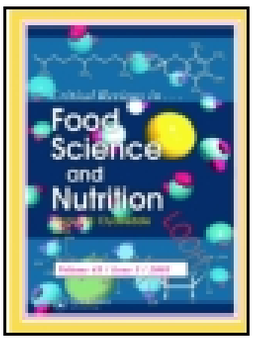

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Cheesomics: the future pathway to understanding cheese flavour and quality

Roya Afshari, Christopher J. Pillidge, Daniel A. Dias, A. Mark Osborn & Harsharn Gill

To cite this article: Roya Afshari, Christopher J. Pillidge, Daniel A. Dias, A. Mark Osborn & Harsharn Gill (2018): Cheesomics: the future pathway to understanding cheese flavour and quality, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2018.1512471

To link to this article: https://doi.org/10.1080/10408398.2018.1512471

Published online: 04 Oct 2018.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2018.1512471

Taylor & Francis
Taylor & Francis Group

REVIEW

Cheesomics: the future pathway to understanding cheese flavour and quality

Roya Afshari[a], Christopher J. Pillidge[a], Daniel A. Dias[b], A. Mark Osborn[a], and Harsharn Gill[a]

[a]School of Science, RMIT University, Bundoora, Victoria, Australia; [b]School of Health and Biomedical Sciences, RMIT University, Bundoora, Victoria, Australia

ABSTRACT

Cheese is a fermented dairy product, harboring diverse microbial communities (microbiota) that change over time and vary depending on the type of cheese and their respective starter and adjunct cultures. These microorganisms play a crucial role in determining the flavor, quality and safety of the final product. Exploring the composition of cheese microbiota and the underlying molecular mechanisms involved in cheese ripening has been the subject of many studies. Recent advances in next generation sequencing (NGS) methods and the development of sophisticated bioinformatics tools have provided deeper insights into the composition and potential functionality of cheese microbiota far beyond the information provided by culture-dependent methods. These advances, which include rRNA gene amplicon sequencing and metagenomics, have been complemented and expanded in recent years by the application of metatranscriptomics, metaproteomics and metabolomics. This paper reviews studies in which application of these meta-omics technologies has led to a better understanding of the microbial composition and functionality of cheese and highlights opportunities by which the integration of outputs from diverse multi-omics analytical platforms (cheesomics) could be used in the future to advance our knowledge of the cheese ripening process and identify biomarkers for predicting cheese flavor, quality, texture and safety, and bioactive metabolites with potential to influence human health.

KEYWORDS

Cheese; microbiota; next generation sequencing; omics analysis; cheese ripening

Introduction

Cheese is a complex biological ecosystem, harboring diverse microbial communities. Collectively known as the cheese microbiota, these communities originate from starter cultures, raw milk (especially in traditional or non-pasteurized cheeses), adjunct cultures, and adventitious microorganisms that originate from equipment and the cheese making plant environment (Fox 2000). The contribution of the cheese microbiota to flavor development and the quality of the cheese is of critical significance, as many of the final characteristics of a cheese are due to the complex dynamics and interactions between the cheese microorganisms, growth substrates and proteins in milk and the cheese environment. However, the growth and interaction between cheese microorganisms during processing and ripening is the least controllable of all of the cheese making steps (Cocolin and Ercolini 2007). In many cases, variation in the final characteristics can be observed within the same variety of cheese made in the same dairy plant, or in different dairies despite the cheese being made following the same manufacturing conditions (Cocolin and Ercolini 2007).

Given the profound importance of the cheese microbiota in determining cheese quality, considerable efforts have been devoted to identifying species variation in cheese microbiota using traditional and molecular methods (reviewed in Quigley et al. 2011). Advances in high-throughput sequencing (HTS) methods are transforming our understanding of cheese microbiology following extraction and sequencing of total DNAs (or PCR-amplified DNA) from cheese samples. These advances have enabled a clearer picture to emerge of the cheese microbiota and (further) the detection of microbial taxa, which had been previously overlooked because of limitations of culture-based methods which underestimate levels of microbial diversity (Hugenholtz et al. 1998). However, there is now a need to go beyond determining just the microbial composition and instead, focus on the functionality of complete cheese microbiota in relation to the cheese environment and in relation to important cheese attributes such as flavor, texture, quality and nutrition. This could be achieved by the integrated application of multi-omics analytical platforms and data processing systems (Raes and Bork 2008). This paper aims to review application of individual omics technologies in cheese and their integration via a systems biology approach which we have termed "cheesomics" (Figure 1).

Non-culture-based methods and cheese microbiota

Molecular (DNA-based) approaches for analyzing complex microbial communities were introduced in the early 1990s, with the use of single-strand conformation polymorphism, terminal-restriction fragment length polymorphism, differential gradient gel electrophoresis, temperature gradient gel

CONTACT Harsharn Gill harsharn.gill@rmit.edu.au School of Science, RMIT University, Bundoora, Victoria, Australia.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.

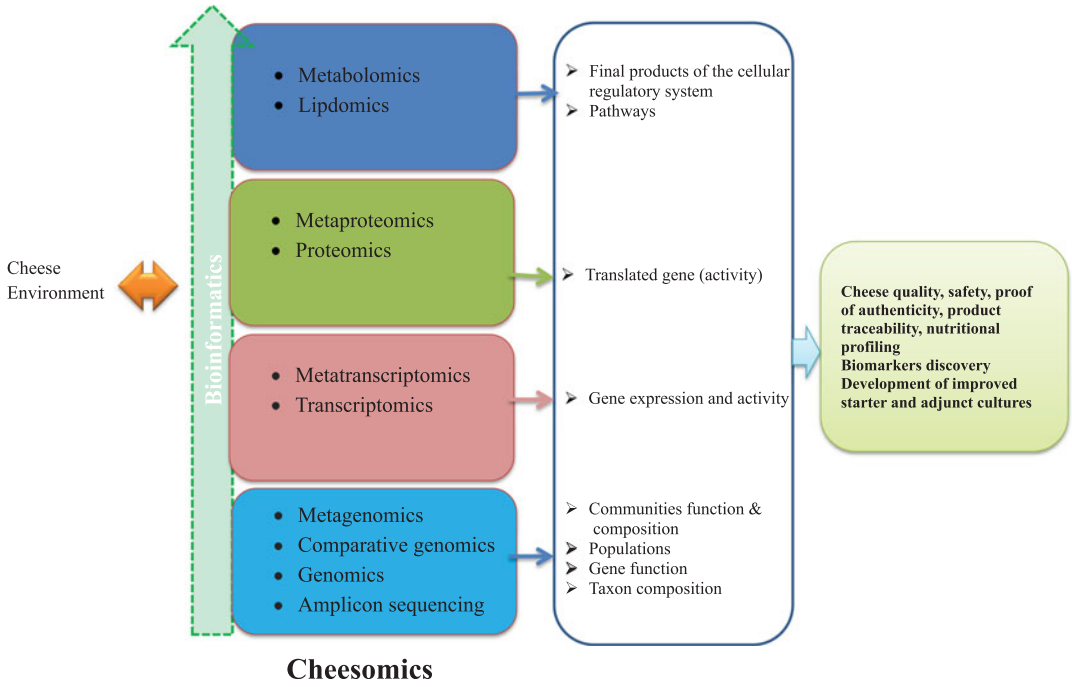

Figure 1. Conceptual framework for cheesomics.

electrophoresis and (SSCP, T-RFLP, DGGE, TGGE respectively), using PCR and specific primers targeting the variable regions of the 16S rRNA gene (Nocker et al. 2007). This resulted in DNA fingerprints that represented dominant microbial populations and/or taxa. This approach could be coupled to the use of cloning and sequencing of (limited) multiple short DNA fragments excised from DGGE/TGGE gels to allow further insights into the identity of taxa within the cheese microbial community (Metzker 2010). Subsequently, the development of high-throughput NGS methods (beginning with 454- then followed by Illumina-sequencing) enabled efficient and cost-effective sequencing of mixed-amplicon DNA sequences following PCR amplification targeting regions of the rRNA operon (Solieri et al. 2013; van Dijk et al. 2014). Nowadays, this method is commonly referred to as "microbial diversity profiling" or "microbial community profiling" (MCP). Its earliest applications were in analyzing environmental samples and the gut microbiome – for example see reviews by Hamady and Knight (2009) and Rastogi and Sani (2011) – whereas application of this and DNA sequencing methods in general to analyze microbiota in foods was carried out at a later stage (Quigley et al. 2011).

Although MCP provides broad taxonomic coverage, it also has shortcomings. Chief among these is the lack of taxonomic depth, since, like the DGGE methods that preceded it, MCP relies on distinguishing bacterial species by comparisons of their variable 16S rRNA gene regions, or for eukaryotes (fungi) the 18S rRNA gene or specific rRNA internal transcribed spacer (ITS) regions. Such approaches do not consider variations at population or strain level. There are also PCR-related errors, for example amplification bias, which may lead to over- or under-representation of specific taxa (De Filippis et al. 2017; Tessler et al. 2017), sequence contamination from reagents and/or the labaroatory (Salter et al. 2014) and also the difficulty in obtaining truly universal primers for all species, especially for fungi (De Beeck et al. 2014). Nevertheless, for cheese and raw milk studies, MCP has been commonly employed and has also revealed species hitherto not observed using traditional culture-based methods (even though the latter methods have often relied on molecular-based methods to identify isolates; see review by Quigley et al. 2011).

In addition to MCP, the introduction of metagenomics (including shotgun) approaches entailing sequencing of the entire collective genome of microbial communities within samples (Handelsman 2004; Quince et al. 2017) enables analysis of the functional capacity within microbiota. This approach has been made possible largely as the cost of sequencing has fallen dramatically (Mardis 2011) and as computing power enabling large-scale "data mining" and analysis of vast numbers of sequence reads has improved (Metzker 2010). Metabolic functional potential of microbial genomes within complex communities can be inferred from metagenomic sequencing (Wolfe et al. 2014; Escobar-Zepeda et al. 2016). Metagenomics can be used to assess the community both in terms of taxonomic profiling and in (predicted) functional profiling (Franzosa et al. 2015).

Based on DNA analysis alone, little or no information can be obtained regarding levels of actively transcribed genes (or of variations in levels of gene expression) in a microbial community. Moreover, MCP and metagenomics approaches do not distinguish live, dead, and damaged (metabolically inactive) cells. However, an inclusive and unbiased analysis of the total gene products, including mRNA transcripts (transcriptomics), proteins (proteomics) and metabolites (metabolomics), can provide deeper insights into complex microbial communities by analysis of actively expressed genes, expressed proteins and enzymatically active proteins and by comparison of these to the microbial metagenome

and metabolome. This holistic approach is often referred to as *systems biology* (Raes and Bork 2008).

Amplicon high-throughput sequencing (HTS) to determine cheese microbial diversity

Amplicon HTS of bacterial and fungal taxonomic markers (such as PCR-amplified 16S or 18S rRNA genes, respectively), following PCR of DNA extracted from cheese, has been widely used to characterize microbial communities in cheese and other food ecosystems (for a recent review see De Filippis et al. 2017). For cheese, most of these studies have focused on the microbial diversity and succession to assess how external factors (e.g. changes in the cheese making process, ingredients, or sampling points) affect the microbial composition, and/- or the dynamics of microbial communities within cheese milk and during the fermentation process and throughout curd maturation and during the shelf life of the cheese (Table 1). Analysis of changes in the microbiota that occur during fermentation enhances our understanding of community dynamics including the fate of the starter and adjunct cultures, and changes in the adventitious or spoilage-associated microorganisms during both fermentation and storage (Ercolini 2013).

