Towards an Ecosystem Approach to Cheese Microbiology

BENJAMIN E. WOLFE and RACHEL J. DUTTON

FAS Center for Systems Biology, Harvard University, Cambridge, MA 02138

ABSTRACT  Cheese is an ideal environment to serve as a model for the behavior of microbes in complex communities and at the same time allow detailed genetic analysis. Linking organisms, and their genes, to their role in the environment becomes possible in the case of cheese since cheese microbial communities have been “in culture” for thousands of years, with the knowledge of how to grow these organisms passed down by generations of cheesemakers. Recent reviews have described several emerging approaches to link molecular systems biology to ecosystem-scale processes, known as ecosystems biology. These approaches integrate massive datasets now available through high-throughput sequencing technologies with measurements of ecosystem properties. High-throughput datasets uncover the “parts list” (e.g., the species and all the genes within each species) of an ecosystem as well as the molecular basis of interactions within this parts list. Novel computational frameworks make it possible to link species and their interactions to ecosystem properties. Applying these approaches across multiple temporal and spatial scales makes it possible to understand how changes in the parts lists over space and time lead to changes in ecosystems processes. By manipulating the species present within model systems, we can test hypotheses related to the role of microbes in ecosystem function. Due to the tractability of cheese microbial communities, we have the opportunity to use an ecosystems biology approach from the scale of individual microbial cells within a cheese to replicated cheese microbial communities across continents. Using cheese as a model microbial ecosystem can provide a way to answer important questions concerning the form, function, and evolution of microbial communities.

While microbes have traditionally been studied as individuals in the laboratory, they exist not as individuals in nature but as parts of complex, multispecies communities. Microbial communities have long been acknowledged as crucial for the proper functioning of our global ecosystem and have recently been implicated in influencing human health, both positively and negatively (1, 2). Understanding how microbial ecosystems function not only will advance our knowledge of some of the most important, and most ancient, organisms on this planet, microbes, but also could uncover ways to eliminate or manipulate these communities to our advantage.

However, progress in understanding microbes in the context of their natural environment is currently facing a dilemma. On one hand, advances in sequencing technologies have led to massive amounts of information on the genome sequences of microbes from the environment, yet there is little way to do detailed studies of these organisms because, often, they cannot be grown in the laboratory (3, 4). Without detailed studies, the roles of the microbes, and the functions encoded by their genomes, remain enigmatic. On the other hand, while genetic studies of model organisms have been highly successful in elucidating the molecular and biochemical basis for cellular life, they leave much to be discovered about how microbes function within the context of the complex communities in which they exist outside the laboratory. Thus, what is currently needed in microbiology is a way to combine these two approaches to microbiology. This dilemma could be addressed by finding simplified, experimentally
tractable microbial ecosystems, which can serve as models for the behavior of microbes in complex communities and at the same time allow detailed genetic analysis. A potential solution to this problem is to return to one of the origins of modern microbiology: food.

Scientific studies based on the microbial inhabitants of food were the beginning of modern microbiology and biochemistry thanks to the pioneering research of scientists such as Louis Pasteur. In the time since Pasteur, microbes isolated from foods, such as Saccharomyces cerevisiae, were established as model organisms for laboratory studies. However, microbes from foods often exist not as individuals but as parts of multispecies communities. Multispecies communities of microbes play important roles in many foods (e.g., wine, pickles, coffee, and chocolate) (5). In fact, the impact of microbial growth is most dramatic in one of the world’s most cherished foods: cheese. Much of the diversity in flavors, smells, and textures of the hundreds of different varieties of cheeses is directly related to the diversity of microorganisms, and their associated rich assembly of metabolic capacities, that can grow in and on cheeses.

Cheese represents an environment in which the study of microbial communities is simplified in a number of ways. Cheese is the habitat for two different communities of microbes. The first is found inside the cheese, containing primarily lactic acid bacteria, and the second is found on the surface of the cheese, where a diverse collection of microbes make up the rind. In both of these cases, the communities are greatly reduced in complexity compared to many other microbial habitats, such as the human body, in which, for example, the gut can contain hundreds of species per gram of intestinal content (6).

