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Microbiome Assembly in Fermented Foods

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Abstract

For thousands of years, humans have enjoyed the novel flavors, increased shelf-life, and nutritional benefits that microbes provide in fermented foods and beverages. Recent sequencing surveys of ferments have mapped patterns of microbial diversity across space, time, and production practices. But a mechanistic understanding of how fermented food microbiomes assemble has only recently begun to emerge. Using three foods as case studies (surface-ripened cheese, sourdough starters, and fermented vegetables), we use an ecological and evolutionary framework to identify how microbial communities assemble in ferments. By combining in situ sequencing surveys with in vitro models, we are beginning to understand how dispersal, selection, diversification, and drift generate the diversity of fermented food communities. Most food producers are unaware of the ecological processes occurring in their production environments, but the theory and models of ecology and evolution can provide new approaches for managing fermented food microbiomes, from farm to ferment.

Contents

1. INTRODUCTION

Fermented foods and beverages rely on the growth of microbial communities to produce diverse flavors and aesthetics (63, 92). Various types of microbial cultures and different combinations of plant and animal materials have been used to create hundreds of different ferments around the world for many centuries (65, 102). Not only do these foods play key economic and cultural roles in many societies (1, 29, 89, 93), but they also have the potential to provide a range of health benefits (62–64, 111).

With the widespread application of high-throughput sequencing, a deluge of descriptive microbiome studies have cataloged the microbial diversity of fermented foods made around the world (2, 12, 13, 25, 26, 46, 57–61, 75, 82, 86, 91, 106, 109, 116). This foundational work has been helpful in determining what bacteria, fungi, and other microbes live in these microbial foods and how microbial composition of fermented foods correlates with specific food production techniques or parameters. Historically, each of these studies focused on individual foods produced in specific regions and used just a single technique to describe fermented food microbial diversity (14, 15, 28). More recently, larger-scale studies have attempted to provide more comprehensive surveys (tens or hundreds of samples from many geographic regions) of the full spectrum of fermented food diversity and/or have used more integrative approaches that combine genomics, metabolomics, sensory tools, and experimental manipulations (6, 40, 54, 55, 58, 113, 115).

One major gap in our current understanding of fermented food microbial diversity is the lack of a mechanistic and predictive framework for how microbiomes in fermented foods assemble. We

have a very strong understanding of the patterns of microbial diversity in fermented foods, but we do not understand what ecological and evolutionary processes control the formation of most fermented food microbiomes. Much of the vast literature on fermented food microbes has taken a food technology approach, where specific process parameters or ingredients are manipulated to understand how to control food quality (43). Many of these studies have not explicitly considered the natural history, ecology, and evolution of the microbes in fermented foods to dissect patterns and processes in these microbiomes. Our main argument in this review is that tools and concepts from ecology and evolution can provide novel and synthetic frameworks for understanding and managing fermented food microbiomes.

To help build a mechanistic and generalizable understanding of how fermented food microbiomes assemble and function, several ecological and evolutionary approaches have emerged in fermented food research over the last decade (8, 22, 33, 35, 38, 114). Our goal is to synthesize major findings from these approaches and highlight some key open questions in the ecology and evolution of fermented food microbial communities. Using a widely adopted community assembly framework (73, 110), we aim to answer the following questions:

- How do top-down approaches used by fermented food producers (recipes, technical specifications, etc.) drive the assembly of microbial communities in fermented foods?
- What are the most important ecological and evolutionary processes shaping fermented food microbiome diversity?
- How do these processes vary across different types of fermented foods?
- How can we use ecological processes to control the quality and safety of fermented foods?

Our goal is to create a shared understanding and vocabulary among microbiologists, ecologists, evolutionary biologists, food scientists, and fermented food producers. We hope to facilitate the application of ecological principles to fermented food production and the use of fermented food systems to learn general principles of microbial community assembly.

Before we review what is known about microbiome assembly in fermented foods, we need to clearly define several terms that will be used throughout this manuscript. We adopt the International Scientific Association for Probiotics and Prebiotics consensus statement definition of fermented foods as "foods made through the desired microbial growth and enzymatic conversions of food components" (63). The fermented food microbiome includes all microbes (bacteria, fungi, viruses, and other types of microbes) that live in and on the food throughout the whole life of the product, from raw ingredients to final consumed food.

The key ecological term that we will use throughout this manuscript is community assembly or microbiome assembly. Community assembly refers to the processes that determine the types of organisms and their abundances within a community. One component of community assembly is the species pool, the collection of all species available to join a community. In fermented foods, the species pool can consist of microbes in the raw ingredients, the food production environment, and perhaps even the people producing the food (12, 30, 70, 84). The other main component of community assembly is environmental filters: abiotic factors like temperature and pH that can determine what species from the species pool can persist in a habitat.

The community assembly framework that we will apply to fermented food microbiomes was developed by Mark Vellend (110) and has been widely adopted by microbial ecologists (73). This framework consists of four main processes: dispersal, selection, diversification, and drift (**Figure 1**). Dispersal includes the various processes that impact how propagules move from a species pool and establish within a microbial community. Selection includes how both the abiotic environment and biotic environment (microbial interactions) can impact the fitness of individual species and ultimately shape species abundances within communities. Diversification is the