The first major comprehensive investigation of the cheese microbiota using non-culture-based methods involved a study of 60 Irish soft, hard and semi-hard cheeses which were analyzed by HTS of pooled 16S rRNA gene amplicons (Quigley et al. 2012). This study reported the detection of several novel bacterial genera in cheese, such as *Prevotella* and *Arthrobacter*. It was further revealed that the bacterial community composition depended upon the cheese type, the origin of the milk, production technology and the ingredients used. Following this landmark study, other studies assessed the microbial diversity of specific types of cheeses using 16S rRNA gene amplicon HTS (Table 1). For example, Wolfe et al. (2014) described amplicon sequencing of 137 cheeses produced in 10 countries, providing an overview of the microbial diversity (bacterial and fungal) in cheeses (specifically cheese rinds) and across wide geographical regions. Twenty-four culturable genera of bacteria and fungi were identified as the dominant microbial community members. Two bacterial genera, *Yaniella* and *Nocardiopsis* had never been reported in food ecosystems before. One genus, a halotolerant *γ-Proteobacteria* that had rarely been reported in individual cheeses (Quigley et al. 2012; Bokulich and Mills 2013; Mounier et al. 2009), was also described as being widespread in cheese communities. Correlation analysis of taxa to rind types (bloomy, natural, and washed) and moisture, but not to geography, suggests the potential importance of these two factors upon the structure of cheese microbial communities (Wolfe et al. 2014).

It has been recognized that microbial diversity is affected by the cheese variety and the manufacturing process such as the coagulation type (lactic versus rennet) or the draining method. For instance, bacterial and fungal diversity within 60 cheeses belonging to 12 traditional French cheese varieties were described by Dugat-Bony et al. (2016) (76 bacterial and 44 fungal phylotypes were identified across 12 cheese varieties). Fungal communities, which showed less variation than bacterial communities, were dominated by *Geotrichum candidum*, *Debaromyces hansenii* and *Candida sake*, while the dominance of bacteria such as *Psychrobacter* in some varieties provided new insights into the prevalence of psychrophilic bacteria in cheeses. These psychrophilic bacteria were mostly dominant in the rind samples (Dugat-Bony et al. 2016). In summary, amplicon-based HTS investigations mostly based on PCR-amplification and sequencing of 16S rRNA-gene or 18SrRNA-gene, or other rRNA regions, have revealed the complexity of cheese microbiota and how technological and environmental conditions can impact the microbial diversity profile, which in turn influences cheese flavor, aroma and quality.

(Meta)omics insights to go beyond cheese microbiota analysis

Cheese ripening is a complex phenomenon. Microbes play a crucial role in this, but to understand the ripening process and its impact on cheese flavor, aroma, quality and safety, it is necessary to go beyond the study of just microbial taxonomic composition and diversity. Instead, efforts need to focus on elucidating the roles of different cheese microflora and their interactions to produce specific metabolites. These insights will enable increasingly sophisticated application of cultures for enhancing cheese flavor and shelf life, accelerating and improving ripening and reducing spoilage, as well as improving overall cheese quality and optimizing the whole cheese making process. These goals can be achieved through a systems biology approach encompassing metagenomics, metatranscriptomics, metaproteomics and metabolomics.

(Meta)genomics

Genomics can be defined as the sequence analysis of complete genomes, to reveal the genetic structure and to predict functions that are encoded within the genome of an organism. Most genome sequences are currently obtained by high throughput shotgun sequencing (Rittmann et al. 2008). Completed annotated genomes, where gene functions are predicted, are fundamental to the interpretation of corresponding transcriptomics and proteomics data. In 2001, *Lactococcus lactis ssp. lactis* IL1403 was the first lactic acid bacterium to have its genome sequence determined (Bolotin et al. 2001). In subsequent years, the complete genome sequences of *L. lactis* ssp. *cremoris* SK11 (Makarova et al. 2006) and *L. lactis* ssp. *cremoris* MG1363 (Wegmann et al. 2007) were obtained. Sequencing of other lactic acid bacteria (LAB) species followed such that thousands of LAB genome sequences are now available (Douillard and De Vos 2014; Kok et al. 2017). Comparative genomics analyses of these sequences have provided in-depth insights into gene functions and evolution within the LAB. Since the introduction of HTS methods, however, many genome sequences in the major databases are partial (incomplete), consisting of multiple contigs. Additionally, bacterial genomes from

Table 1. Microbial diversity profiling and comparison of dominant microbial taxa within cheese microbiota.

Type of cheese	Sampling details	Target region	Dominant microorganisms (Fungi*)	Reference
Ricotta cheese	whole cheeses	16S rRNA (V3-V4)	*Bacillus* *Paenibacillus* *Clostridium*	Sattin et al. (2016)
Cheddar cheese	cheese made from milk under different heat treatments	16S rRNA (V1-V3)	*Streptococcaceae*	Lee et al. (2016)
Traditional French cheese (washed and natural rind)	12 different cheese varieties (core and rind)	16S rRNA (V3-V4) And ITS2	*Geotrichum** *Debaryomyces** *Psychrobacter* (rind) *Lactococcus* (core) *Streptococcus* (core)	Dugat-Bony et al. (2016)
Mexican Cotija cheese	whole cheeses from different farmhouses	16S rRNA and single-copy marker genes	*Lactobacillus* *Leuconostoc* *Weissella*	Escobar-Zepeda et al. (2016)
Pico cheese	whole cheeses from different manufacturers	16S rRNA (V3–V4)	*Lactococcus* *Streptococcus* *Acinetobacter* *Enterococcus*	Riquelme et al. (2015)
Poro cheese	whey, curd and whole cheese (7 and 60 d)	16S rRNA (V1-V3)	*Streptococcus salivarius* *Lactobacillus delbrueckii*	Aldrete-Tapia et al. (2014)
Cheese rinds	cheese rinds made in 10 different countries	16S rRNA (V4), ITS1f and ITS2	*Brevibacterium* *Staphylococcus* *Halomonas* *Debaryomyces** *Galactomyces**	Wolfe et al. (2014)
Belgian soft cheese (washed rind)	cheese rind and core made from raw and pasteurized milk	16S rRNA	*Lactococcus lactis* (core) *Psychrobacter* (core) *Staphylococcus equorum* (core) *Corynebacterium casei* (rind) *Psychrobacter* (rind) *Lactococcus lactis* ssp. *cremoris* (rind) *Staphylococcus equorum* (rind)	Delcenserie et al. (2014)
Hard cheese	three whole cheeses from three different facilities	16S rRNA and 18S rRNA (V3 to V7)	Actinobacteria or Proteobacteria *Staphyloccocus* *Scopulariopsis**	Schornsteiner et al. (2014)
Artisan raw ewe's milk cheese	whole cheeses from two farm makers (during 90 d)	16S rRNA	*Lactococcus lactis* *Enterococcus* spp. *Streptococcus parauberis*	Fuka et al. (2013)
Irish artisanal cheese (soft, semi-hard and hard cheeses)	whole cheeses	16S rRNA (V4)	*Lactococcus*	Quigley et al. 2012
Latin-style cheese	whole cheeses	16S rRNA (V1-V3)	*Exiguobacterium Clostridiaceae*	Lusk et al. (2012)
Traditional mozzarella cheese	raw milk, intermediate product and whole cheese	16S rRNA (V1-V3)	*Acinetobacter* (raw milk) *Streptococcus* and *Lactobacillus* (cheese)	Ercolini et al. (2012)
Danish semi-hard raw milk cheese	whole cheeses	16S rRNA (V3 and V4)	*Lactococcus Lactobacillus Streptococcus*	Masoud et al. (2011)

uncultured microorganisms can be assembled from metagenomics datasets, whereby environmental DNA is directly sequenced (Loman and Pallen 2015; Jovel et al. 2016). By 2014, there were more than 30,000 sequenced bacterial genomes publically available and thousands of metagenome projects in progress (Land et al. 2015), with a recent study reporting sequencing of an additional 1003 reference genomes as part of the Genomic Encyclopedia of Bacteria and Archaea (GEBA) initiative (Mukherjee et al. 2017). This wealth of information, combined with a comprehensive understanding of gene function and increased computing power today further informs our capacity to interpret metagenomics and other omics datasets.

The genome sequences of non-lactic acid bacteria species such as *Propionibacterium freudenreichii* ssp. *shermanii* and *Brevibacterium linens* that are also involved in cheese flavor have also been completed (Falentin, Deutsch, et al. 2010). Comparative genomics of *P. freudenreichii* ssp. *shermanni* CIRM-BIA1 and *Propionibacterium acnes* KPA171202 revealed the absence of most virulence-related genes of strain KPA171202 in CIRM-BIA1, which is a confirmation of the safe status of *P. freudenreichii* (Falentin, Deutsch, et al. 2010). More recently, the genomes of 213 *Lactobacillus* strains and associated genera were sequenced (Sun et al. 2015). This study identified 60 cell envelope proteinases, which are important for cleaving casein during growth in milk and in production of cheese flavor, and 48 glycoside hydrolases, which are important in carbohydrate metabolism. Such investigations provide new insights for developing new starter cultures for cheese production to improve and diversify the flavor profiles of cheese and other fermented foods

Cheeses, particularly artisanal cheeses, contain a very diverse mix of bacterial species, some of which have not yet been sequenced and/or the relevant data have not yet been made publicly available. Recently, an effort was made to construct draft sequences of 117 bacteria isolated from different cheeses which had been made at a normal cheesemaking standard or at a higher quality production standard. These draft sequences belonged to 67 genera including *Kluyvera*, *Luteococcus* and *Marinilactibacillus,* which at that time were missing from the public database (Almeida et al. 2014). This study nearly doubled the number of available genome sequences of different species isolated from dairy products. However, additional studies are needed to extend the database in order to provide a complete view of the cheese ecosystem. One interesting consequence of the discovery of new cheese bacterial species has been the challenge in determining which of these can be considered "safe" to use in commercial food cultures (Bourdichon et al. 2012).

Metagenomics data (based on shotgun HTS of complete metagenomes), though more expensive, can simultaneously provide more complete information with respect to the taxonomic profile of microbiota and also can provide insight into the functional genetic potential and the associated metabolic pathways that might contribute to the specific characteristic of cheese such as flavor (Wolfe et al. 2014; De Filippis et al. 2016). In addition to amplicon sequencing as previously described, Wolfe et al. (2014) performed a metagenomics analysis on cheese rind communities. Putative functional genes and their associated metabolic pathways were resolved in three types of cheese rinds (bloomy, natural and washed), which could explain some specific characteristics of flavor in washed rind cheeses such as sweaty and pungent aromas. The metagenomic profiling of the three types of cheese rinds indicated the potential contribution of some unusual species including, *Pseudoalteromonas haloplanktis* and *Psychrobacter immobilis* to the characteristics of these cheese products that were not associated with the inoculated starter cultures. Metagenomics profiling has similarly been used to predict the metabolic capacity of the Mexican cotija cheese microbiota (Escobar-Zepeda et al. 2016), particularly in relation to both the metabolism of branched chain amino acids, for the production of a wide range of flavor compounds and free fatty acids. This study also identified genes associated with bacteriocin production and immunity which affected the growth dynamics of individual species within the consortium. Further studies are needed to distinguish microorganisms at the strain level, not just species level, since flavor impacts depend upon specific strain properties (Walsh et al. 2017). Metagenomics studies such as those described above provide comprehensive information about the functional potential of microorganisms, but do not provide information on their *actual* functional activity. One strategy to connect the functional potential of the cheese microbiota, revealed by genomics and metagenomics, to functional activity of the microbiota is through the analysis of RNA transcripts (transcriptomics) (Franzosa et al. 2015).

