genes. Dominating LAB species were established after five days of fermentation. *Lactococcus lactis* had an important activity at the beginning of the biological process, whereas *P. pentosaceus* dominated during most of the process (Weckx et al. 2010).

Functional properties of the fermented food microbiota can also be investigated by metagenomics. Indeed functional diversity can be explored through genetic screening of genes of interest. For example, a study focused on 33 genes involved in probiotic and nutritional functions involved in survival, gastro tract, starch metabolism, folate and riboflavin biosynthesis (Turpin et al. 2011). In addition, a few studies have investigated the expression of targeted genes of interest in food matrix. For instance, the expression of gene encoding for amylase has been monitored in Pearl millet slurries (Oguntoyinbo and Narbad 2012, Humblot et al. 2014).

Metatranscriptomic analysis of fermented food microbiota still presents technical locks. For instance, the extraction of RNA from food matrix remains difficult (Ulve et al. 2008). Indeed such complex matrixes contain fats, polysaccharides and inhibitors that make difficult the extraction of good quality and quantity RNA. In addition, a lack of basic sequence data and annotation remains. Sequence information available in databases is still limited to some bacteria (lactobacilli, staphylococci and food pathogens).

Because metagenomics data include a huge proportion of genes encoding classical cell functions with little interest for food microbiota, sequencing of metagenomes should focus on specific parts (key enzymes for flavor compound production, toxin synthesis, or specific amino acid degradation) to be able to monitor, in addition to variations of species diversity, changes in microbial activity.

4. Is There a Fermentative Flora Signature of Terroir? Traceability Tools

The French notion of 'terroir' (the product identity), which is at the heart of the AOC (Appellation d'Origine Contrôlée), is directly linked to the food quality and origin. The European equivalent is the PGI, for Protected Geographical Indication, related to the European Regulation No 1151/2012 which promotes and protects names of quality agricultural foods (Capozzi et al. 2012). Many recent studies support the idea that biogeographical characteristics of terrestrial microorganisms may lead to regionalized properties associated with valuable crops, and so, underline the existence of a microbial biogeography or 'microbial terroir' (Renouf et al. 2006, Fierer 2008, Bokulich et al. 2013, Gilbert et al. 2014). As an example, the aquaculture farms in Vietnam were differentiated by their bacterial ecology showing the relation between terroir and presence of bacteria. The bacterial ecology was shown to stay stable on different fish samples collected at 6 months intervals (Le Nguyen et al. 2008). Similar studies were realized on fruits (El Sheikha et al. 2009, 2011) and marine salts (Dufossé et al. 2013), showing the robustness of the approach. Taking into account the importance

of the native microbial community in spontaneous fermentative process and the fact that this microbial ecology could be linked to the terroir, it leaves open to question whether fermentative microbes could be used as a potential signature of food terroir.

The resurgence of biogeography research, particularly the biogeography of microorganisms, is mainly due to the technological advances in molecular biology over the last decade. These advances led DNA markers to become one of the most effective instruments to traceability (Galimberti et al. 2013). The choice of appropriate molecular tool will depend on different parameters (quantity/quality/integrity of DNA, degree of genetic variations of the analysed species, etc.). In a general way, these microbial DNA analysis tools are characterized by the use of specific DNA markers (usually universal) based on PCR amplification techniques, such as digestion with specific restriction enzymes or electrophoretic analysis (RFLP, RAPD—Random Amplified Polymorphic DNA, AFLP—Amplified Fragment Length Polymorphism, DGGE, etc.). Over the last years, PCR-DGGE analysis has been very popular in the study of food safety and traceability, notably of fermented foods (Peres et al. 2007, Dalmacio et al. 2011, Hosseini et al. 2012, Zheng et al. 2012). An interesting study realized by Mauriello and his colleagues (2003) showed an example of PCR-DGGE application proving that the effect of microbial diversity of natural starter cultures on traditional dairy products and its evolution during fermentation may represent important proof of authenticity for the traceability of origin and mode of production. Another molecular tool application on fermented foods was done on wine grapes by using a high-throughput, short-amplicon sequencing technique (Bokulich et al. 2013). Using this method, more than five million reads for bacterial 16S rRNA coding sequences and more than 3.2 million reads for fungal ITS sequences were generated and, *in silico*, analysed. This study showed different microbial profiles according to vine-growing regions, giving thus evidence for a link between microbiota and regional, varietal, and climatic factors across multi-scale viticultural areas.