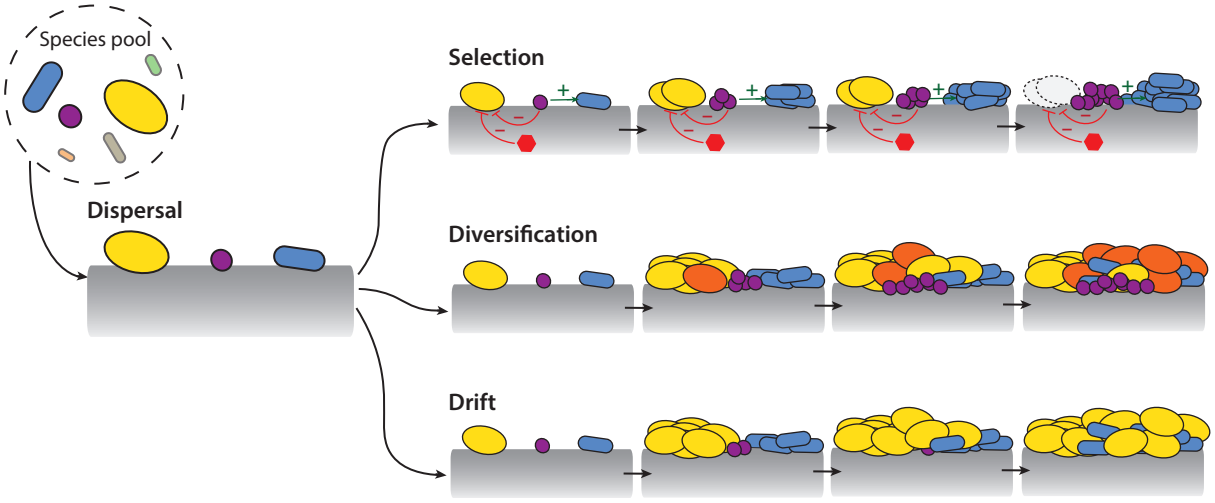

Figure 1

Dispersal, selection, diversification, and drift shape the diversity of ecological communities, including fermented food microbiomes. This framework is based on processes described in References 73 and 110 and applied here to fermented food microbiomes. Round shapes of different colors represent cells of different microbial species. In dispersal, microbial cells arrive at the fermented food substrate from a broader species pool. In selection, the abiotic environment (nutrients and stressors, represented by red hexagons) and biotic environment (microbial interactions, as indicated by lines with plus and minus signs) combine to impact the fitness of individual cells and subsequently the growth or decline of populations. In diversification, novel genetic and/or phenotypic lineages arise from within the local community (indicated by the emergence of the orange-colored, oval cells from initial genotypes of the yellow-colored, oval cells). Drift causes stochastic changes in species abundances within microbial communities and may explain why some rare microbes go extinct within ferments.

generation of new genetic diversity within microbial communities that can shape community composition. Drift, perhaps the most elusive and hard to measure of the four processes, is the change in microbial species abundances within communities over time due to demographic stochasticity.

2. THREE FERMENTED FOODS SERVE AS MICROBIOME ASSEMBLY CASE STUDIES

This review will not comprehensively describe the diversity of fermented food microbiomes, as this has been done elsewhere (34, 41, 42, 85, 105–108). Instead, we will focus on microbial communities of three different fermented foods to illustrate various microbiome assembly concepts: fermented vegetables, surface-ripened cheeses, and sourdough starters (**Figure 2**).

Fermented vegetables are produced when vegetables are chopped up, salted, and placed into anaerobic conditions (**Figure 2*a***). These are some of the easiest ferments because no advanced equipment is needed and specialized starter cultures are not required. Lactic acid bacteria that naturally occur on the vegetables (known as the phyllosphere microbiome) are enriched in the anaerobic conditions and produce organic acids that acidify the fermentation environment. A huge range of vegetables are fermented, but some of the most widely consumed products, including sauerkraut and kimchi, have a large proportion of cabbage as their base ingredient (27). While various genera of lactic acid bacteria are the dominant members of most fermented vegetables, yeasts can sometimes be found in these products (19, 50, 95). The main nutrients available for microbial fermentation are carbohydrates (fructose, galactose, etc.) released from plant cells during preparation of the ferment. Salt and acidification by lactic acid apply strong abiotic selection to

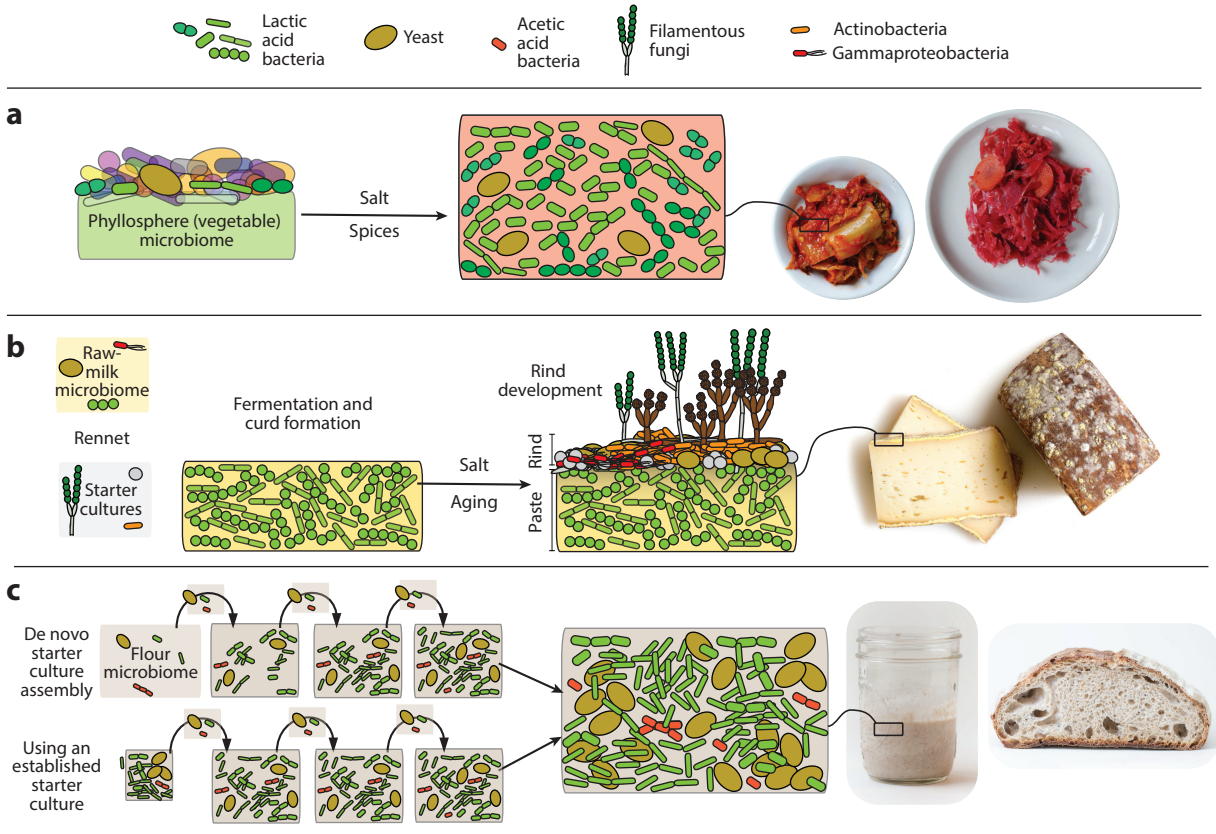

Figure 2

Microbiomes of fermented vegetables, surface-ripened cheeses, and sourdough serve as case studies to understand microbiome assembly across fermented foods. (*a*) Vegetables with lactic acid bacteria living in the phyllosphere are chopped up and placed in an anaerobic environment with salt and spices to create kimchi, sauerkraut, and other fermented vegetables. (*b*) In the production of surface-ripened cheeses, milk, rennet, and cultures are mixed together to first transform milk into curds. These curds are then aged to allow a rind community to form on the surface. (*c*) Sourdough starters can be formed in two ways. De novo starters develop from the dormant microbial cells present in flour. When water is added to the flour and it is repeatedly subcultured, a community of lactic acid bacteria, yeasts, and acetic acid bacteria develops. Alternatively, an already established starter culture community can be used and maintained by repeatedly adding it to fresh flour to provide new carbohydrates and other nutrients to fuel microbial fermentation. Photos of fermented vegetables in panel *a* by Benjamin Wolfe. Photo of the cheese in panel *b* reproduced with permission from Adam DeTour. Photos of the sourdough starter and bread in panel *c* reproduced with permission from Lauren Nichols.

limit the growth of undesirable bacteria. After initial mixing of ingredients, most fermented vegetables are closed fermentations, limiting opportunities for additional dispersal after fermentation has initiated.