(Meta)transcriptomics

Most transcriptomic studies on cheese-related bacteria to date have centered on laboratory studies of *Lactococcus lactis* as this organism is a common component of starter cultures used in cheese production. Transcriptional responses of *L. lactis* during oxidative stress were first investigated by Duwat et al. (2001), who showed that certain respiratory genes in *L. lactis* were activated and that (surprisingly) cell survival increased when cells were exposed to O_2 late in the growth phase. In subsequent studies microarrays were also used to determine gene expression of *L. lactis* (Kuipers et al. 2002). More recently, several investigations have been conducted to determine gene expression characteristics of *L. lactis,* particularly those important for selection of starters for cheese making. For example, the role of several transcriptional regulators (CodY, CcpA) which control expression of genes regulating carbon and nitrogen metabolism, were identified during the growth transition phases (den Hengst et al. 2005; Zomer et al. 2007). Responses toward physicochemical stresses were also targeted. For instance, responses of *L. lactis* ssp. *lactis* IL1403 against a carbon source were investigated, which showed that there were three different types of transcriptomic responses to glucose depletion (global responses, specific responses, and previously undescribed pleiotrophic responses involving cell competence, prophage induction and other cell functions). Transcription in relation to carbon source and amino acid starvation was also

investigated by Dressaire et al. (2008), who showed that transcription varied in relation to the growth rate. In another lactococcal species, *Lactococcus chungangensis* originally isolated from activated sludge, functional genes related to cheese flavor development were upregulated whilst this microorganism was grown under laboratory conditions (Konkit et al. 2014). The up-regulation of genes associated with the degradation of casein, stress response and the ability to use a variety of substrates, and (conversely) the down-regulation of certain transporter genes which are known to play a role in intrinsic drug resistance, were demonstrated relative to gene expression in *L. lactis* IL1403 by comparative microarrays.

Cheese model systems have been also used to evaluate the transcriptional profile of *L. lactis* in response to cheese-making conditions, which are harsh for microorganisms in terms of pH, moisture content and nutrient depletion (Cretenet et al. 2011; Larsen et al. 2016). Results of these studies showed that expression of genes involved in acidic- and oxidative stress responses was induced under control of CcpA. A comparative transcriptomic study was carried out to reveal core and specific gene expression profiles to stresses encountered by four strains of *L. lactis* ssp. *lactis* during the cheddar cheese manufacturing process (Taïbi et al. 2011). In that study, core gene expression shared by all four strains involved genes related to either stress response or carbohydrate and amino acid metabolism. In contrast, the strain-specific responses involved regulation of oligopeptide transporter genes which were suggested as predictive markers for selecting starter strains, since these are associated with amino acid degradation for the production of flavor. In another study, analysis of six strains of *L. lactis* identified different transcriptional profiles in cheese made with ultrafiltered milk relative to normal milk, despite the strains sharing a large core genome of almost 2,000 genes (Tan-A-Ram et al. 2011).

Recently, transcriptomic approaches have been used to identify metabolic pathways of nonstarter LAB during cheesemaking and ripening. In one study, the transcriptional profile of *Lactobacillus rhamnosus* in a cheese-like medium revealed how the use of alternative metabolic pathways such as pyruvate degradation and ribose catabolism assists species to adapt under conditions of carbon source deficiency (Lazzi et al. 2014). With regards to other (non-LAB) cheese bacteria, the role of *P. freudenreichii* in cheese ripening and cheese flavor development has also been investigated using transcriptomic profiling. In microbiologically-controlled Emmental cheese batches, the maximum activity of key enzymes involved in flavor formation (determined by microarray studies) was observed at the end of the cold ripening stage and this remained constant during the first two weeks in a warm room (Falentin, Postollec, et al. 2010). In conditions mimicking both cheese ripening and cold storage, *P. freudenreichii* was metabolically active with the expression of different genes involved in the formation of cheese flavor compounds remaining unchanged during cold storage (4 °C) when compared to warm storage (30 °C) (Dalmasso et al. 2012). The functional role of *P. freudenreichii* was investigated further by combining genome- and transcriptome-sequencing of *P. freudenreichii* ssp. *shermanii* identifying genes associated with flavor development during the ripening of Swiss cheese (Ojala et al. 2017). This analysis showed that carbon central metabolism and propionate production along with amino acid catabolism by *P. freudenreichii* ssp. *shermanii* during warm room ripening was associated with the development of flavor, although noticeable metabolic activity was also observed during cold room ripening (Ojala et al. 2017).

Although many transcriptomics studies have led to an improved understanding of the functional genes differentially expressed in various microorganisms during cheese ripening, and in food more generally, they have largely focused on individual cultivable microorganisms in relation to their corresponding genome context. In contrast, very few studies to date have attempted to examine the complete gene expression of microorganisms within a complex community such as cheese. A key problem has been development of reliable protocols to recover all the expressed RNA from cheese without losses due to RNA degradation (Monnet et al. 2008). The most recent advances in profiling the entire mRNA of microbiota, known as metatranscriptomics, has provided greater dynamic and functional insight into microbial gene expression in cheese environments, at least for species for which reference genomes are available (Lessard et al. 2014; De Filippis et al. 2016; Monnet et al. 2016). In one such study, the metatranscriptomic analysis of a surface ripened cheese, which was produced using two LAB species (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*), two yeasts (*Debaromyces hansenii* and *Geotrichum candidum*), and one ripening bacterium (*Brevibacterium aurantiacum*) was undertaken (Monnet et al. 2016). *B. aurantiacum* transcripts were detected at lower levels than for transcripts from other taxa, suggesting lower activities on the surface of the cheese. Following the analysis of rRNA depleted-RNA, *G. candidum* accounted for the largest proportion of the transcribed short sequence reads, indicating this species to be the most active from day 5–35 of ripening. Only minor changes were observed for the transcriptomes of LAB. The up-regulation of amino acid catabolism genes in *G. candidum* and *D. hansenii* from day 14–35 and at day 35, respectively, suggested their contribution to amino acid degradation later during ripening (Monnet et al. 2016). In another study, a metatranscriptome analysis of an industrial camembert cheese during a 77 day ripening period identified the fungal transcripts from two cheese ripening species, *Penicillium camemberti* and *Geotrichum candidum,* the sequences for which had not been previously available in public databases (Lessard et al. 2014).

In general, transcriptomics has often been used as a proxy for the direct analysis of proteins (*i.e.* determining protein functional activity), because the characterization of proteins by proteomics tools such as mass spectrometry is complex, time consuming and expensive. In addition, gene expression can be detected at extremely low levels, in contrast to most expressed proteins (Rittmann et al. 2008). However, the number of mRNA transcripts often correlates

poorly with the abundance of the corresponding proteins for reasons such as variable half-lives of mRNA (Bernstein et al. 2002), allosteric changes in mRNA (Mandal and Breaker 2004), uneven protein-synthesis rates, and the possibility of post-translational modifications (PTMs) of proteins (Ekman et al. 2008). Similarly, a meta-analysis investigating relationships between metabolic activity and gene expression in natural environmental samples based on RT-qPCR data showed a negligible relationship between gene expression and functional activity (Rocca et al. 2015). Consequently, direct analysis of intact proteins and peptides, in addition to transcriptomics, is considered a valuable complementary tool for investigating the functional activity of microbiota (Rittmann et al. 2008).

(Meta)proteomics

Analysis of genes and transcripts are useful for determining the presence and functional potential and activity of microorganisms. However, identification and quantification of proteins which can be obtained through a proteomics approach provides evidence at the post-translational level of the activity and function of microorganisms and their associated enzymes. Proteomics is the large-scale study of the total protein content in complex mixtures or biological samples (Gagnaire et al. 2009), and can serve as a bridge to link the genome and transcriptome data to biological function (Wu et al. 2016). 2D-gel electrophoresis and mass spectrometry are fundamental tools to perform proteomics on dairy products, with mass spectrometry now the most widely-applied technique for accurate quantitative and qualitative protein analysis (Conto 2017). Mass spectrometry-based proteomics relies on appropriate protein fractionation and protein or peptide ionization and MS-fragmentation (Zhang et al. 2013). The fragmentation pattern of a peptide displays its amino acid sequence and any post-translational modifications (PTMs). Peptides are assigned to full length proteins by comparison to reference databases notably SWISS-PROT and PFAM (Franzosa et al. 2015). Such studies, for example, have revealed extensive heterogeneity and the presence of different isoforms of milk proteins (e.g. A_1 and A_2 β-caseins) by the application of proteomics methods to dairy products (for reviews see (O'donnell et al. 2004; Manso et al. 2005; Casado et al. 2009; Roncada et al. 2012; Korte and Brockmeyer 2017).

In cheese, proteolysis is one of the key biochemical processes occurring during ripening. This phenomenon can be affected by many factors such as processing conditions, duration of ripening and seasonal variations in milk composition (Conto 2017). To monitor these phenomena and also to monitor product authenticity (*e.g.* for Protected Designation of Origin or PDO status), proteomics has been applied to different cheeses. For instance, proteomic studies identified peptides which could serve as possible markers for quality control and characterization of the Brazilian artisanal "Coalho" cheese (Silva et al. 2016). Alternatively, a proteomic analysis was used to detect the irregular replacement of milk with whey in Minas Frescal Brazilian cheese (Magenis et al. 2014). Variation in hydrolysis of caseins and casein-derived low molecular weight products in cheddar cheeses, that affect product quality over different climatic seasons, have also been demonstrated using proteomics (Hinz et al. 2012).

Proteomics of milk proteins and LAB in relation to fermented dairy products have been reviewed by Manso et al. (2005) and Gagnaire et al. (2009). More recently, Angelis et al. (2016) reviewed proteomics of *Lactobacillus* species, specifically. Jardin et al. (2012) used cheese model systems to investigate the proteome (mostly proteins involved in central metabolism or stress response proteins) of specific dairy bacteria during cheese making. Yvon et al. (2011) also applied proteomics to show how initial proteolysis in cheese affected the proteome. In a more recent study, proteomics was used to detect differences in protein expression between two commercial strains of *Lb. delbrueckii* (one ssp *bulgaricus*, the other ssp. *lactis*) and one novel strain (also subspecies *lactis*) isolated from a traditional fermented fresh cheese. Proteins related to amino acid metabolism were exclusively expressed by *Lb. delbrueckii* ssp. *bulgaricus*, whereas those related to maltose and glutathione metabolism were expressed by *Lb. delbrueckii* ssp. *lactis*. Sugar metabolism and fatty acid biosynthesis genes were specific to the novel strain (Zanni et al. 2017). The authors also determined the probiotic properties of these microorganisms using the organism *Caenorhabditis elegans* as a feeding model. The relationship between the proteomes of such cheese microorganisms and cheese flavor remains an area where more research is needed.