In conclusion, it is too early to conclude about the existence of a microbial signature of terroir, but research carried out until now shows interesting results in this direction. The increasing development of culture-independent methods drives research forward for a better understanding of the dynamics of microbial ecosystems in fermented foods.

5. Applications of Metagenomics towards Starter Selection

The use of microbial inoculants to enhance nutritional and quality properties (shelf-life, texture, aroma, etc.) or to hasten the process is widely applied in food industry. But, in the majority of the developing countries, the fermentation process relies largely on empirical techniques, characterized by an incomplete process control. As a consequence, low yields and variable quality products are obtained. From traditional food fermentations, microbial starters can be selected and re-used further. Microbial starter selection criteria are plurals. They include, not only the ability to use various substrates, the formation of metabolism products and the physiology of strain, but also food safety requirements and other quality expectations (Holzapfel 2002). Microbial starters gain to be characterized phenotypically and

genetically, for technological, safety and probiotic features (Ammor and Mayo 2007). The advances in this field towards an enhancement of fermented food quality often involve back-sloping or the inoculation of raw materials with a residue from a previous batch, but sometimes remain insufficient to ensure food safety. (For a comprehensive review of biotechnology issues in developing countries, see FAO 2010.) In addition, current applications of these starters are numerous in the field of food safety and show an increasing interest to understand manufacturing processes of local fermented foods (Benito et al. 2007, Capozzi and Spano 2011, Zdolec et al. 2013). It was demonstrated in case studies, such as yoghurt, that microbe-microbe interaction could have a positive impact on product quality of fermented food by exchange of 'information' and metabolites. Indeed, the Quorum Sensing phenomenon can be shared between species occupying the same niche (Smid and Lacroix 2013) and so, can play a role in complex communities of microbes involved in the fermentation process.

Molecular biology tools are very helpful in describing and understanding biological and biochemical phenomena related to fermentation. As they do not require the isolation and cultivation of microorganisms, they reflect the 'true' microbial composition of foods. By highlighting the main microbial species involved in the process of fermentation, metagenomic approaches are likely to unravel new fermentative species, thus helping in the selection of new starter strains (Díaz Ruiz and Wacher 2003, Cocolin and Ercolini 2008). The development and the application of metagenomic tools significantly contributed to elucidate the microbial community involved in many fermented foods. Applications of metagenomics to study the microbial ecology of fermented foods are becoming more and more numerous, but very few are related to the selection of starters. Some of them point the potentials of dominant strains, well adapted to the ecological niche, in the production of a safe fermented product.

An authoritative list of microorganisms with documented use in food has been established since 2002 from a joint project between the International Dairy Federation (IDF) and the European Feed and Food Culture Association (EFFCA) (Mogensen et al. 2002a, 2002b). The group of Microbial Food Cultures (MFC) satisfies the regulation EC no. 178/2002 to ensure manufacturers the safe use of microorganisms in food (Hansen 2002, Bourdichon et al. 2012). Most of the cultures are lactic acid bacteria (LAB) but yeasts (e.g., *Saccharomyces*) and molds (e.g., *Penicillium*) have been found for several foodstuffs (cereals, meats, milk, etc.). The use of these microorganisms to obtain safe fermented foods is based on their QPS (Qualified Presumption of Safety) status, similar in concept and purpose to the GRAS status (Generally Recognized as Safe) which is used in the USA. The QPS approach is maintained by EFSA (European Food Safety Authority) since 2007, according to European regulation 178/2002.

Molecular tools may have a significant role to play in QPS risk assessment. The detection, by metagenomics approaches, of a given genus or species in many fermented foods strengthens the body of knowledge of this genus/species and thus contributes to its acceptance as QPS.

6. Metagenomics for Safety Improvement of Fermented Food

Stress conditions associated to food fermentations are related to environmental stress and to biochemical modifications resulting from microbial metabolism. These conditions result in the selection of dominant flora but also can induce viable-but-nonculturable (VBNC) and/or non-viable states of microorganisms (Giraffa and Neviani 2001). For example, in wine, the spoilage yeast *Brettanomyce bruxellensis* can enter this stage and exert its aromatic deviation a long time after bottling (Serpaggi et al. 2012).

On one hand, the microbial dynamics observed during fermentation gives fermented foods an effective protection against pathogens (Adams and Mitchell 2002, Adams and Nicolaides 2008). It is also implied in the removal of toxic compounds from raw materials (Hammes and Tichaczek 1994). Competition and/or antagonism between microorganisms can be mediated through inhibitory molecules (peptides, bacteriocins, acids) that exhibit specific and potentially interesting properties.