Surface-ripened cheeses, such as Camembert, Limburger, and clothbound Cheddars, are aged to intentionally grow a microbial biofilm on their surface. After milk has been fermented by lactic acid bacteria and the resulting curds are shaped into a form, the cheese is salted and placed in an aging facility (cave or other climate-controlled environment) where a community of bacteria, yeast, and filamentous fungi forms an aerobic aging community on the cheese surface (**Figure 2*b***). This microbiome decomposes the cheese curd and through enzymatic activity and other processes adds additional flavor as the cheese ripens for weeks, months, or sometimes years. Cheese makers

can use a mix of industrial starter cultures to inoculate cheeses to grow defined microbes in their products. Cheese rinds are relatively open systems where microbes in raw milk or in the aging environment can easily colonize the cheese surface and establish in a rind community. A wide range of community types have been characterized for surface-ripened cheeses, ranging from just a few taxa in some highly inoculated cheeses to more than 20 species in some washed-rind cheeses (44, 94, 113). Most easy-to-metabolize carbohydrates are scarce after the lactic fermentation, and microbes compete intensely for other resources including amino acids, lipids, and iron (68). Salt provides a strong abiotic selection mechanism that only allows salt-tolerant microbes to flourish (68).

Sourdough starters form when flour is made into a wet dough and a microbial community is enriched through successive rounds of refreshing the dough with flour (**Figure 2*c***). These starters contain lactic acid bacteria, acetic acid bacteria, and yeasts that produce organic acids, carbon dioxide, and other metabolites that give sourdough unique flavor and structural properties. Many bakers obtain an established sourdough starter from a colleague or commercial source, leading to the widespread dispersal of sourdough microbial communities across wide geographic distributions (55). De novo sourdough starter community formation is also possible because viable yeast and lactic acid bacteria cells are present in raw flours. Once a starter community is established, the sourdough microbiome tends to be a relatively closed system where the same microbial community is propagated repeatedly. Sourdough starter communities range from two to ten species, with many reproducible community types forming across wide geographic regions (55). Variation in sourdough community structure has been correlated with functional properties of the bread, including aromas, dough rise, and bread structure (55, 87, 112). Competition for carbohydrates (e.g., maltose) and peptides as well as acid and alcohol production provide strong abiotic selection in sourdough starters (26).

All these foods contain multispecies communities with co-occurring bacteria and fungi, but there are some striking contrasts in production processes that could impact how their communities assemble. For example, the "openness" of each community, based on how they are aged or produced, can affect dispersal of microbial cells into the system. Fermented vegetables and cheese rinds generally experience a single round of community formation, whereas sourdough is repeatedly subcultured as the starter is fed and maintained over many generations. Repeated rounds of community assembly have the potential to impact diversification processes that may not happen in the short timescales of cheese and fermented vegetable production. Each of these varying properties will be explored in each microbiome process described below.

3. MICROBIAL DISPERSAL IN FERMENTED FOODS

3.1. Dispersal of Starter Cultures and in Spontaneous Ferments

The first step in fermented food microbiome assembly is the introduction of microbes to the substrate via immigration. Dispersal of microbes into fermented foods can be facilitated and manipulated through inoculation with starter cultures. In some cases, fermenters control dispersal through mixing individual microbial species with known abundances in industrial starter cultures. For example, in the production of surface-ripened cheeses, industrial starter cultures with pure cultures of bacteria, yeasts, or molds can be added to help improve ripening (5, 76). Alternatively, dispersal may be manipulated using starters of unknown composition that have produced desired ferments in the past, as is the case for sourdough.

In contrast to heavily inoculated ferments, spontaneous ferments rely on uncontrolled immigration from any number of species pools. Before the advent of industrial starter culture production, many ferments were spontaneous and relied on the naturally widespread distribution

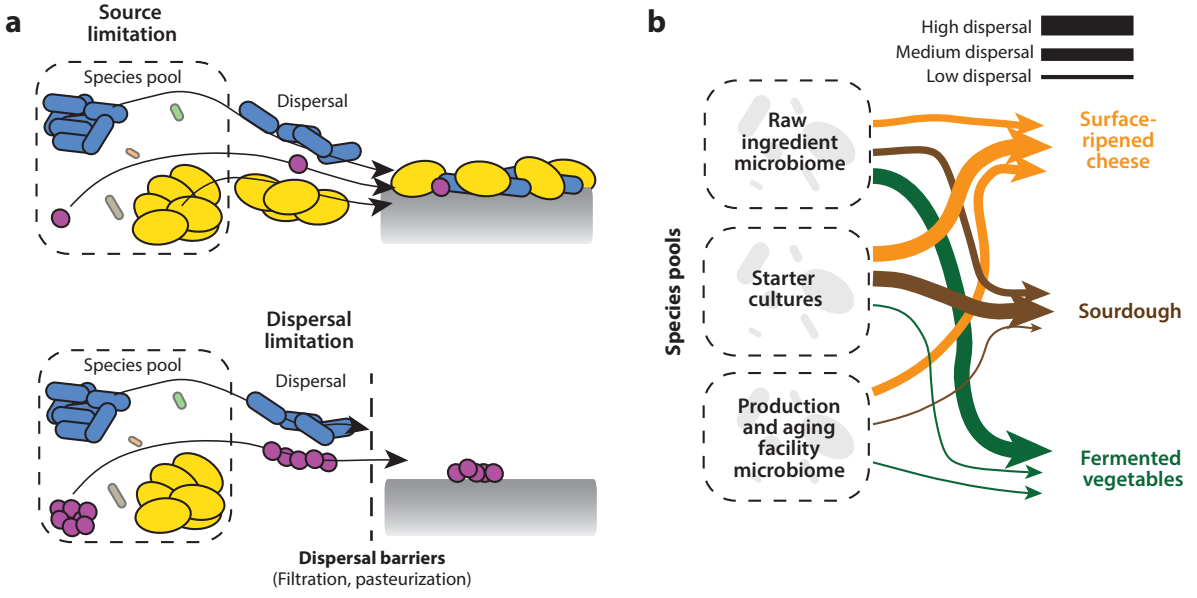

Figure 3

Dispersal processes in fermented food microbiomes. Circular shapes of different colors represent cells of different microbial species. (*a*) Both source (*top*) and dispersal (*bottom*) limitation can explain the abundances of microbes in fermented food microbiomes. In source limitation, there is a low abundance of propagules in the species pool, causing limited dispersal to the fermented food environment. In dispersal limitation, dispersal agents (humans, wind, animals, etc.) are not available to move propagules of a species from a species pool to a fermented food substrate. (*b*) Different species pools that can serve as sources of fermented food microbiomes and their relative contributions to cheese, sourdough, and fermented vegetable microbiome assembly. Figure adapted from Reference 66.

of microbes in species pools and their dispersal to fermentation substrates (106). Most fermented vegetables, such as sauerkraut and kimchi, are still often produced using spontaneous fermentation (78, 106). In these ferments, raw materials are gathered and processed and the microbes that live on those materials serve as the inoculum for fermentation. Spontaneous ferments can also rely on dispersal from species pools beyond raw ingredients (69).