While studies targeting specific microorganisms in cheese are of value, an improved understanding of how microorganisms function in a community and respond to various harsh conditions encountered during cheese-making can only be achieved by metaproteomics. Metaproteomics is the large scale study of the entire protein complement of microbial communities within an environment at a given time point (Wilmes and Bond 2004). In contrast to pure strain proteomic data, interpreting metaproteomic data in animal food products is challenging because of the simultaneous presence of cow (or other animal) milk proteins and proteins from mixed cheese microbiota. For this reason such investigations are limited. One such study examined the effects of lysosome treatment during cheese making on the function of a microbial community within the hard cheese Grana Padana. It was shown that several enzymes, such as acetate kinase from Clostridia associated with late blowing defect in cheese, decreased as a result of the lysozyme treatment (Soggiu et al. 2016). In summary, proteomics is a valuable complementary tool that can provide additional information related to gene and transcript data. Application of proteomics to study LAB has elucidated specific mechanisms that these microorganisms use to adapt to different environmental conditions. Moreover, development in sensitive MS analytical instrumentation and sophisticated bioinformatics tools is facilitating application of a metaproteomic approach to unravel the mechanisms that LAB use and their interactions with other microorganisms in the cheese environment.

In the current omics era, studies to date have focused mostly on one or other omics technologies without understanding a true systems biology approach. However, such

data interpretations are by their nature limiting. For example, a change in an mRNA transcript or protein does not always correlate with a change in the level of a metabolite, and an increase in mRNA does not always correlate with an increase in the corresponding protein's level. Furthermore, once a protein is translated, it may not become enzymatically active (Sumner et al. 2003). In contrast, the metabolome reflects the biochemical consequences of microbial communities and the result of changes in microbial phenotypes and their function (Putri et al. 2013). Metabolites are the final products of the cellular regulatory system and can be altered as a response by the biological system in relation to genetic and environmental changes (Fiehn et al. 2000).

Metabolomics

Metabolomics is a multidisciplinary field of research combining analytical chemistry, biochemistry and bioinformatics which aims to identify all endogenous small molecules biosynthesised and modified in a cell, tissue or whole organism. Typically, it is used to detect, identify, quantify and/or semi quantify low-molecular weight metabolites (<1500 Da) in a biological system in response to an environmental, abiotic, biotic or disease state (Roessner et al. 2011). Typically, there are two main approaches - targeted or untargeted analysis. A targeted metabolomics approach focuses on the analysis of specific metabolites or groups of metabolites (eg. TCA metabolites) that is identified and quantified to determine the effects of metabolism upon a specific treatment (Spratlin et al. 2009). An untargeted metabolomics approach is generally used for "discovery" where it is unknown what metabolites will respond to a biological treatment, disease or stress. In this approach metabolites are often left unidentified until it has been determined that the "unknown" is responding to a specific treatment. Ultimately, this approach allows for the comprehensive identification of all endogenous metabolites (metabolome) from a sample under a given set of conditions (Goodacre et al. 2004).

Metabolomics relies on the efficient extraction of endogenous metabolites followed by chromatographic separation and mass spectral detection, and identification, (semi- and/or quantification) multivariate statistics to decipher changes in metabolic pathways (Dias et al. 2016). Metabolite separation is carried out using various analytical techniques such as, high performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), gas chromatography (GC) and capillary electrophoresis (CE) coupled to mass spectrometry (MS) (for reviews see Johanningsmeier et al. 2016; Dias et al. 2016). Gas chromatography mass spectrometry (GC-MS), classified as the "*work-horse*" in metabolomics, is limited to the analysis of compounds (through chemical derivatisation) of <1,000 Da, and can comprehensively resolve in excess of 400 volatile compounds (or compounds which can be made volatile through the process of chemical derivatisation) to measure metabolite classes such as: sugars, sugar alcohols, sugar phosphates, amino and organic acids, amines, sterols and fatty acids typically involved in primary metabolism and fundamental biological processes (e.g. glycolysis,) (Dias and Koal 2016). In contrast, liquid chromatography-mass spectrometry (LC-MS) is a complementary analytical platform used to identify metabolites that have higher molecular weights and is best suited to the identification of compounds involved in secondary metabolism (Dias and Koal 2016).

Metabolomics studies related to cheese (and foods generally) have recently been performed to determine the chemical diversity of metabolites in cheese to understand how it can reflect "cheese quality" originating from the action of starter and other (e.g. adjunct) bacteria, added ingredients (salt, enzymes), the manufacturing process and the complex metabolic reactions that take place during the maturation process (Ochi, Bamba, et al. 2012; Pisano et al. 2016). The chemical fingerprint of cheeses obtained by metabolomics can be used to discriminate closely related cheese varieties, detect fraudulent PDO cheeses, or gain information for improving cheese quality. For example, metabolic fingerprinting using high resolution (HR)-MS-NMR of Mozzarella di Bufala Campana (MBC) cheese, produced from the buffalo milk in the Campana region of Italy, has been used to assess the quality and traceability of this PDO cheese to prevent either adulterated or false PDO sales (Mazzei and Piccolo 2012). The discrimination of MBC cheeses from two different geographical regions was determined based on differences in the metabolite levels of galactose, lactose, acetic acid and glycerol. Freshness of the MBC cheese was indicated by an increase in the concentration of isobutyl alcohol, lactic acid and acetic acid with aging. Metabolomics based on GC-MS in combination with microbial culture-dependent methods was similarly applied to Italian Buffalo Mozzarella cheese in order to assess its designation of origin (PDO) status (Pisano et al. 2016). Italian mozzarella cheese produced with buffalo and cow milk was compared in terms of metabolite profiles and microbial diversity. It was found that metabolite pools reflected differences in production protocols and also the microbial composition of these cheeses.

Metabolomics based on GC-TOF-MS has also been used to create sensory predictive models for cheeses such as Cheddar, Gouda, and Parmigiano Reggiano (Ochi, Naito, et al. 2012). These authors further showed that GC-FID can provide a sensory predictive model which is comparable to that obtained by GC-TOF-MS. Indeed, metabolomics based on GC-FID is a relatively inexpensive alternative approach, since GC-FID is easier to operate and maintain compared to GC-MS (Ochi, Naito, et al. 2012). Similarly, metabolite analyses by GC-MS have been coupled to olfactometry to identify the volatile profile of various cheeses (Brattoli et al. 2013; Fuchsmann et al. 2015).

In addition to whole cheese analyses, metabolomics studies have also focused on specific metabolites produced by cheese microbiota. Some of these investigations have been aimed to evaluate the ability of cheese microbiota to produce flavor compounds, while others have sought to explore the growth requirements of nonstarter LAB, and to understand cellular fluxes in the production of key flavor compounds. Characterisation of microorganisms in terms of their ability to produce aroma- and taste-active compounds is important because it provides information useful for

diversification of cheese flavor, which can be obtained using new LAB or other strains. This is highly desirable as pasteurized milk containing reduced natural microflora levels is mostly used to manufacture cheese, especially at large commercial scales. Metabolomics approaches have been critical to interrogate the precise contributions of microorganisms to produce flavor compounds. A summary of specific volatile compounds produced by cheese-associated microorganisms either in cheese model systems or cheese-based media is shown in Table 2. Sgarbi et al. (2013) investigated the aroma characterization of different *Lb. casei* and *Lb. rhamnosus* strains grown on media containing products of lysed cells or on cheese as a substrate. It was suggested that the growth of NSLAB on products of lysed cells required the catabolism of amino acid and fatty acids, while pyruvate catabolism was the predominant pathway supporting the growth of NSLAB on products of cheese as substrate. This study demonstrated that the metabolic capabilities and the total volatiles produced (the volatilome) by NSLAB strains can also be used to identify wild strains suitable for use as adjunct cultures (Sgarbi et al. 2013).

In another study, the Grana-Padano cheeses, made in two different dairies were found to exhibit variations in volatile profiles after 13 months of ripening with respect to the microbial composition and starter lysis (Lazzi et al. 2016). Increased levels of ketones, alcohols, hydrocarbons, acetic acid and propionic acid were associated with the cheese having a complex microbial composition, and where NSLAB (*Lb. rhamnosus*/*Lb. casei*) were dominant. In contrast, the production of benzaldehyde, citric and pyroglutamic acid and free fatty acids was linked to the cheese which had greater lysed starter cells. In a recent study by Pogačić et al. (2016) the metabolic fingerprints of volatiles produced by *Lactobacillus* and *Leuconostoc* in a curd-based slurry medium mimicking cheese conditions revealed strain to strain variation. *Lb. rhamnosus* and *Lb. paracasei* were major producers of diacetyl, acetoin, acids and esters, while *Leuconostoc* spp. were major producers of alcohols and esters. Such information is valuable for informing strain selection of adjunct cultures.

Metabolomics has also been used to differentiate non-lactic acid bacteria (Actinobacteria, *P. freudenreichii*, and *Hafnia alvei*) as well as LAB strains for their ability to produce aroma compounds in a curd-based medium mimicking semi-hard cheese conditions. Fifty-two aroma compounds were identified, of which 49 differed in abundance between different bacteria (Pogačić et al. 2015). Metabolomic profiling of volatiles has been also applied to Gram negative bacteria (*Psychrobacter celer* and *H. alvei)* growing on a soft cheese model system (Irlinger et al. 2012). Large inter-species and intra-species diversity of dairy Propionibacteria (76 strains of *P. freudenreichii*, *P. jensenii*, *P. thoenii* and *P. acidipropionici*) in their ability to produce different aroma compounds in a curd based medium has also been demonstrated by volatile compound profiling (Table 2). The ability of strains of *P. freudenreichii* to produce various aroma compounds was higher than that found in other bacteria. Moreover, variation between strains of the same species to produce the same compound was observed, with differences between strains as high as ~500-fold, suggesting the potential of dairy propionibacteria to modulate cheese flavor (Yee et al. 2014).

Metabolic fingerprinting has also been done based on the analysis of nonvolatiles. A combined GC/LC-MS study of volatile and nonvolatile metabolites of a model cheese inoculated by *L. lactis* ssp. *lactis* revealed that the metabolome varied over time (0–48 h) and identified a number of biomarkers including creatine, uric acid and L-carnitine that had not previously been reported in milk or in cheese (Le Boucher et al. 2013). A later sophisticated LC–HR-MS metabolomics study by the same group demonstrated that variations in the cheese metabolome could be affected by the spatial distribution of *L. lactis* colonies at different stages of cheese ripening (Le Boucher et al. 2015). The authors identified 26 group of compounds that were affected by the spatial distribution of bacterial colonies, including amino acids, organic acids, vitamins and two proteolysis products. Some of these compounds had not been reported earlier in cheese (such as 2-oxoadipic acid and adipic acid).

Finally, metabolomics has led to a better understanding of the growth requirements and the metabolic cellular flux in cheese microbiota. For instance, analysis of volatiles produced by *Lactobacillus paracasei* ATCC334 during growth in a cheddar cheese extract aged eight months demonstrated that the ensuing carbon limitation did not restrict the growth of this strain in cheese for up to eight months, since other undefined metabolic pathways were employed by *Lb. paracasei* to maintain its energy balances (Budinich et al. 2011). Similarly, metabolomics based on LC-MS and head space (HS)-GC-MS methods were used to investigate the sulfur metabolic network and the response to sulfur availability of *Brevibacterium aurantiacum*, which is used as a cheese-ripening culture (Forquin et al. 2011). The authors stated that the production and/-or accumulation of the reducing thiol molecules, either extracellularly or intracellularly, could be an adaptive feature of its biotope in response to growth on the cheese surface and exposure to oxygen.