Metagenomics is potentially a tool of choice to assess the survival of pathogens, toxinogens or spoilage species over fermented food elaboration process. A possible limit of the metagenomics approach is its failure to detect the less numerous microorganisms in an environment containing a dominant microbiota. Interestingly, the first study suggesting this application investigated microbial diversity in *Potopoto*, a maize dough used as weaning food, and in *Dégué*, a millet-based paste, by PCR-TGGE and by comparing DNA extraction techniques (Abriouel et al. 2006). The detection of *Escherichia coli* and of *Bacillus* species, which potentially included *Bacillus cereus*, over product elaboration, questioned the survival conditions of these microbiological hazards. Similarly, in other cereal fermented food traditionally prepared in Africa, the presence of *Clostridium perfringens* and of *B. cereus* was evidenced from a clone library while the identification from the main bands from PCR-DGGE profile did not showed these species (Oguntoyinbo et al. 2011). In another study, a hazard related to the detection of *Proteus mirabilis* and *Staphylococcus* spp. was pointed out by ARDRA analysis applied to isolates from soybean fermented food and from fermented pork samples whereas PCR-DGGE detected uncultivated *Clostridium* spp. and *Staphylococcus* spp. from fermented fish products (Singh et al. 2014). Thus, the discrepancy of results between classical microbiological methods and molecular methods should be more deeply investigated. Meanwhile the most reliable approach is to use both approaches as complementary tools.

On the other hand, the application of metagenomics in evaluating the impact of process modification on fermentation microbiota and safety issues is highly relevant. Salt reduction strategies for sauerkraut, in a more general view of reducing sodium intake, relied on the decrease of sodium chloride content, with or without its partial replacement by other mineral salts (calcium chloride, magnesium chloride and potassium chloride) (Wolkers-Rooijackers et al. 2013). The consequences of these changes on sensorial properties were investigated. In parallel, PCR-DGGE and classical microbiology were used to assess fermentative and safety issues resulting from this process changes. It was thus checked that fermentative profiles and *Enterobacteriaceae* population did not significantly change with salt content and composition. Another study focused on the relationships between mycotoxin contamination and the

fungal communities associated to food products, such as coffee (Durand et al. 2013, Nganou et al. 2014).

7. Conclusion

Metagenomics approaches are very powerful in exploring the microbial ecology of complex ecosystems such as fermentative foods. Different biological questions can now be addressed by global or targeted methods (functional metagenomics or metatranscriptomics). However, several considerations should be taken into account when undertaking metagenomics studies.

Technical issues should be carefully addressed in order to anticipate biases that can occur in the generated data. Nucleic acid extraction (DNA/RNA) yields are subjected to variations according to the composition of the food matrix and the microbial species present in the ecosystem. To obtain a reliable view of the ecosystem, the reproducibility of extraction and PCR has to be ensured. In addition, before arriving at a conclusion about a microbial feature or signature in a fermentation ecosystem, it is required that several studies be performed in the same ecosystem.

While whole community NGS approaches are not severely impacted by technical biases, targeted approaches are subjected to biases (as for every PCR-based method). Preferential amplification selects sequences and impairs the representativeness of amplified sequences with regard to the initial sample. Both nucleic acid extraction and PCR amplification reaction biases can produce a fraction of truncated or misleading data. Therefore, it is recommended data obtained from such approaches be complemented with data obtained from different approaches (molecular and cultural methods). In all, the production of metagenomic data needs to be correlated with all data available (pH, temperature, Aw, chemical composition, etc.) to produce meaningful and useful data for the comprehension of complex ecosystems.

Recently, metagenomic approaches have been used to study the interactions between human gut and food microbiota (Donovan et al. 2012, Faith et al. 2011). Obesity being associated with a particular gut microbiota (depletion of Bacteroidetes) (Ley et al. 2006), the simultaneous investigation of fermented food diet and gut microbiota by metagenomics would result in a better knowledge of the interactions between these microbiota and could be helpful in fighting against obesity. Moreover, products that benefit for human health could be developed. In the future, one can dream of the application of metagenomics, and more specifically metatranscriptomics, to detect in an ecosystem, the presence of mRNA synthesized from genes encoding amino acid decarboxylases involved in biogenic amine pathways or encoding enzymes involved in mycotoxin formation. The same tools could be applied to investigate the formation of bacteriocins in an ecosystem.

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