3.2. Propagule Limitation as a Dispersal Framework for Fermented Foods

One useful framework for dissecting dispersal in fermented foods is propagule limitation, where initial community dispersal processes can be broken into two distinct parts: source limitation and dispersal limitation (66) (**Figure 3**). The power of compartmentalizing the dispersal process is that it enables researchers and food fermenters to pinpoint exact areas where this process could influence microbial community structure (20, 66, 81). In this review, propagules are microbial cells, spores, or any microbial entity that is capable of dispersal and regrowth from a parent population into a new habitat.

Source limitation occurs when a low abundance or a complete absence of propagules in feeder communities (species pools) limits the abundance of microbial species in a particular habitat (**Figure 3*a***). Source limitation is particularly important for fermented foods that are spontaneously fermented, because the fermentation of those foods relies on microbes naturally present in the environment. For example, a recent study from our group provided evidence for source limitation in vegetable fermentation (66). When we surveyed various farms throughout New England for the presence of lactic acid bacteria that drive fermentation of vegetables, we found that they

are generally quite rare in local species pools and are variable from one farm to another. This work suggests that source limitation of lactic acid bacteria can lead to variable fermentation outcomes, depending on the specific microbiome of the farm environment. The widespread use of large quantities of starter cultures in many ferments circumvents the challenges of source limitation.

Characterizing and managing the species pools that contribute to specific fermented foods can be essential for understanding how source limitation would influence community structure (**Figure 3*b***). Much of the research on where microbes are present in fermented food environments has focused on pathogens as part of food safety surveillance programs (23, 72, 77). This is critical for maintaining safe food production practices, but ignoring the beneficial microbes in fermentation environments means we have a limited understanding of how microbes disperse to and among ferments. The source-tracking approach developed for pathogens has helped to begin to fill this gap. For example, a study of a cheese aging facility in California mapped the microbial communities throughout the facility and the final cheese product and determined that microbes in the production environment were abundant on the surfaces of washed-rind cheeses (14). Similarly, a study of a fermented vegetable production facility determined that the raw cabbage– and vegetable-handling surfaces could be the main sources of inoculum for the final fermented product (30). This illustrates that unlike classic ecological theory, the species pool reflects not just the surroundings of the fermented food but also the material contributing to the substrate, akin to a seed bank in plant ecology.

Dispersal limitation occurs when dispersal agents (wind, insects, humans, etc.) or actively dispersing microbes (e.g., motile bacteria) are unable to move propagules of microbial species from the species pool to a developing community. Dispersal limitation can occur at multiple scales within ferments. At small scales, microbes may not be able to disperse to all portions within an individual ferment, leading to spatial structure within the final microbial community. An example would be lactic acid bacteria in vegetable ferments not being able to grow throughout a vat of fermenting cabbage, leading to patchiness of acidity and variation in flavor. At medium scales, microbes may not be able to move from a species pool within a production facility to the fermentation substrate. For example, fermented food aging environments use specialized filtration to reduce dispersal of microbes within the production facility.

At large spatial scales, dispersal limitation could be a driver of geographically distinct microbial communities that form across the same type of fermented food product. If some microbial species that impart distinct flavor or other properties in ferments are restricted in their dispersal at large spatial scales, ferments made in particular regions may have unique properties. One way to test for this pattern is to identify whether similarity in community composition is correlated with geographic distances between production locations, commonly called distance-decay relationships in ecology (100). In our own work, we have found limited evidence for dispersal limitation in surface-ripened cheeses and sourdough starters. When we sampled both fermented foods across wide geographic regions, we did not find that community dissimilarity increased with geographic distance between ferments. This lack of large-scale dispersal limitation makes sense considering how the starter cultures used in these ferments are distributed around the globe, which effectively provides a globally shared species pool. In contrast, spontaneous ferments and ferments inoculated with locally enriched cultures show stronger signals of large-scale dispersal limitation. For example, several studies of microbial communities associated with wine grapes have demonstrated regional differences in species and strains of microbes that could contribute to fermentation (16, 37). Similarly, a study of fermented milk matsoni inoculated with undefined cultures that were serially propagated within a facility found distinct bacteria and fungi at broad geographic scales (10).

4. ABIOTIC SELECTION IN FERMENTED FOOD MICROBIOMES

4.1. Abiotic Selection Can Differentially Prevent and Promote Microbes in Fermented Foods

Once microbial propagules have dispersed to a food substrate, abiotic selection plays a key role in structuring microbiome assembly. Abiotic conditions can prevent or facilitate the establishment of newly arrived taxa. For example, salt is a widely used abiotic selection tool that directly limits the growth of many undesirable organisms and promotes the growth of salt-tolerant fermenting microbial species. Abiotic selection can also change species interactions and the outcomes of competition to indirectly shape the community. Because it is relatively easy to manipulate environmental aspects of ferments, abiotic selection is the most widely manipulated and studied component of fermented food microbiome assembly. Salt concentration, temperature, and availability of nutrients such as simple sugars are some of the most common tools producers use to shift community composition in ferments.

Despite this wealth of information on how to control the growth of microbes in ferments, much of this research has been compartmentalized to specific food production processes or environments. Few studies have explored how abiotic selection, from farm to ferment, can shape the assembly of fermented food microbiomes. We argue that abiotic selection varies dramatically based on the timing and location of production. The fermentation process has three broad phases—in the raw ingredient environment, the production environment, and the fermentation and aging environment—that can each provide different abiotic selection pressures that serve as distinct filters for microbes (**Figure 4**). The sequential nature of these phases means that the final fermentation community is a product of all three filters. In the following sections we evaluate the selection pressures provided by each phase.

4.2. Abiotic Selection During Ingredient Production Determines the Microbes Available for Fermentation

Before raw ingredients arrive at a fermentation production facility, abiotic selection can play a role in determining what microbes are available for the fermentation (**Figure 4*a***). As the raw ingredients are grown, processed, packaged, and distributed, they experience various abiotic selection pressures that filter out some microbes and favor others. This process is especially important for plant-based ingredients where microbes associated with plant materials (often lactic acid bacteria) are the main fermentation microbes.

Abiotic selection of ingredient microbiomes can be driven by human activities on the farm where the plant or animal material used for fermentation is grown. For example, in a farm-to-ferment study of sourdough, organic versus conventional farming practices influenced the bacterial communities of wheat milled for flour and ultimately made into sourdough starters (87). The abiotic environments created by these farming practices drove differences in sourdough starter composition and affected key quality properties of the bread, including crust color.