In summary, metabolomics offers a powerful approach towards understanding the contribution of cheese microbiota in the production of different metabolites, and in particular, those with flavor attributes. This research also provides new insights to understand the contribution and role that cheese microbiota plays towards cheese quality. Moreover, combining metabolomics with other meta-omics platforms and microbial taxa profiling using HTS methods can provide new information particularly, regarding the effects of microbial taxa with low relative abundance in cheese microbiota, which normally are undetected by traditional microbiological cultivation methods. This facilitates the possibility of using "new" bacteria (non-LAB strains) as novel starter or adjunct cultures to improve or diversify the flavor of cheese, pending safety evaluation, as noted previously.

Integration of meta-omics technologies gives more in-depth insights

Until recently, most studies have focused on the application of one or other individual omics approaches to investigate

Table 2. Volatile compounds and their associated microorganisms identified by metabolomics in cheese-based growth media and a cheese model.

Volatile compounds	Microorganism (species/strains)	Growth medium	Reference
Methylthioacetate, DMTS	*Hafnia alvei* or *Psychrobacter celer*	A cheese model (smear soft cheese)	Irlinger et al. (2012)
Methylthiobutyrate	*P. celer*		
3 &2-Heptanone, 2-pentanone, 2-nonanone	*P. celer*		
Benzeneacetaldehyde	*P. celer*		
Octanoic acid	*P. celer*		
Methyl ester	*P. celer*		
Dimethylundecane	*Lactobacillus rhamnosus*	Cheese broth and lysed cell medium	Sgarbi et al. (2013)
Benz aldehyde	*Lb. rhamnosus* & *Lactobacillus casei*		
8-Octadecenal	*Lb. casei* 1247 strain & *Lb. rhamnosus*		
2-Decenal	*Lb. rhamnosus*1216		
Nonanal	*Lb. rhamnosus* 1473		
2-Nonanone	*Lb. rhamnosus* 1473		
Diacetyl, acetoin, ethanol	*Lb. casei* 1247& *Lb. casei*		
Dimethyl sulfide	*Propionibacterium freudenreichii*	A curd-based medium	Yee et al. (2014)
DMS, DMTS	*Propionibacterium acidipropionici*		
Short chain fatty acids , ethyl esters, 2-methylbutanoic acid, 3- & 2-methylbutanol	*P. freudenreichii*		
Ethyl propanoate	*Propionibacterium jensenii* *Propionibacterium thoenii* & *P. freudenreichii*		
2-Methylbutanal, 3-methylbutanal	*P. jensenii* & *P. thoenii*		
Ethyl hexanoate, 3-methylbutyl hexanoate, ethyl butanoate	*Brachybacterium* strains	A curd-based medium	Pogačić et al. (2015)
Sulfur compounds (DMDS,DMS,DMTS)	*H. alvei*		
Aldehydes including benzaldehyde	*Lactobacillus fermentum* & *Lactobacillus helveticus*		
Diacetyl	*Microbacterium. gubbeenense* & *Lactobacillus paracasei*		
Ethanol, hexanol, secondary alcohols	*Lb. fermentum*		
Acetic acid	*P. freudenreichii*		
Propionic acid, methylproponal, methylbutanol	*H. alvei*		
2 & 3 Methylbutanoic acid	*Lb. paracasei*	A curd-based medium	Pogačić et al. (2016)
2 & 3-Methylbutanol	*Lb. rhamnosus* LSL212		
Diacetyl, acetoin, 1-hydroxy-2-propanone	*Lb. rhamnosus* strains & *Lb. paracasei* CIRM248		
2,3-Pentanedione	*Lb. paracasei* CIRM849 & *Lb. rhamnosus* CIRM1436		
DMDS and DMTS	*Lactobacillus mesenteroides*		
1-propanol and 1-pentanol	*Lactobacillus sakei* CIRM467T		

microbial associations and microbial influence on cheese quality and/or flavor. Very few studies have combined different omics approaches simultaneously. However, it is now recognized that a "systems biology" approach, whereby combined omics studies are applied, provides comprehensive information on cheese flavor development and food quality in general (Cocolin et al. 2017; Ferrocino et al. 2018; Walsh et al. 2017). For example, Dugat-Bony et al. (2015) used a combination of metagenomics, metatranscriptomics and biochemical analysis to analyze an experimental surface-ripened cheese ecosystem composed of nine bacterial and three yeast species over a one month ripening period. The biomarker genes which were representative of the most active species at various stages of ripening were identified and the major contributions of most dominant species identified. *L. lactis* and *Kluyveromyces lactis* were revealed to be most active during the initial stages of ripening, followed by *Geotrichum candidum, Debaromyces hansenii* and acid-sensitive bacteria such as *Corynebacterium casei* and *Hafnia alvei*. The rapid consumption of lactose and the production of lactate *by Lb. casei*, which was consequently consumed by *G. candidum*, were reflected by an increase in the amount of lactate dehydrogenase transcripts for *G. candidum*. The results of mapping RNA-Seq reads to the *G. candidum* genes also demonstrated the major contribution of this species to fat and protein degradation. In the later stages of ripening, amino acid degradation-related genes of *G. candidum*, *C. casei* and *H. alvei* were expressed, which correlated with their late development in the surface ripened cheese (Dugat-Bony et al. 2015). In a further study, the metatranscriptome and metabolome data showed how the functional diversity of a pasta-filata cheese microbiota could be influenced by different ripening conditions (De Filippis et al. 2016); transcriptomics analysis identified 651 genes that were differentially expressed between cores of two cheeses ripened at normal (16 °C) and higher (20 °C) temperature. Proteases, peptidases, dipeptide transporters, amino acid permeases, amino acid catabolism, fatty acid β-oxidation, and biosynthesis genes were all over-expressed in the core of cheese ripened at higher temperatures. Metabolomic data was also consistent with the metatranscriptome data, suggesting the higher ripening temperature promoted the development of flavor compounds in the cheese (De Filippis et al. 2016).

Further studies using combined "meta-omics" technologies are needed to better understand cheese ripening and maturation over time. This will require the application of bioinformatics and other computational tools to bioinformatically relate different omics datasets (network-based analysis, as described by Gwinner et al. 2017), to determine the correlations and associations between specific bacteria and fungi and to cheese related attributes. Newer omics subdisciplines such as lipidomics, which is the large scale study of cellular lipids, (Han 2009), have been applied to milk in some studies (Li et al. 2017; Sokol et al. 2015; Dallas et al. 2014) and will also need to be included in future cheese studies.

Cheesomics and human health

Cheese consumption is associated with a range of health benefits, including reducing obesity, improving bone health, lowering blood pressure and in reducing the risk of type-2 diabetes, cardiovascular disease and dental caries (St-Onge, Farnworth, Jones 2000; Cheng et al. 2005; Aune et al. 2013; Telgi et al. 2013; Chen et al. 2017). These beneficial effects are associated with the presence of specific nutrients and bioactive components that are produced during cheese ripening (Gobbetti, Minervini, Rizzello 2007; Walther et al. 2008; Rizzello et al. 2005; Marco et al. 2017; Martinez-Villaluenga, Peñas, Frias 2016). Cheesomics offers unique opportunities to discover novel bioactive metabolites in cheese with potential to impact human health and shed light on mechanisms by which these effects are mediated. For example, recent omics studies have revealed the presence of novel bioactive peptides and amino acids such as gamma amino butyric acid (GABA), and other bioactive compounds in cheese (Santiago-López et al. 2018; Afshari et al. manuscript in preparation).

Furthermore, the microbiota of ripened cheese is dominated by nonstarter lactic acid bacteria (NSLAB) and many of these have the potential to positively influence health. Indeed, several NSLAB isolated from cheese have been found to exhibit unique health-promoting properties and are currently being used as probiotics worldwide (Prasad et al. 1998; Gill et al. 2001; da Cruz et al. 2009; Settanni and Moschetti 2010). Cheesomics, in combination with knowledge of proteins and genes linked with probiotic activity, provides a powerful tool for discovery of new strains. It could also be used to identify toxic metaboloites and microbes that are likely to pose food safety risk and have negative impact on human health.

Conclusion and future prospects

It has long been known that the total cheese microbiota is of major importance in the manufacture of cheese, as its composition and function affects the final organoleptic properties, quality and safety of cheese. However, success in improving or accelerating cheese ripening demands in-depth knowledge of not only the cheese microbiota, but also its combined effects on the cheese. Thanks to recent advances in omics analytical technologies, our knowledge of this is progressing beyond traditional microbiological culturing, MCP and metagenomics studies, to include (meta)transcriptomics, (meta)proteomics, metabolomics, and other types of omics studies. Concurrently, there has been development of sophisticated computational algorithms, with increasing size and application of DNA, protein and MS databases, such as Genbank, KEGG, the human metabolome database (HMBD) and METLIN. The future pathway to understand cheese ripening at the molecular level will be the *simultaneous* application of these multi-omics platforms and the integration of very large datasets using advanced data processing systems, that is, a systems biology approach which we have termed "cheesomics". There is the tantalizing possibility that this will lead to the identification of key biomarker profiles early in the cheese making process that will enable accurate prediction and control of cheese ripening outcomes for a variety of cheese types. Cheesomics also has the potential to discover and unravel the mechanisms by which cheese nutrients, metabolites and bacteria exert beneficial effects on human health.

References

Aldrete-Tapia, A., M. C. Escobar-Ramírez, M. L. Tamplin, and M. Hernández-Iturriaga. 2014. High-throughput sequencing of microbial communities in poro cheese, an artisanal Mexican cheese. *Food Microbiology* 44:136–41.

Almeida, M., A. Hébert, A.-L. Abraham, S. Rasmussen, C. Monnet, N. Pons, C. Delbès, V. Loux, J.-M. Batto, P. Leonard, et al. 2014. Construction of a dairy microbial genome catalog opens new perspectives for the metagenomic analysis of dairy fermented products. *BMC Genomics* 15 (1):1101.

Angelis, M., M. Calasso, N. Cavallo, R. Di Cagno, and M. Gobbetti. 2016. Functional proteomics within the genus lactobacillus. *Proteomics* 16 (6):946–62.

Aune, D., T. Norat, P. Romundstad, and L. J. Vatten. 2013. Dairy products and the risk of type 2 diabetes: A systematic review and dose-response meta-analysis of cohort studies. *The American Journal of Clinical Nutrition* 98 (4):1066–83.

Bernstein, J. A., A. B. Khodursky, P.-H. Lin, S. Lin-Chao, and S. N. Cohen. 2002. Global analysis of mRNA decay and abundance in *Escherichia coli* at single-gene resolution using two-color fluorescent DNA microarrays. *Proceedings of the National Academy of Sciences of the United States of America* 99 (15):9697–702.

Bokulich, N. A., and D. A. Mills. 2013. Facility-specific "house" microbiome drives microbial landscapes of artisan cheesemaking plants. *Applied and Environmental Microbiology* 79 (17):5214–23.

Bolotin, A., P. Wincker, S. Mauger, O. Jaillon, K. Malarme, J. Weissenbach, S. D. Ehrlich, and A. Sorokin. 2001. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. lactis IL1403. *Genome Research* 11 (5):731–53.

Bourdichon, F., S. Casaregola, C. Farrokh, J. C. Frisvad, M. L. Gerds, W. P. Hammes, J. Harnett, G. Huys, S. Laulund, and A. Ouwehand. 2012. Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology* 154 (3):87–97.