The goal of linking organisms, and their genes, to their role in the environment also becomes possible in the case of cheese. Achieving this goal in other systems has been particularly difficult because typically less than 1% of microbes from any given environment grow in a laboratory setting (3). However, cheese microbial communities have been “in culture” for thousands of years, with the knowledge of how to grow these organisms passed down by generations of cheesemakers. In addition, genetic systems have been established for a number of cheese-associated microbes, such as the lactic acid bacteria (7). Cheese microbial communities have several other attributes which make them attractive for study. The large numbers of replicates produced within a batch and between batches make the collection of large datasets and statistical analysis possible. The ease of sample collection and the abundance of microbial cells within the samples also simplify analysis. Another key benefit of these communities is that their formation can be monitored over time.

Given the experimental tractability of cheese microbial communities, they can serve as a model for applying a systems biology approach to the study of microbial communities. Recent reviews have described several emerging approaches to link molecular systems biology to ecosystem scale processes (8). Called ecosystems biology, these approaches integrate massive datasets now available through high-throughput sequencing technologies with measurements of ecosystem properties. High-throughput datasets uncover the “parts list” (e.g., the species and all the genes within each species) of an ecosystem as well as the molecular basis of interactions within this parts list. Novel computational frameworks make it possible to link species and their interactions to ecosystem properties. Applying these approaches across multiple temporal and spatial scales makes it possible to understand how changes in the parts lists over space and time lead to changes in ecosystems processes. By manipulating the species present within model systems, we can test hypotheses related to the role of microbes in ecosystem function. Due to the tractability of cheese microbial communities, we have the opportunity to use an ecosystems biology approach from the scale of individual microbial cells within a cheese to replicated cheese microbial communities across continents (Fig. 1). Therefore, using cheese as a model microbial ecosystem can provide a way to answer questions concerning the form, function, and evolution of microbial communities.

CELLULAR-SCALE STUDIES

How cells sense and respond to their environment, obtain and utilize nutrients, and grow and reproduce are fundamental questions in cell biology and genetics. Despite their relatively simple appearance, microbial cells are incredibly powerful in their ability to adapt to diverse environments and carry out diverse metabolic processes. Because of their ability to grow with relative ease under laboratory conditions, certain microbes, such as Escherichia coli and Saccharomyces cerevisiae, have become models for studying cell biology and genetics. Studies of these and other model organisms have led to a vast amount of knowledge concerning cellular processes. Yet, there remains much to be discovered about processes at the cellular level within microbes.

Take, for example, E. coli, which is probably the most extensively studied organism on the planet. E. coli has approximately 4,000 genes in its genome, yet we still do not know the function of a more than a quarter of these
FIGURE 1  Cheese as a model for microbial ecosystems biology. A conceptual overview of the fundamental biological processes that occur within cheese microbial ecosystems is shown. Emerging systems biology approaches provide the potential to dissect these processes across multiple spatial scales, ranging from individual microbial cells growing within a cheese to microbial communities distributed across a cheesemaking region.

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genes (9). Although many of the key pathways involved in the metabolism and reproduction of E. coli, as well as the pathways which regulate these processes, have been mapped out in great detail, we do not have a clear and predictive model of exactly how a cell integrates all of this information and makes the changes necessary in response to variations in the environment within the laboratory or within a host. We know relatively little about the mechanisms of metabolism, growth, and regulation in other microbial species compared to E. coli, including those involved in cheese production.

In order to make progress in our understanding of microbes at the cellular level, microbiologists are applying both traditional and new approaches in a wide range of ways. Genetic and cell biological studies of microbes beyond the standard genetic workhorses have led to the identification of novel cellular pathways. One fascinating example is the discovery of a method for cell-cell communication called quorum sensing in the marine bacterium Vibrio fischeri. Quorum sensing, which relies on the production and detection of small molecules by cells, allows microbes (both bacteria and fungi) to count their own numbers within a population and, in some cases, detect other species of microbe present in their local environment (10, 11). Doing so allows microbial cells to alter their growth and behavior in a wide range of ways. In the case of Vibrio fischeri, at high cell density, the cells turn on a pathway that leads to the production of light. There have now been many studies highlighting other biological pathways regulated by quorum sensing in microbes, including the formation of biofilms and regulation of enzyme production, both of which are important in cheese. Because the use of quorum sensing appears to be widespread and controls diverse cellular pathways, more detailed study of this process in cheese-associated bacteria and fungi could reveal new and interesting uses of quorum sensing in cheese.