Abiotic selection of fermentation microbes on farms can also be driven by intrinsic processes generally beyond human control. We examined the role of the plant environment on the distribution of lactic acid bacteria on vegetables used for fermentation (66). Despite their critical roles in driving the successful fermentation of sauerkraut, kimchi, and other vegetable ferments, lactic acid bacteria are typically quite low in abundance in the phyllosphere of plants (117). Why they are so rare on plants and how this impacts fermentation outcomes are largely unknown. Using a gnotobiotic cabbage system in which we could inoculate highly controlled combinations of bacteria on plant leaves (67), we determined that most lactic acid bacteria struggled to survive in the phyllosphere when they were grown alone, and many slowly died over time. Other plant-associated

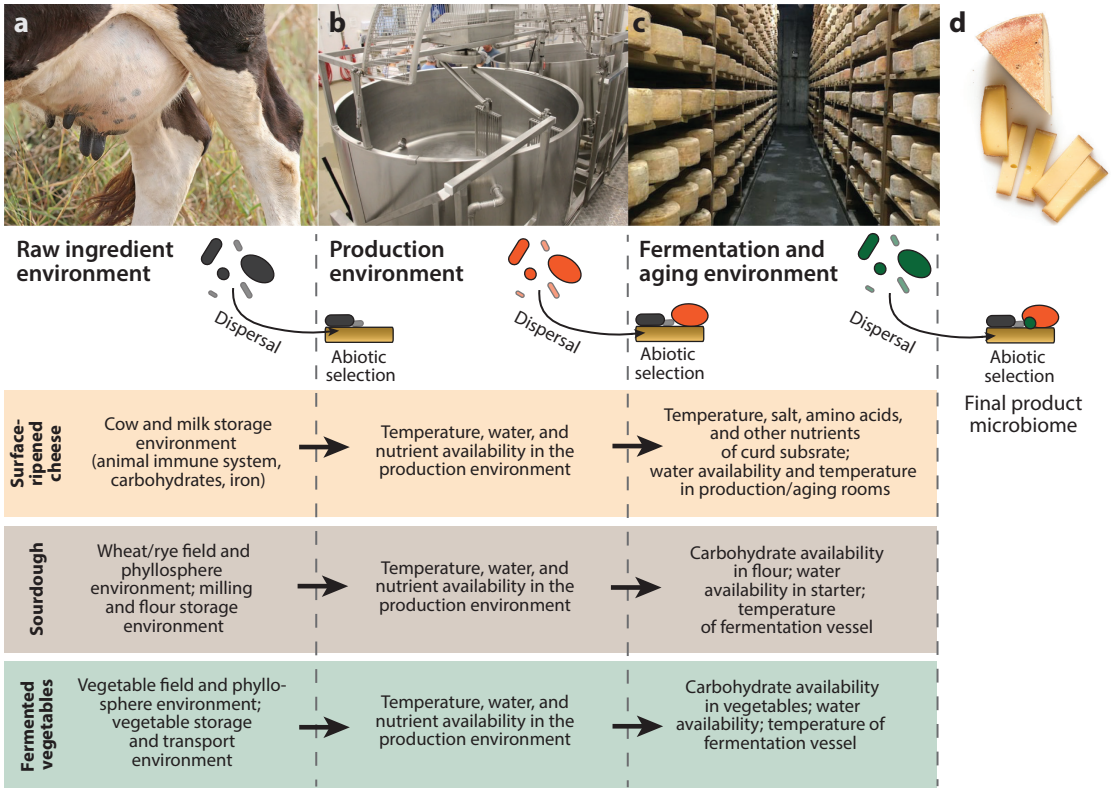

Figure 4

Abiotic selection, from farm to ferment. Using three case studies, this figure illustrates how final microbiomes in fermented foods result from multiple rounds of abiotic selection at different stages in production. We visually illustrate this with surface-ripened cheeses. Note that the abiotic selection examples provided are not comprehensive and may vary across specific products and production practices. Cow teat photo (*a*) by Muhammad Mahdi Karim and used under GNU Free Documentation License. Cheese vat (*b*) and cheese shelf (*c*) photos reproduced with permission from Jasper Hill Farm. Cheese photo (*d*) reproduced with permission from Adam DeTour.

microbes that are adapted to the low-nutrient, dry, and high-light conditions of the phyllosphere grew quite well. These experiments demonstrate that abiotic selection in the phyllosphere environment can shape the pool of microbial species available for spontaneous ferments.

Beyond abiotic selection on the farm, how raw materials are packaged and distributed may impact microbial quality of the raw ingredients. For example, Kable et al. (47) found that raw milk sampled from two dairy farms across seasons hosted a consistent core milk microbiome. Once milk was transported, the abiotic selection pressures accompanying different transportation modes (silos and tanker trucks) dramatically altered milk microbiomes. This milk was not intended for raw-milk cheese production, but the study illustrates how selection during raw ingredient distribution can alter fermentation potential.

Most studies of abiotic selection have focused on the ferment itself, and not the raw materials used for fermentation. As the examples above illustrate, ecological processes can shape the microbial composition of raw materials used in fermentation and that microbial composition can in turn impact fermentation outcomes. Additional studies that consider farm-to-ferment abiotic selection will continue to make these systems-level connections more apparent. These types of studies are

also needed to identify management strategies that can be used to alter the microbial quality of raw ingredients used in fermentation. If we can diagnose the timing and location in production during which microbiomes are most variable, fermented food producers can prioritize controlling abiotic selection pressures at that specific stage to ensure a consistently reproducible product.

4.3. Abiotic Selection in Fermentation Production Facilities Creates a Built Environment Species Pool That Affects Ferments

The second major abiotic filter in the production of fermented foods is determined by the built environment (**Figure 4*b***). The built environment has its own microbiome independent from the raw ingredients and cultures used in fermentation. Microbiome community composition and diversity in such built environments are tightly correlated with abiotic factors like temperature, relative humidity, source of ventilation air, and rate of airflow (52). Prior to production, an existing "house" microbiome already experiences a suite of abiotic selection pressures that determines the local species pool of microbes that can interact with ferments (12).

Several studies illustrate how the built environment microbiome can interact with fermented food microbiomes. In a study of a surface-ripened cheese production facility, environmental organisms found throughout the facility were also common on washed-rind cheeses (14). The investigators found evidence for spatial variation of microbes associated with different niches within the facility, suggesting that different environments in the facility were selecting for specific microbial taxa. A more recent study of a different surface-ripened cheese facility also found that each processing room in the facility harbored unique microbial community compositions, which contributed to the microbes surveyed on ingredients used for cheese production (103). To explore the origin of microbes within a sauerkraut production facility, a recent study found that food surfaces hosted distinct microbial communities relative to different locations within the facility (30). Similar built environment and ferment interactions have been demonstrated for sake and beer (11, 15).