Brattoli, M., E. Cisternino, P. R. Dambruoso, G. De Gennaro, P. Giungato, A. Mazzone, J. Palmisani, and M. Tutino. 2013. Gas chromatography analysis with olfactometric detection (GC-O) as a useful methodology for chemical characterization of odorous compounds. *Sensors* 13 (12):16759–800.

Budinich, M. F., I. Perez-Díaz, H. Cai, S. A. Rankin, J. R. Broadbent, and J. L. Steele. 2011. Growth of *Lactobacillus paracasei* ATCC 334 in a cheese model system: A biochemical approach. *Journal of Dairy Science* 94 (11):5263–77.

Casado, B., M. Affolter, and M. Kussmann. 2009. OMICS-rooted studies of milk proteins, oligosaccharides and lipids. *Journal of Proteomics* 73 (2):196–208.

Chen, G.-C., Y. Wang, X. Tong, I. M. Y. Szeto, G. Smit, Z.-N. Li, and L.-Q. Qin. 2017. Cheese consumption and risk of cardiovascular disease: a Meta-analysis of prospective studies. *European Journal of Nutrition* 56 (8):2565–75.

Cheng, S., A. Lyytikäinen, H. Kröger, C. Lamberg-Allardt, M. Alén, A. Koistinen, Q. J. Wang, M. Suuriniemi, H. Suominen, A. Mahonen, et al. 2005. Effects of calcium, dairy product, and vitamin d supplementation on bone mass accrual and body composition in 10–12-y-old girls: a 2-y randomized trial. *The American Journal of Clinical Nutrition* 82 (5):1115–26.

Cocolin, L., and D. Ercolini. 2007. *Molecular techniques in the microbial ecology of fermented foods*. New York, NY: Springer.

Cocolin, L., M. Mataragas, F. Bourdichon, A. Doulgeraki, M.-F. Pilet, B. Jagadeesan, K. Rantsiou, and T. Phister. 2017. Next generation microbiological risk assessment Meta-omics: The next need for integration. *International Journal of Food Microbiology*. doi:10.1016/j.ijfoodmicro.2017.11.008

Conto, F. 2017. *Advances in dairy products*. Hoboken, NJ: John Wiley & Sons.

Cretenet, M., V. Laroute, V. Ulvé, S. Jeanson, S. Nouaille, S. Even, M. Piot, L. Girbal, Y. Le Loir, P. Loubière, et al., and M. Cocaign-Bousquet. 2011. Dynamic analysis of the *Lactococcus lactis* transcriptome in cheeses made from milk concentrated by ultrafiltration reveals multiple strategies of adaptation to stresses. *Applied and Environmental Microbiology* 77 (1):247–57.

da Cruz, A. G., F. C. A. Buriti, C. H. B. de Souza, J. A. F. Faria, and S. M. I. Saad. 2009. Probiotic cheese: Health benefits, technological and stability aspects. *Trends in Food Science and Technology* 20 (8):344–54.

Dallas, D. C., V. Weinborn, J. M. de Moura Bell, M. Wang, E. A. Parker, A. Guerrero, K. A. Hettinga, C. B. Lebrilla, J. B. German, and D. Barile. 2014. Comprehensive peptidomic and glycomic evaluation reveals that sweet whey permeate from colostrum is a source of milk protein-derived peptides and oligosaccharides. *Food Research International* 63:203–9.

Dalmasso, M., J. Aubert, V. Briard-Bion, V. Chuat, S.-M. Deutsch, S. Even, H. Falentin, G. Jan, J. Jardin, M.-B. Maillard, et al. 2012. A temporal-omic study of *Propionibacterium freudenreichii* CIRM-BIA1T adaptation strategies in conditions mimicking cheese ripening in the cold. *PLoS One* 7 (1):e29083.

De Beeck, M. O., B. Lievens, P. Busschaert, S. Declerck, J. Vangronsveld, and J. V. Colpaert. 2014. Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. *PLoS One* 9 (6):e97629.

De Filippis, F., A. Genovese, P. Ferranti, J. A. Gilbert, and D. Ercolini. 2016. Metatranscriptomics reveals temperature-driven functional changes in microbiome impacting cheese maturation rate. *Scientific Reports* 6 (1):21871.

De Filippis, F., E. Parente, and D. Ercolini. 2017. Metagenomics insights into food fermentations. *Applied Microbiology and Biotechnology* 10 (1):91–102.

Delcenserie, V., B. Taminiau, L. Delhalle, C. Nezer, P. Doyen, S. Crevecoeur, D. Roussey, N. Korsak, and G. Daube. 2014. Microbiota characterization of a Belgian protected designation of origin cheese, herve cheese, using metagenomic analysis. *Journal of Dairy Science* 97 (10):6046–56.

Den Hengst, C. D., S. A. van Hijum, J. M. Geurts, A. Nauta, J. Kok, and O. P. Kuipers. 2005. The *Lactococcus lactis* CodY regulon: Identification of a conserved cis-regulatory element. *The Journal of Biological Chemistry* 280 (40):34332–42.

Dias, D. A., O. A. Jones, D. J. Beale, B. A. Boughton, D. Benheim, K. A. Kouremenos, J.-L. Wolfender, and D. S. Wishart. 2016. Current and future perspectives on the structural identification of small molecules in biological systems. *Metabolites* 6 (4):46.

Dias, D. A., and T. Koal. 2016. Progress in metabolomics standardisation and its significance in future clinical laboratory medicine. *EJIFCC* 27 (4):331.

Douillard, F. P., and W. M. De Vos. 2014. Functional genomics of lactic acid bacteria: from food to health. *Microbial Cell Factories* 13 (1):S8.

Dressaire, C., E. Redon, H. Milhem, P. Besse, P. Loubière, and M. Cocaign-Bousquet. 2008. Growth rate regulated genes and their wide involvement in the *Lactococcus lactis* stress responses. *BMC Genomics* 9 (1):343.

Dugat-Bony, E., L. Garnier, J. Denonfoux, S. Ferreira, A.-S. Sarthou, P. Bonnarme, and F. Irlinger. 2016. Highlighting the microbial diversity of 12 french cheese varieties. *International Journal of Food Microbiology* 238 :265–73.

Dugat-Bony, E., C. Straub, A. Teissandier, D. Onésime, V. Loux, C. Monnet, F. Irlinger, S. Landaud, M.-N. Leclercq-Perlat, P. Bento, et al. 2015. Overview of a surface-ripened cheese community functioning by meta-omics analyses. *PloS One* 10 (4):e0124360.

Duwat, P., S. Sourice, B. Cesselin, G. Lamberet, K. Vido, P. Gaudu, Y. Le Loir, F. Violet, P. Loubière, and A. Gruss. 2001. Respiration capacity of the fermenting bacterium *Lactococcus lactis* and its positive effects on growth and survival. *Journal of Bacteriology* 183 (15):4509–16.

Ekman, M., P. Tollbäck, and B. Bergman. 2008. Proteomic analysis of the cyanobacterium of the azolla symbiosis: identity, adaptation, and NifH modification. *Journal of Experimental Botany* 59 (5):1023–34.

Ercolini, D. 2013. High-throughput sequencing and metagenomics: Moving forward in the culture-independent analysis of food microbial ecology. *Applied and Environmental Microbiology* 79 (10):3148–55.

Ercolini, D., F. De Filippis, A. La Storia, and M. Iacono. 2012. Remake' by high-throughput sequencing of the microbiota involved in the production of water buffalo mozzarella cheese. *Applied and Environmental Microbiology* 78 (22):8142–5.

Escobar-Zepeda, A., A. Sanchez-Flores, and M. Q. Baruch. 2016. Metagenomic analysis of a Mexican ripened cheese reveals a unique complex microbiota. *Food Microbiology* 57:116–27.

Falentin, H., S.-M. Deutsch, G. Jan, V. Loux, A. Thierry, S. Parayre, M.-B. Maillard, J. Dherbécourt, F. J. Cousin, J. Jardin, et al. 2010. The complete genome of *Propionibacterium freudenreichii* CIRM-BIA1 T, a hardy actinobacterium with food and probiotic applications. *PloS One* 5 (7):e11748.

Falentin, H., F. Postollec, S. Parayre, N. Henaff, P. Le Bivic, R. Richoux, A. Thierry, and D. Sohier. 2010. Specific metabolic activity of ripening bacteria quantified by real-time reverse transcription PCR throughout emmental cheese manufacture. *International Journal of Food Microbiology* 144 (1):10–9.

Ferrocino, I., A. Bellio, M. Giordano, G. Macori, A. Romano, K. Rantsiou, L. Decastelli, and L. Cocolin. 2018. Shotgun metagenomics and volatilome profile of the microbiota of fermented sausages. *Applied and Environmental Microbiology* 84 (3):e02120–17.

Fiehn, O., J. Kopka, P. Dörmann, T. Altmann, R. N. Trethewey, and L. Willmitzer. 2000. Metabolite profiling for plant functional genomics. *Nature Biotechnology* 18 (11):1157

Forquin, M.-P., A. Hébert, A. Roux, J. Aubert, C. Proux, J.-F. Heilier, S. Landaud, C. Junot, P. Bonnarme, and I. Martin-Verstraete. 2011. Global regulation of the response to sulfur availability in the cheese-related bacterium *Brevibacterium aurantiacum*. *Applied and Environmental Microbiology* 77 (4):1449–59.

Fox, P. 2000. *Fundamentals of cheese science*. New York, NY: Springer Science & Business Media.

Franzosa, E. A., T. Hsu, A. Sirota-Madi, A. Shafquat, G. Abu-Ali, X. C. Morgan, and C. Huttenhower. 2015. Sequencing and beyond: Integrating molecular 'omics' for microbial community profiling. *Nature Reviews Microbiology* 13 (6):360–72.

Fuchsmann, P., M. T. Stern, Y.-A. Brügger, and K. Breme. 2015. Olfactometry profiles and quantitation of volatile sulfur compounds of Swiss tilsit cheeses. *Journal of Agricultural and Food Chemistry* 63 (34):7511–21.

Fuka, M. M., S. Wallisch, M. Engel, G. Welzl, J. Havranek, and M. Schloter. 2013. Dynamics of bacterial communities during the ripening process of different croatian cheese types derived from raw ewe's milk cheeses. *PLoS One* 8 (11):e80734.

Gagnaire, V., J. Jardin, G. Jan, and S. Lortal. 2009. Invited review: Proteomics of milk and bacteria used in fermented dairy products: From qualitative to quantitative advances. *Journal of Dairy Science* 92 (3):811–25.

Gill, H. S., K. J. Rutherfurd, M. L. Cross, and P. K. Gopal. 2001. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. *The American Journal of Clinical Nutrition* 74 (6):833–9.

Gobbetti, M., F. Minervini, and C. G. Rizzello. 2007. Bioactive peptides in dairy products. In *Handbook of food products manufacturing*, ed. Y. H. Hui., 489–517. Hoboken, NJ: John Wiley & Sons, Inc.

Goodacre, R., S. Vaidyanathan, W. B. Dunn, G. G. Harrigan, and D. B. Kell. 2004. Metabolomics by numbers: Acquiring and understanding global metabolite data. *Trends in Biotechnology* 22 (5):245–52.

Gwinner, F., G. Boulday, C. Vandiedonck, M. Arnould, C. Cardoso, I. Nikolayeva, O. Guitart-Pla, C. V. Denis, O. D. Christophe, J. Beghain, et al. 2017. Network-based analysis of omics data: The LEAN method. *Bioinformatics (Oxford, England)* 33 (5):701–9.

Hamady, M., and R. Knight. 2009. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Research* 19 (7):1141–52.