The understanding of microbial diversity at the cellular level has been greatly aided by tremendous advances in genome sequencing. To date, there are over 1,500 fully sequenced, publicly available microbial genomes (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). The majority of these genomes have been sequenced in just the past 5 years. While microbiologists have long recognized that microbes are enormously diverse, the availability of these genome sequences allows us to begin to understand the genetic underpinnings of this known diversity and has revealed how much we still have to learn about microbes. For example, a recent study sequenced over 50 phylogenetically diverse bacterial and archaeal genomes, and for every new genome sequenced, the researchers discovered, on average, over 1,000 new protein families (12).

Yet the large-scale sequencing of microbial genomes does far more than highlight the gaps in our knowledge. It has significantly increased our ability to decipher the function and evolution of proteins in the cell through the use of comparative genomics approaches. For example, by comparing the compositions of genomes of cheese microbes to those of closely related species that are not specialized to the cheese environment, it was possible to understand underlying processes of genome evolution that have led to the specialization of certain microbes to cheeses (13). Differences in genome size and content between species of cheese microbes could have important outcomes in the way different microbes are able to grow in or on cheese, in acidification during cheesemaking, and in the many flavor- and aroma-generating metabolic pathways active during the aging of cheese. Thus, determining the genomic differences between species can reveal why certain microbes have a particular impact on cheese and has the potential to lead to new information about these processes.

As a complement to the genomic approaches described above, new methods for quantitative detection and identification of mRNA transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics) can be used to decipher how cells work and behave within the context of specific environments (14). In each of these approaches, the total population of a class of molecules within the cell can be extracted and studied in a single experiment. In this way, global changes in the levels and types of molecules present can be measured in response to changes in the environment, or between different strains or species of microbes. For example, one can measure the production of volatiles by a specific cheese-associated microbe and understand how this changes at different growth phases or under different growth conditions such as nutrient source, pH, temperature, and salinity. Changes in the metabolite profile can be compared to the profile of both proteins and mRNA within the cell under the same conditions. Thus, these approaches can lead more directly to information on the functional roles of organisms or pathways. In the case of microbes for which genetic tools are available, such as the lactic acid bacteria, these types of experiments can inform hypotheses about gene function that can then be tested directly. One disadvantage to these approaches is that they require the ability to grow isolated species in a laboratory. As described above, many environmental microbes resist cultivation in the laboratory. However, a wide range of methods for performing similar types of studies on microbes within the context of their native
environment and within a community of species are being developed and are described in the following sections.

COMMUNITY-SCALE STUDIES

While the approaches described above highlight the major advances in the ability to understand individual microbes, there are few examples in nature where a single species exists in isolation. Microbes usually grow in the presence of other species. Whether this is just a single other species or hundreds of other species, the biology that takes place within communities could differ significantly from what is observed in pure culture studies in a laboratory. Thus, it is important to take growth within multispecies communities into consideration when attempting to understand microbial processes.

The first challenge in studying community-level biology is the identification of species present within the community. This challenge has only recently been overcome due to advances in DNA sequencing, as well as conceptual advances in classifying and comparing species based on DNA sequences. Instead of relying on the growth of microbes in pure culture in the laboratory, microbiologists now have the ability to identify all species in an environmental sample using community sequencing approaches (15). This sequence-based versus culture-based analysis of microbes has revolutionized our view of the microbial world, as well as the way in which microbes are studied, and importantly, this type of analysis can be applied to any type of environmental sample, including cheese.

The sequence-based approach to the identification of species most commonly relies on determining the sequence of the genes encoding rRNA (16). In bacteria, the 16S rRNA gene is used, and in fungi, sequences between rRNA genes (internal transcribed spacers) are typically used. For these studies, total community genomic DNA is extracted from a sample, and the rRNA gene of interest is amplified using PCR. After this step, the mixed population of amplified sequences can either be cloned to make a plasmid library for sequencing or, using next-generation sequencing methods, be sequenced directly without the need for cloning. Next-generation technologies allow for the simultaneous sequencing of thousands to millions of pieces of DNA, at a fraction of the cost per base pair of traditional Sanger sequencing (17).

In addition to the identity of species within a sample, these newer technologies have recently been used to determine not only the ribosomal DNA sequences but also the total genomic sequences present within communities. This type of metagenomic analysis provides a sort of genetic blueprint of a community, outlining the genetic and metabolic potential within a community (6, 18, 19). These sequence-based technologies can be used to define the species diversity within cheeses, to monitor the succession within cheese communities over time, and to describe the genetic potential of a community.