4.4. Both Fermentation Conditions and Substrate Types Determine Microbial Community Composition of Ferments

Once ingredients for a ferment are mixed and placed in a controlled fermentation environment, fermentation conditions (temperature and salt concentration) and substrate (nutrients and other resources available in raw food materials) play the final role in abiotic selection of community composition (**Figure 4*c***). Numerous studies of cheese, fermented vegetables, sourdough, and other ferments have documented how microbial community diversity at the initial stages of fermentation dramatically decreases as selection only allows certain microbes to persist within fermentation communities (31, 32, 118).

Because fermentation substrates (raw food ingredients) affect nutrient availability for microbial growth, they can serve as one of the most important abiotic drivers of microbial community composition across different fermented food products. The types of carbon and nitrogen sources as well as macro- and micronutrients vary widely across the many materials that are fermented, including milk, flour, and fresh vegetables (43). This variation in resource availability selects for microbes that are adapted to efficiently utilize different nutrients. Some of the best data to support the importance of substrate in driving microbial composition in fermented foods come from a recent broad survey of many fermented foods with divergent substrates. Specifically, Leech at al. (58) surveyed 58 fermented food types across multiple countries and categorized them as brine, coconut kefir, dairy, soy, and sugar substrates. They found little evidence for geography explaining

microbial community composition, whereas substrate type, and more specifically its nutrient composition, explained the most variation in fermented food microbial community composition.

At a finer scale within specific fermented food types, abiotic selection due to production practices may play a larger role than substrate in shaping microbial community composition. For example, our lab found that rind communities from cheeses sampled across North America and Europe were consistently dominated by 24 species and that community composition of these species was tightly correlated with cheese rind type—but not geography, milk treatment, or milk source (113). This suggests that abiotic pressures such as temperature during ripening, salt concentrations, and water availability are the most relevant filters for cheese-rind microbial communities. Likewise, Kamimura et al. (49) found that production conditions and starter cultures were the strongest predictors of microbial community composition in cheese.

5. BIOTIC SELECTION IN FERMENTED FOOD MICROBIOMES

5.1. Identifying Biotic Selection in Fermented Foods

While microbes are dispersing to a fermented food and being filtered out by abiotic selection, biotic selection can play an important role in determining winners and losers within fermented food microbiomes. Historically, the role of biotic selection in fermented foods has received less attention compared to abiotic selection. It is much easier to alter salt concentrations or temperature in a ferment than it is to change how and when microbial species interact. However, microbial interactions are increasingly acknowledged as important drivers of microbiome composition and fermented food quality and safety (22, 45, 79, 96, 97, 114). In addition to dispersal and abiotic selection, interactions may provide critical tools for management of fermented food microbiomes.

One reason that biotic selection has received less attention is that it is experimentally more challenging to characterize how biotic interactions alter community assembly in fermented foods compared to abiotic selection. One approach is to use patterns of species co-occurrence across large sequencing surveys to predict which species may have positive or negative interactions. There are many caveats to this approach, including the potential for two species to have a significant pattern of occurrence across many fermented foods simply because they have the same abiotic niche (22). However, survey-based approaches can help generate hypotheses about species interactions for targeted manipulative studies. In our work with both surface-ripened cheeses and sourdough starters, we observed striking patterns of co-occurrence from metagenomic sequencing surveys that we were able to recapitulate in lab experiments with cocultures (51, 55, 113).

Another more intensive approach is to simply measure the outcomes of experimental interactions between microbial species as a screen (8). By growing microbes in pairwise combinations or as whole communities where microbes are added or removed, it is possible to identify microbial species with strong interaction phenotypes that can alter microbiome assembly. Because we have a good understanding of the nutrients and growth factors required by many of the microbes in fermented foods, most can be readily cultured in the lab for these types of interaction studies. This approach does not necessarily start with in situ patterns of diversity, and these interactions measured in the lab may not translate to the real world of food production. But it can pinpoint the biotic levers within communities that could be used by food producers to manipulate communities and alter product quality.

5.2. Direct and Substrate-Mediated Interactions in Fermented Foods

We find it helpful to divide microbial interactions in fermented foods into two broad categories: direct interactions and substrate-mediated biotic interactions (**Figure 5**). Direct interactions

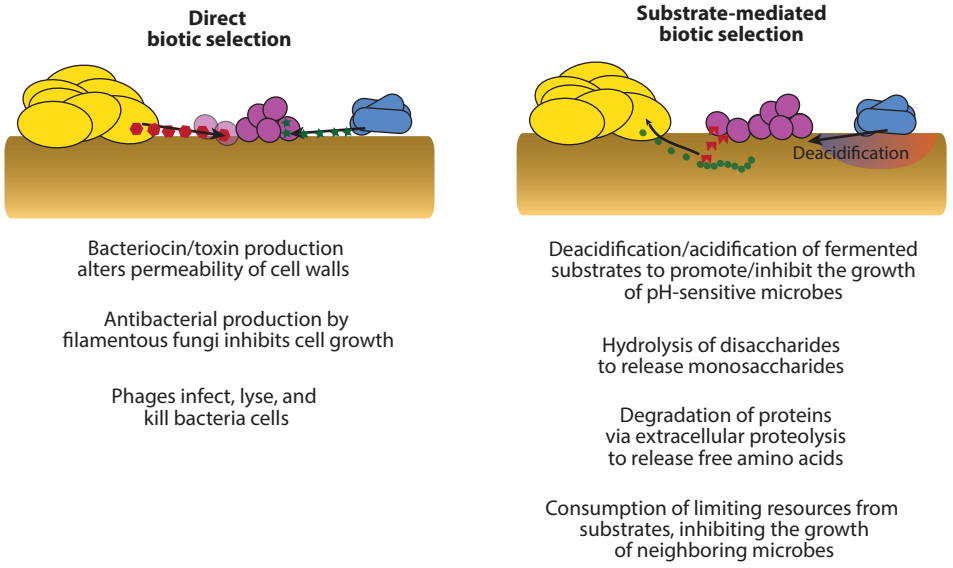

Figure 5

Biotic selection in fermented foods. Two broad categories of biotic selection—direct and substrate-mediated—dominate the types of interactions between species in fermented foods. Substrate-mediated interactions are especially important in fermented foods where microbes are breaking down and transforming raw food substrates during fermentation. The green circles represent a resource being broken down into available subunits via an enzyme pictured by red triangles on the right. A variety of examples illustrate the two broad types of biotic selection. These are not exhaustive lists. Circular shapes of different colors represent cells of different microbial species. Red hexagons and green stars indicate potential negative and positive mediators of direct microbial interactions.

include cases where a microbe produces a metabolite or other substance that directly inhibits or promotes the growth of a neighboring microbial species. The outcome of the interaction is not dependent on the producer microbe modifying the environment because the metabolite is produced directly by its cells and directly interacts with neighboring cells. A classic example of this is the production of bacteriocins by lactic acid bacteria that cause damage to the cell membranes of neighboring bacteria (24, 53).