Han, X. 2009. Lipidomics: Developments and applications. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* 877 (26):2663.

Handelsman, J. 2004. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews* 68 (4):669–85.

Hinz, K., P. M. O'Connor, B. O'Brien, T. Huppertz, R. P. Ross, and A. L. Kelly. 2012. Proteomic study of proteolysis during ripening of cheddar cheese made from milk over a lactation cycle. *The Journal of Dairy Research* 79 (2):176–84.

Hugenholtz, P., B. M. Goebel, and N. R. Pace. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology* 180 (18):4765–74.

Irlinger, F., S. A. Y. In Yung, A.-S. Sarthou, C. Delbès-Paus, M.-C. Montel, E. Coton, M. Coton, and S. Helinck. 2012. Ecological and aromatic impact of two gram-negative bacteria (*Psychrobacter celer* and *Hafnia alvei*) inoculated as part of the whole microbial community of an experimental smear soft cheese. *International Journal of Food Microbiology* 153 (3):332–8.

Jardin, J., D. Mollé, M. Piot, S. Lortal, and V. R Gagnaire. 2012. Quantitative proteomic analysis of bacterial enzymes released in cheese during ripening. *International Journal of Food Microbiology* 155 (1–2):19–28.

Johanningsmeier, S. D., G. K. Harris, and C. M. Klevorn. 2016. Metabolomic technologies for improving the quality of food: Practice and promise. *Annual Review of Food Science and Technology* 7:413–38.

Jovel, J., J. Patterson, W. Wang, N. Hotte, S. O'Keefe, T. Mitchel, T. Perry, D. Kao, A. L. Mason, K. L. Madsen, and G. K.-S. Wong. 2016. Characterization of the gut microbiome using 16S or shotgun metagenomics. *Frontiers in Microbiology* 7:459

Kok, J., L. A. van Gijtenbeek, A. de Jong, S. B. van der Meulen, A. Solopova, and O. P. Kuipers. 2017. The evolution of gene regulation research in *Lactococcus lactis*. *FEMS Microbiology Reviews* 41 (1): S220–S43.

Konkit, M., J.-H. Kim, N. Bora, and W. Kim. 2014. Transcriptomic analysis of *Lactococcus chungangensis* sp. nov. and its potential in cheese making. *Journal of Dairy Science* 97 (12):7363–72.

Korte, R., and J. Brockmeyer. 2017. Novel mass spectrometry approaches in food proteomics. *TrAC Trends in Analytical Chemistry* 96:99–106.

Kuipers, O. P., A. De Jong, R. J. Baerends, S. A. Van, A. L. Z. Hijum, H. A. Karsens, C. D. Den Hengst, N. E. Kramer, G. Buist, and J. Kok. 2002. *Transcriptome analysis and related databases of Lactococcus lactis. Lactic acid bacteria: Genetics, metabolism and applications*. Berlin, Germany: Springer.

Land, M., L. Hauser, S.-R. Jun, I. Nookaew, M. R. Leuze, T.-H. Ahn, T. Karpinets, O. Lund, G. Kora, T. Wassenaar, et al. 2015. Insights from 20 years of bacterial genome sequencing. *Functional & Integrative Genomics* 15 (2):141–61.

Larsen, N., S. Moslehi-Jenabian, B. B. Werner, M. L. Jensen, C. Garrigues, F. K. Vogensen, and L. Jespersen. 2016. Transcriptome analysis of *Lactococcus lactis* subsp. *lactis* during milk acidification as affected by dissolved oxygen and the redox potential. *International Journal of Food Microbiology* 226:5–12.

Lazzi, C., M. Povolo, F. Locci, V. Bernini, E. Neviani, and M. Gatti. 2016. Can the development and autolysis of lactic acid bacteria influence the cheese volatile fraction? The case of Grana Padano. *International Journal of Food Microbiology* 233:20–8.

Lazzi, C., S. Turroni, A. Mancini, E. Sgarbi, E. Neviani, P. Brigidi, and M. Gatti. 2014. Transcriptomic clues to understand the growth of *Lactobacillus rhamnosus* in cheese. *BMC Microbiology* 14 (1):28

Le Boucher, C., F. Courant, S. Jeanson, S. Chereau, M. B. Maillard, A. L. Royer, A. Thierry, G. Dervilly-Pinel, B. Le Bizec, and S. Lortal. 2013. First mass spectrometry metabolic fingerprinting of bacterial metabolism in a model cheese. *Food Chemistry* 141 (2):1032–40.

Le Boucher, C., F. Courant, A.-L. Royer, S. Jeanson, S. Lortal, G. Dervilly-Pinel, A. Thierry, and B. Le Bizec. 2015. LC–HRMS fingerprinting as an efficient approach to highlight fine differences in cheese metabolome during ripening. *Metabolomics* 11 (5):1117–30.

Lee, J. Y., J. Y. Joung, Y.-S. Choi, Y. Kim, and N. S. Oh. 2016. Characterisation of microbial diversity and chemical properties of cheddar cheese prepared from heat-treated milk. *The International Dairy Journal* 63:92–8.

Lessard, M.-H., C. Viel, B. Boyle, D. St-Gelais, and S. Labrie. 2014. Metatranscriptome analysis of fungal strains *Penicillium camemberti* and *Geotrichum candidum* reveal cheese matrix breakdown and potential development of sensory properties of ripened camembert-type cheese. *BMC Genomics* 15 (1):235.

Li, Q., Y. Zhao, D. Zhu, X. Pang, Y. Liu, R. Frew, and G. Chen. 2017. Lipidomics profiling of goat milk, soymilk and bovine milk by UPLC-Q-Exactive Orbitrap mass Spectrometry. *Food Chemistry* 224: 302–9.

Loman, N. J., and M. J. Pallen. 2015. Twenty years of bacterial genome sequencing. *Nature Reviews Microbiology* 13 (12):787

Lusk, T. S., A. R. Ottesen, J. R. White, M. W. Allard, E. W. Brown, and J. A. Kase. 2012. Characterization of microflora in Latin-style cheeses by next-generation sequencing technology. *BMC Microbiology* 12 (1):254.

Magenis, R. B., E. S. Prudêncio, L. Molognoni, and H. Daguer. 2014. A control method to inspect the compositional authenticity of minas frescal cheese by gel electrophoresis. *Journal of Agricultural and Food Chemistry* 62 (33):8333–9.

Makarova, K., A. Slesarev, Y. Wolf, A. Sorokin, B. Mirkin, E. Koonin, A. Pavlov, N. Pavlova, V. Karamychev, N. Polouchine, et al. 2006. Comparative genomics of the lactic acid bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 103 (42):15611–6.

Mandal, M., and R. R. Breaker. 2004. Gene regulation by riboswitches. *Nature Reviews. Molecular Cell Biology* 5 (6):451–63.

Manso, M., J. Léonil, G. Jan, and V. Gagnaire. 2005. Application of proteomics to the characterization of milk and dairy products. *The International Dairy Journal* 15 (6-9):845–55.

Marco, M. L., Heeney, D. S. Binda, C. J. Cifelli, P. D. Cotter, B. Foligné, M. Gänzle, R. Kort, G. Pasin, A. Pihlanto, E. J. et al. 2017. Health benefits of fermented foods: Microbiota and beyond. *Current Opinion in Biotechnology* 44:94–102.

Mardis, E. R. 2011. A decade's perspective on DNA sequencing technology. *Nature* 470 (7333):198.

Martinez-Villaluenga, C., E. Peñas, and J. Frias. 2016. Bioactive peptides in fermented foods: Production and evidence for health effects. *Fermented foods in health and disease prevention* 23–47.

Masoud, W., M. Takamiya, F. K. Vogensen, S. Lillevang, W. A. Al-Soud, S. J. Sørensen, and M. Jakobsen. 2011. Characterization of bacterial populations in Danish raw milk cheeses made with different starter cultures by denaturating gradient gel electrophoresis and pyrosequencing. *The International Dairy Journal* 21 (3):142–8.

Mazzei, P., and A. Piccolo. 2012. 1H HRMAS-NMR metabolomic to assess quality and traceability of mozzarella cheese from Campania buffalo milk. *Food Chemistry* 132 (3):1620–7.

Metzker, M. L. 2010. Sequencing technologies – The next generation. *Nature Reviews. Genetics* 11 (1):31–46.

Monnet, C., E. Dugat-Bony, D. Swennen, J.-M. Beckerich, F. Irlinger, S. Fraud, and P. Bonnarme. 2016. Investigation of the activity of the microorganisms in a reblochon-style cheese by metatranscriptomic analysis. *Frontiers in Microbiology* 7:536.

Monnet, C., V. Ulvé, A.-S. Sarthou, and F. Irlinger. 2008. Extraction of RNA from cheese without prior separation of microbial cells. *Applied and Environmental Microbiology* 74 (18):5724–30.

Mounier, J., C. Monnet, N. Jacques, A. Antoinette, and F. Irlinger. 2009. Assessment of the microbial diversity at the surface of livarot cheese using culture-dependent and independent approaches. *International Journal of Food Microbiology* 133 (1-2):31–7.

Mukherjee, S., R. Seshadri, N. J. Varghese, E. A. Eloe-Fadrosh, J. P. Meier-Kolthoff, M. Göker, R. C. Coates, M. Hadjithomas, G. A. Pavlopoulos, D. Paez-Espino, et al. 2017. 1,003 Reference genomes of bacterial and archaeal isolates expand coverage of the tree of life. *Nature Biotechnology* 35 (7):676.

Nocker, A., M. Burr, and A. K. Camper. 2007. Genotypic microbial community profiling: A critical technical review. *Microbial Ecology* 54 (2):276–89.

O'Donnell, R., J. W. Holland, H. C. Deeth, and P. Alewood. 2004. Milk proteomics. *The International Dairy Journal* 14 (12):1013–23.

Ochi, H., T. Bamba, H. Naito, K. Iwatsuki, and E. Fukusaki. 2012. Metabolic fingerprinting of hard and semi-hard natural cheeses using gas chromatography with flame ionization detector for practical sensory prediction modeling. *The Journal of Bioscience and Bioengineering* 114 (5):506–11.

Ochi, H., H. Naito, K. Iwatsuki, T. Bamba, and E. Fukusaki. 2012. Metabolomics-based component profiling of hard and semi-hard natural cheeses with gas chromatography/time-of-flight-mass spectrometry, and its application to sensory predictive modeling. *The Journal of Bioscience and Bioengineering* 113 (6):751–8.

Ojala, T., P. K. S. Laine, T. Ahlroos, J. Tanskanen, S. Pitkänen, T. Salusjärvi, M. Kankainen, S. Tynkkynen, L. Paulin, and P. Auvinen. 2017. Functional genomics provides insights into the role of *Propionibacterium freudenreichii* ssp. *shermanii* JS in cheese ripening. *International Journal of Food Microbiology* 241:39–48.

Pisano, M. B., P. Scano, A. Murgia, S. Cosentino, and P. Caboni. 2016. Metabolomics and microbiological profile of Italian mozzarella cheese produced with buffalo and cow milk. *Food Chemistry* 192: 618–24. doi: 10.1016/j.foodchem.2015.07.061.

Pogačić, T., M.-B. Maillard, A. Leclerc, C. Hervé, V. Chuat, F. Valence, and A. Thierry. 2016. *Lactobacillus* and *leuconostoc* volatilomes in cheese conditions. *Applied Microbiology and Biotechnology* 100 (5): 2335–46.