One of the major differences between life on a petri dish in a laboratory and life within a community consists of the interactions that take place between microbes. Interactions, whether they are of an antagonistic, cooperative, or neutral nature, determine many of the characteristics of any given community, such as the number and type of species present. Microbe-microbe interactions must take place at every stage of a community, from the establishment of a community, its maintenance, and, eventually, its decline. Understanding the nature and extent of these interactions within the cheese microbial community could lead to insights on how to manipulate the microbial communities in desirable ways. Most microbe-microbe interactions known to date are mediated by small molecules (20). Despite the fact that microbial communities found on cheeses produce a rich assortment of small molecules, many of which we perceive as tastes and aromas, the potential biological roles of these compounds has received little study.

One technique for observing species interactions is coculturing, in which two microbes are grown in close proximity and compared to individual growth (21). In this way, changes in the growth rate (either positive or negative) or development can be measured. Small molecules involved in interactions may be identified by high-performance liquid chromatography or gas chromatography and mass spectroscopy. A classic example of the application of this technique is in antibiotic discovery. Most antibiotics in use today originate from microbially produced small molecules. In nature, these molecules likely play roles not just in competition but also in signaling and cell-cell communication between and within species (22, 23). Thus, the above-mentioned studies examining community-level interactions not only may reveal new pathways for cell-cell interactions but also could lead to the discovery of new antibiotics (24).

ECOSYSTEM-SCALE STUDIES

In addition to understanding the factors that control the establishment and maintenance of diversity in microbial communities, one major research goal in microbial ecology is to link the composition of microbial communities to ecosystem function. By examining how
patterns in community diversity correlate with ecosystem processes, it is possible to tease apart the relative roles of different community members in specific processes. Most previous studies linking species composition to function have assessed correlations between the diversity of “bar-coding genes” (taxonomic diversity) and an ecosystem parameter, such as carbon and nitrogen availability (25, 26). Other studies have used a functional-gene approach, linking the diversity and abundance of specific functional genes with ecosystem processes (27, 28). These targeted approaches are useful for understanding specific processes, but it is impossible to understand how the functioning of complex, multispecies gene networks within communities contributes to the transformation of resources within an ecosystem because only a small subset of genes are targeted by these approaches. Newly developed systems-level approaches that utilize next-generation sequencing technologies allow microbial ecologists to link whole-community genomic diversity and global gene expression patterns with ecosystem function. These approaches will be critical in moving towards an ecosystems approach to cheese microbial communities.

Metatranscriptomics, the large-scale sequencing of expressed transcripts of entire microbial communities, is currently emerging as a powerful systems-level tool that can link microbial community function with composition (29). While metagenomic studies (described above) explore the taxonomic diversity of microbial communities and provide insight on the metabolic potential of microbial communities, metatranscriptomic studies measure the diversity of expressed genes within a microbial community, providing information on what species and genes are active within an environment. The resulting massive datasets provide a large-scale snapshot of the diversity of genes expressed within an ecosystem. By mapping sequenced transcripts to genes within sequenced genomes or metagenomes, it is possible to understand the relative contributions of different microbial species to specific components of community metabolism (30). Transcript sequences can also be used to construct models of metabolic pathways relevant to nutrient cycling and measure changes in transcript abundances in these pathways in relation to environmental parameters (31). Metatranscriptome studies also provide an opportunity to discover novel genes and regulatory elements with previously unknown functions (32, 33).

How can these new metatranscriptomics approaches developed for marine, terrestrial, and human gut microbial communities advance our understanding of cheese microbiology? Our current assessment of the community metabolism of cheese microbial communities is limited to the genomic potential described from sequencing the genomes or transcriptomes of a few microbial species in isolation (as discussed above) or based on monitoring the expression of individual functional genes (34). These targeted approaches have provided initial glimpses at the potential function of microbes isolated from cheese. Metatranscriptomic has the power to uncover the vast gene networks of multispecies cheese communities and reveal how these transcriptional patterns relate to transformation of resources within cheese ecosystems. For example, despite the relatively simple and well-understood resource base within cheese ecosystems, a mechanistic understanding of how community metabolism of cheese microbial communities relates to the transformation of this resource base is lacking. One of the most important biochemical processes driving the production of flavor compounds in cheese is proteolysis (as reviewed in reference 35). Individual proteases have been isolated and characterized from many cheese microbes, and total community protease activity has been measured within cheese ecosystems (36, 37, 38, 39, 40, 41, 42), but how genes in the proteolytic systems in multispecies cheese communities correlate with proteolysis and amino acid catabolism has not been explored. Metatranscriptomics could be used to determine which microbial species contribute most to protein transformations within cheese and how interactions between gene networks of different species contribute to proteolysis. A similar approach could be used to map out gene expression networks that contribute to the transformation of lactose and that of lipids, two other important biochemical processes in cheese development.