Substrate-mediated interactions (sometimes referred to as indirect interactions) occur when a microbial species alters the environment in a manner that impacts the fitness of neighboring microbial species. In these cases, there is no direct secretion of chemicals that directly alter the growth of other microbes. Instead, a microbe produces a metabolite or grows in a particular way that alters the environment and that subsequently can change the growth of other microbes. A classic example of substrate-mediated interactions in fermented foods is the secretion of extracellular enzymes that break down the food substrate and release nutrients that other microbes can use (51).

Distinguishing between these two types of interactions is important for understanding how biotic interactions can impact microbiome assembly in fermented foods. Direct interactions tend to be specific and can impact only part of the microbial community. For example, bacteriocins produced by lactic acid bacteria in vegetable ferments often target a narrow spectrum of microbes and would only negatively impact a subset of the community. In contrast, substrate-mediated interactions are often more generalized and can impact a wide swath of microbial diversity in ferments. For example, the deacidification of cheese curd by fungi in surface-ripened cheeses promotes the

growth of a range of *Proteobacteria*, *Actinobacteria*, and other pH-sensitive bacteria (71, 113). The breakdown of proteins and release of aminos acids by extracellular proteases can positively impact a range of species in sourdough, cheese, and other ferments (36, 51).

5.3. Advancing Biotic Selection Research in Fermented Foods

A major limitation of biotic selection research in fermented foods is that most work (including most of our own research) has focused on the causes and consequences of pairwise interactions, and not all potential interactions within a community. A main reason for this bias is that mapping out interaction networks within microbial communities is simplified by focusing first on how pairs of species affect each other's fitness. But when whole fermented food communities are assembling in real-world ferments, many species are interacting with one another simultaneously, and higher-order interactions may completely change the outcomes of pairwise interactions (104). In order to make biotic selection a useful tool for managing ferments, we need to understand how interactions unfold in more complex communities (79).

To help advance biotic selection research in ferments, one approach is "leave-one-out" or species addition types of experiments. By constructing experimental communities that exclude or include individual species one at a time, we can measure the resulting impact of a species on the rest of the community (71). This helps to quickly identify highly impactful or interactive species that could have major roles in shaping the entire microbiome assembly process, as well as capturing the consequences of both direct and indirect interactions. For example, when we added or subtracted different fungal species from experimental cheese rind communities, we found that *Proteobacteria* species were often the most responsive to the presence of certain fungi (21, 119).

Another major gap in our understanding of biotic selection in fermented foods is the mechanisms underlying most interactions. We frequently observe that one species alters the growth of another species or a whole fermented food community, but we typically do not understand what is driving these interaction outcomes. With mechanistic insights we can potentially engineer better ferments that contain species with compatible biotic selection. For example, building on the "leave-one-out" type of experiments described above with methods like RNA sequencing or random barcode transposon site sequencing, it is often possible to identify the molecular or cellular underpinnings of microbial interactions. In our own work with cheese rinds, we have used these approaches to determine that flagellar motility in bacteria is critical for their growth promotion by filamentous fungi (119) and that fungi relieve bacteria of amino acid and iron limitation (51, 80).

6. DIVERSIFICATION OF MICROBIAL SPECIES AND COMMUNITIES IN FERMENTED FOODS

As dispersal introduces populations from the species pool and selection filters some of them out, diversification can add novel genetic and phenotypic diversity to a community, generally on a longer time frame. When new mutations arise and are established, the community of a ferment no longer reflects merely a filtered subset of the local species pool. Many fermented food microbial communities are reassembled from the same inoculum source. For example, sourdough starters are maintained over months or years by repeatedly refreshing the dough nutrients by adding fresh flour. This repeated subculturing of the entire microbial community also happens with many other ferments, including kombucha, kefir, vinegar, and many others (8, 54). The sustained growth of microbial populations in relatively stable environments with ample resources creates the potential for microbes to adapt to the fermented food substrate and generate new strains with novel ecological functions. Microbial adaptation to fermented food environments may have caused the domestication of numerous microbes used in fermentation (38).

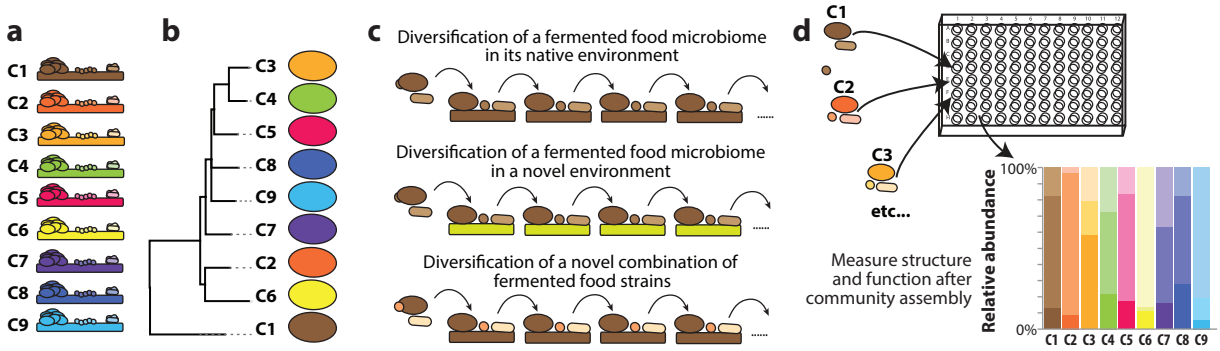

Figure 6

Approaches for measuring diversification of fermented food microbiomes. (*a*) Taxonomically similar fermented food microbial communities (represented by C1–C9) may exist in unique production facilities. In this scenario, there are nine communities that each have the same three microbial species. (*b*) Comparative genomic approaches can help highlight genomic diversification of individual microbial strains from across ferments. (*c*) Experimental evolution has the potential to illustrate the mechanisms of whole-community diversification within fermented foods. Different types of whole-community evolution experiments could be used to understand diversification mechanisms in ferments, including adaptation to native or novel substrates and adaptation with novel combinations of strains (strains originating from different locations or products). (*d*) Assembling experimental ferments from distinct microbial strains can help illustrate the consequences of diversification within ferments.

Despite this great potential for evolution within fermented food microbial communities, we are unaware of studies that have tracked the genomic or phenotypic evolution of fermented food microbes in situ. This would require repeatedly sampling the same fermented food community over many months or years as it was being used in food production. There are many challenges of this approach, including costs of lost product by the producer and difficulties capturing the best temporal and spatial scales of sampling. To overcome these issues, several other approaches have been used to provide evidence for diversification within fermented food microbiomes.

One widely used observational approach to study diversification is to measure the genomic and phenotypic diversity of strains within a microbial species and correlate that with abiotic properties of the fermented food environment (**Figure 6*a*,*b***). Numerous studies have revealed extensive strain diversity within widespread fermentation microbes such as *Saccharomyces cerevisiae* (33), *Penicillium roqueforti*, *Penicillium camemberti* (90), *Debaryomyces hansenii*, *Aspergillus oryzae* (39), *Lactiplantibacillus plantarum* (98), *Fructilactobacillus sanfranciscensis* (88), and many others. These comparative genomic and phenotypic studies have demonstrated the key traits that have allowed each of these species to thrive in fermented food substrates. This type of research reveals that diversification has happened, but it does not reveal specific ecological or evolutionary mechanisms that have facilitated the diversification process.