Pogačić, T., M.-B. Maillard, A. Leclerc, C. Hervé, V. Chuat, A. L. Yee, F. Valence, and A. Thierry. 2015. A methodological approach to screen diverse cheese-related bacteria for their ability to produce aroma compounds. *Food Microbiology* 46:145–53.

Prasad, J., H. Gill, J. Smart, and P. K. Gopal. 1998. Selection and characterization of lactobacillus and bifidobacterium strains for use as probiotics. *The International Dairy Journal* 8 (12):993–1002.

Putri, S. P., Y. Nakayama, F. Matsuda, T. Uchikata, S. Kobayashi, A. Matsubara, and E. Fukusaki. 2013. Current metabolomics: Practical applications. *Journal of Bioscience and Bioengineering* 115 (6): 579–89.

Quigley, L., O. O'Sullivan, T. P. Beresford, R. P. Ross, G. F. Fitzgerald, and P. D. Cotter. 2011. Molecular approaches to analyzing the microbial composition of raw milk and raw milk cheese. *International Journal of Food Microbiology* 150 (2–3):81–94.

Quigley, L., O. O'Sullivan, T. P. Beresford, R. P. Ross, G. F. Fitzgerald, and P. D. Cotter. 2012. High-throughput sequencing for detection of subpopulations of bacteria not previously associated with artisanal cheeses. *Applied and Environmental Microbiology* 78 (16):5717–23.

Quince, C., A. W. Walker, J. T. Simpson, N. J. Loman, and N. Segata. 2017. Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology* 35 (9):833.

Raes, J., and P. Bork. 2008. Molecular eco-systems biology: Towards an understanding of community function. *Nature Reviews. Microbiology* 6 (9):693

Rastogi, G., and R. K. Sani. 2011. Molecular techniques to assess microbial community structure, function, and dynamics in the environment. In *Microbes and microbial technology*, ed. I. Ahmad, F. Ahmad, and J. Pichtel, 29–57. New York, NY: Springer.

Riquelme, C., S. Câmara, M. D L N. Enes Dapkevicius, P. Vinuesa, C. C. G. da Silva, F. X. Malcata, and O. A. Rego. 2015. Characterization of the bacterial biodiversity in pico cheese (an artisanal Azorean food)). *International Journal of Food Microbiology* 192:86–94.

Rittmann, B. E., R. Krajmalnik-Brown, and R. U. Halden. 2008. Pre-genomic, genomic and post-genomic study of microbial communities involved in bioenergy. *Nature Reviews. Microbiology* 6 (8):604.

Rizzello, C., I. Losito, M. Gobbetti, T. Carbonara, M. De Bari, and P. Zambonin. 2005. Antibacterial activities of peptides from the water-soluble extracts of Italian cheese varieties. *Journal of Dairy Science* 88 (7):2348–60.

Rocca, J. D., E. K. Hall, J. T. Lennon, S. E. Evans, M. P. Waldrop, J. B. Cotner, D. R. Nemergut, E. B. Graham, and M. D. Wallenstein. 2015. Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. *The Isme Journal* 9 (8):1693.

Roessner, U., A. Nahid, B. Chapman, A. Hunter, and M. Bellgard. 2011. Metabolomics–the combination of analytical biochemistry, biology, and informatics. In *Comprehensive biotechnology*, ed. M. Moo-Young, 447–459. 2nd ed. Cambridge, MA: Academic Press.

Roncada, P., C. Piras, A. Soggiu, R. Turk, A. Urbani, and L. Bonizzi. 2012. Farm animal milk proteomics. *Journal of Proteome Research* 75 (14):4259–74.

Sattin, E., N. Andreani, L. Carraro, L. Fasolato, S. Balzan, E. Novelli, A. Squartini, A. Telatin, B. Simionati, and B. Cardazzo. 2016. Microbial dynamics during shelf-life of industrial ricotta cheese and identification of a *bacillus* strain as a cause of a pink discolouration. *Food Microbiology* 57:8–15.

Salter, S. J., M. J. Cox, E. M. Turek, S. T. Calus, W. O. Cookson, M. F. Moffatt, P. Turner, J. Parkhill, N. J. Loman, and A. W. Walker. 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology* 12 (1):87.

Santiago-López, L., J. E. Aguilar-Toalá, A. Hernández-Mendoza, B. Vallejo-Cordoba, A. M. Liceaga, and A. F. González-Córdova. 2018. Invited review: Bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption. *Journal of Dairy Science* 101 (5):3742–57.

Schornsteiner, E., E. Mann, O. Bereuter, M. Wagner, and S. Schmitz-Esser. 2014. Cultivation-independent analysis of microbial communities on Austrian raw milk hard cheese rinds. *International Journal of Food Microbiology* 180:88–97.

Settanni, L., and G. Moschetti. 2010. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. *Food Microbiology* 27 (6):691–7.

Sgarbi, E., C. Lazzi, G. Tabanelli, M. Gatti, E. Neviani, and F. Gardini. 2013. Nonstarter lactic acid bacteria volatilomes produced using cheese components. *Journal of Dairy Science* 96 (7):4223–34.

Silva, R. A., V. S. Bezerra, M. D C B. Pimentel, A. L. F. Porto, M. T. H. Cavalcanti, and L. José Luiz Filho. 2016. Proteomic and peptidomic profiling of Brazilian artisanal 'Coalho' cheese. *Journal of the Science of Food and Agriculture* 96 (13):4337–44.

Soggiu, A., C. Piras, S. L. Mortera, I. Alloggio, A. Urbani, L. Bonizzi, and P. Roncada. 2016. Unravelling the effect of clostridia spores and lysozyme on microbiota dynamics in grana padano cheese: A meta-proteomics approach. *Journal of Proteome Research* 147:21–7.

Sokol, E., T. Ulven, N. J. Faergeman, and C. S. Ejsing. 2015. Comprehensive and quantitative profiling of lipid species in human milk, cow milk and a phospholipid-enriched milk formula by GC

and MS/MSALL. *European Journal of Lipid Science and Technology: Ejlst* 117 (6):751–9.

Solieri, L., T. C. Dakal, and P. Giudici. 2013. Next-generation sequencing and its potential impact on food microbial genomics. *Annals of Microbiology* 63 (1):21–37.

Spratlin, J. L., N. J. Serkova, and S. G. Eckhardt. 2009. Clinical applications of metabolomics in oncology: a review. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 15 (2):431–40.

St-Onge, M.-P., E. R. Farnworth, and P. J. Jones. 2000. Consumption of fermented and nonfermented dairy products: Effects on cholesterol concentrations and metabolism. *The American Journal of Clinical Nutrition* 71 (3):674–81.

Sumner, L. W., P. Mendes, and R. A. Dixon. 2003. Plant metabolomics: Large-scale phytochemistry in the functional genomics era. *Phytochemistry* 62 (6):817–36.

Sun, Z., H. M. B. Harris, A. McCann, C. Guo, S. Argimón, W. Zhang, X. Yang, I. B. Jeffery, J. C. Cooney, T. F. Kagawa, et al. 2015. Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. *Nature Communications* 6 (1):8322.

Tan-A-Ram, P., T. Cardoso, M.-L. Daveran-Mingot, S. Kanchanatawee, P. Loubière, L. Girbal, and M. Cocaign-Bousquet. 2011. Assessment of the diversity of dairy *Lactococcus lactis* subsp. *lactis* isolates by an integrated approach combining phenotypic, genomic, and transcriptomic analyses. *Applied and Environmental Microbiology* 77 (3): 739–48.

Taïbi, A., N. Dabour, M. Lamoureux, D. Roy, and G. LaPointe. 2011. Comparative transcriptome analysis of *Lactococcus lactis* subsp. *cremoris* strains under conditions simulating cheddar cheese manufacture. *International Journal of Food Microbiology* 146 (3):263–75.

Telgi, R. L., V. Yadav, C. R. Telgi, and N. Boppana. 2013. In vivo dental plaque pH after consumption of dairy products. *General Dentistry* 61 (3):56–9.

Tessler, M., J. S. Neumann, E. Afshinnekoo, M. Pineda, R. Hersch, L. F. M. Velho, B. T. Segovia, F. A. Lansac-Toha, M. Lemke, R. DeSalle, et al. 2017. Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. *Scientific Reports* 7 (1):6589.

van Dijk, E. L., H. Auger, Y. Jaszczyszyn, and C. Thermes. 2014. Ten years of next-generation sequencing technology. *Trends in Genetics* 30 (9):418–26.

Walsh, A. M., F. Crispie, M. J. Claesson, and P. D. Cotter. 2017. Translating omics to food microbiology. *Annual Review of Food Science and Technology* 8:113–34.

Walther, B., A. Schmid, R. Sieber, and K. Wehrmüller. 2008. Cheese in nutrition and health. *Dairy Science and Technology* 88 (4–5): 389–405.

Wegmann, U., M. O'Connell-Motherway, A. Zomer, G. Buist, C. Shearman, C. Canchaya, M. Ventura, A. Goesmann, M. J. Gasson, O. P. Kuipers, et al. 2007. Complete genome sequence of the prototype lactic acid bacterium *Lactococcus lactis* subsp. *cremoris* MG1363. *Journal of Bacteriology* 189 (8):3256–70.

Wilmes, P., and P. L. Bond. 2004. The application of two-dimensional polyacrylamide gel electrophoresis and downstream analyses to a mixed community of prokaryotic microorganisms. *Environmental Microbiology* 6 (9):911–20.

Wolfe, B. E., J. E. Button, M. Santarelli, and R. J. Dutton. 2014. Cheese rind communities provide tractable systems for *in situ* and *in vitro* studies of microbial diversity. *Cell* 158 (2):422–33.

Wu, Z., P. Wang, J. He, D. Pan, X. Zeng, and J. Cao. 2016. Proteome analysis of *Lactobacillus plantarum* strain under cheese-like conditions. *Journal of Proteomics* 146:165–71.

Yee, A. L., M.-B. Maillard, N. Roland, V. Chuat, A. Leclerc, T. Pogačić, F. Valence, and A. Thierry. 2014. Great interspecies and intraspecies diversity of dairy propionibacteria in the production of cheese aroma compounds. *International Journal of Food Microbiology* 191: 60–8.

Yvon, M., C. Gitton, E. Chambellon, G. Bergot, and V. Monnet. 2011. The initial efficiency of the proteolytic system of *Lactococcus lactis* strains determines their responses to a cheese environment. *The International Dairy Journal* 21 (5):335–45.

Zanni, E., E. Schifano, S. Motta, F. Sciubba, C. Palleschi, P. Mauri, G. Perozzi, D. Uccelletti, C. Devirgiliis, and A. Miccheli. 2017. Combination of metabolomic and proteomic analysis revealed different features among *Lactobacillus delbrueckii* subspecies *bulgaricus* and *lactis* strains while in vivo testing in the model organism caenorhabditis elegans highlighted probiotic properties. *Frontiers in Microbiology* 8:1206.

Zhang, Y., B. R. Fonslow, B. Shan, M.-C. Baek, and J. R. Yates. III. 2013. Protein analysis by shotgun/bottom-up proteomics. *Chemical Reviews* 113 (4):2343–94.

Zomer, A. L., G. Buist, R. Larsen, J. Kok, and O. P. Kuipers. 2007. Time-resolved determination of the CcpA regulon of *Lactococcus lactis* subsp. *cremoris* MG1363. *Journal of Bacteriology* 189 (4): 1366–81.