Applying the metatranscriptomics sampling schemes developed in other microbial ecosystems could uncover the molecular basis of fundamental ecological processes within cheese ecosystems. Sequencing the metatranscriptome of a cheese over time would reveal how community-level gene expression relates to critical transition points during the aging of cheeses, such as rind decodification. Comparing metatranscriptomes across the same cheese type that vary in flavor profiles could uncover novel genes previously unknown to be involved in the production of flavor compounds. Spatial and temporal sampling of metatranscriptomes of cheese microbial communities could reveal how gene expression networks are partitioned over space and time within cheese ecosystems. Comparative transcriptomics between different cheese types could reveal how gene networks within particular cheese microfloras lead to the
processing of resources within cheese ecosystems. Several recent studies in other food fermentations (43, 44) have used microarrays developed for many common lactic acid bacteria to explore community-level expression and provide a glimpse of the exciting potential the transcriptomics can offer to exploring the community metabolism of cheese microbes.

While metatranscriptomics can uncover the diversity and abundance of transcripts within a microbial community, the abundance and activity of proteins within microbial communities are the ultimate determinants of community and ecosystem function. Because the abundance of a particular mRNA transcript does not necessarily correlate with the abundance of the corresponding protein, determining proteins expressed within a microbial community can provide a more direct link to microbial community function than metatranscriptomic approaches (45). Few large-scale metaproteomic studies have been published due to the challenges involved with isolating and identifying all expressed proteins in environmental samples (46), but as new metaproteomic techniques overcome these technological hurdles, metaproteomic approaches can also be applied to cheese microbial communities.

BIOGEOGRAPHIC-SCALE STUDIES

In addition to making rapid advancements in our understanding of the local diversity and function of microbial communities using systems biology approaches, microbial ecologists have begun to explore how microbial diversity and function is structured across multiple spatial scales. Recent studies have clearly demonstrated that the diversity and function of microbial communities are not homogenous over space, even at incredibly small spatial scales in what appear to be homogenous environments (47, 48, 49, 50). At the species level, it has also become clear that some microbial species have distinct biogeographic ranges, just like plants and animals (49). Theoretical approaches are being developed to understand the factors that determine the biogeography of microbes (51).

How can these new advances in microbial biogeography advance our understanding of the diversity and function of cheese microbial communities? Knowledge of how cheese microbial communities are structured over large biogeographic scales may reveal whether microbes contribute to the unique flavor development of cheeses in particular biogeographic regions. It is widely assumed that microbial communities of cheeses are different from one cheesemaker to another and that these biogeographic differences in microbial communities contribute to the terroir of cheese. However, our understanding of the biogeography of cheese microbial communities is surprisingly limited. Numerous studies have described the diversity of microbes on cheese from different regions (52), but most of these studies used vastly different techniques, making it impossible to directly compare diversity of microbes between regions. Large-scale surveys of cheese microbial communities from across many biogeographic regions are needed to uncover the biogeography of cheese microbes.

SUMMARY

Only recently have tools been available for the study of microbial communities that allow the collection and integration of data spanning molecular biology to ecosystem function. Due to their relative simplicity and reproducibility, cheese microbial communities are a very attractive system for applying these new technologies, and they therefore represent an emerging model system for the field of ecosystems biology. The outcome of the types of studies proposed above will represent a significant advance in the understanding of how microbial communities and ecosystems are formed, how they function, and if there are any underlying rules or principles that govern this process. These principles would not be specific to just the cheese environment but, as with any good model system, could illuminate the underpinnings of these processes elsewhere in nature. In addition to serving as a model system for understanding microbial communities, the study of cheese microbiology in such a way should lead directly to insights applicable to every step of the cheesemaking and aging process.

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REFERENCES