Experimental evolution offers an alternative approach that can reveal mechanisms of diversification within fermented food microbial communities. By experimentally passaging fermented food microbes in different abiotic conditions in a simulated fermentation environment, these studies have revealed the phenotypic and genomic mechanisms of adaptation to fermented food substrates (3, 4). Because new strains that are better adapted to a substrate are likely to outcompete their progenitors, these experiments are likely to capture joint diversification and selection in the form of widespread loss or gain of traits. In our own work with *Penicillium* molds from surface-ripened cheeses, we were able to observe rapid (over just a few weeks) loss of many traits (toxin production, pigment production, sporulation) in a strain of *P. biforme* as it was repeatedly subcultured on cheese curd media (9). This experimental evolution process mimics the predicted

domestication of molds used in Camembert production and revealed underlying metabolic and transcriptomic mechanisms that could explain fungal adaptation to the cheese environment.

While most work on diversification in ferments has studied individual microbial species in isolation, species in a community evolve in the context of one another. The mutations that persist as species evolve depend on the effects of other community members. By exposing ferment communities to novel substrates or novel combinations of strains that originated from divergent products, whole-community evolution experiments have the potential to reveal how diversification operates within ferments at the community level (**Figure 6*c***). We are unaware of any studies using this approach in fermented foods, but several studies outside of fermentation have adapted whole communities to novel environments (17, 56, 83). In these experiments, interactions among species and whole-community functions often shift during evolution, including the strength of species interactions and how resources are utilized (56, 83). A directed evolution approach like this may be useful for developing whole fermented food communities that could have novel quality and safety functions (18). For example, evolving dairy starter culture communities to grow on plant "milk" substrates could provide novel starter communities for emerging plant-based cheese products.

Another largely unexplored area in the diversification of fermented food microbiomes is how this diversification can impact microbiome assembly. Most studies in this field have focused on the evolution of an individual species and how adaptation impacts the traits of that species. We are not aware of many studies that have examined how genetic and phenotypic changes resulting from diversification can have ecological impacts on microbiome assembly. A recent study from our group demonstrated how in situ diversification of cheese rind communities can impact microbiome assembly and function (74). We isolated the same three species of bacteria from nine geographically isolated natural-rind cheeses (**Figure 6*d***). When we assembled those nine bacterial communities in a standard lab cheese environment, we observed divergent community compositions and functions of the communities despite identical initial starting conditions (identical cell input densities, identical cheese substrate properties). This demonstrates that as taxonomically identical communities of microbes diversify in different fermented food production environments, they can shift in their assembly dynamics and how they impact the aesthetics and quality of the fermentation. Future studies that use experimental evolution to recreate this community-level diversification will better understand how whole-community evolution has contributed to the unique properties of fermented foods produced in different regions.

7. ECOLOGICAL DRIFT IN FERMENTED FOODS

Drift—changes in population abundance due to the randomness of births and deaths—is perhaps the most difficult process to study in fermented food microbiome assembly. In the absence of selection (i.e., if all species were functionally identical), drift would be the only source of changes in relative abundance of species. When species become functionally distinct, however, the effect of drift diminishes. Trying to experimentally manipulate and measure random births and deaths of species within communities is remarkably challenging. The interaction between drift and other ecological processes makes it hard to disentangle when ecological dynamics within communities are driven by stochastic processes like drift or deterministic processes like abiotic selection (101). By its very definition, drift is unpredictable and is therefore not something that most fermented-food producers may want to occur in their ferments.

Drift has the largest impact when populations are small, i.e., when individual random births and deaths represent larger changes in relative abundance. This means that drift should have a greater impact on community structure during establishment of a new ferment community (when

all populations are small) and may cause the stochastic extinction of seemingly viable species or strains immediately after dispersal or diversification (when that individual population is small) (11, 70). For example, in sauerkraut, kimchi, and other fermented vegetables, the rare and variable abundances of lactic acid bacteria on raw vegetables may cause them to be especially subject to drift in the early stages of fermentation (66). Drift may also impact the composition of ferments that are serially passaged and experience strong population bottlenecks, such as sourdough starters.

To help us better understand the potential contribution of drift in structuring ferment communities, high-resolution time series data from simple ferments are needed. We are unaware of any studies that have followed the same ferment for many generations and tracked species composition over time. If these data from a sourdough or other serially passaged ferment were available, they could then be used in combination with models of drift developed for microbial communities (99).

8. CONCLUSIONS

As we have illustrated above, the dispersal-selection-diversification-drift framework is a useful approach for illuminating the ecology and evolution of fermented food microbiomes. But how can food producers use this framework to manage and manipulate the quality and safety of their products? In addition to very specific examples highlighted throughout the review, the framework has several general applications.

First, this framework can be especially helpful in diagnosing and managing microbial defects in fermented food products. When recipes and quality protocols are followed, most batches of ferments are high quality and contain microbial communities that are considered desirable. But with some frequency, batches of ferments cannot meet consumer expectations and never make it to market (7). In our own work in our lab, we have used the dispersal-selection-diversification-drift framework to solve economically devastating microbial defects. After we identify the microbes that are putative agents of defects in ferments (48), we then consider how all four processes may explain why the microbes in question have become problematic in a product or production facility. For example, we survey species pools throughout the production pipeline to identify how new dispersal processes may be contributing undesirable microbes. We also consider how abiotic or biotic selection might be used to decrease the abundance of microbes associated with quality defects.

Second, by understanding the mechanisms of microbiome assembly, we can also use ecology and evolution to develop novel fermentations. For example, if we want to add novel microbial species to traditional ferments to alter their aesthetics or flavor, we need to understand the ecological and evolutionary constraints operating within that ferment. What abiotic selection pressures will promote or inhibit the establishment of novel species? Are there ways that we can promote diversification of existing strains to facilitate the addition of novel species?

Finally, a strong ecological and evolutionary framework can also provide a conceptual and theoretical foundation for preserving fermented food microbial communities. With new technologies and shifting climates, the biodiversity of microbes in traditional ferments may be changing. By integrating surveys of microbial diversity of previously unexplored ferments with mechanistic studies that identify how fermented food microbial communities assemble, we can develop microbiological, social, and political frameworks that can maintain these economic and culturally significant food microbiomes.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Adesulu A, Awojobi KO. 2014. Enhancing sustainable development through indigenous fermented food products in Nigeria. *Afr. J. Microbiol. Res.* 8:1338–43
2. Arora K, Ameur H, Polo A, Di Cagno R, Rizzello CG, Gobbetti M. 2021. Thirty years of knowledge on sourdough fermentation: a systematic review. *Trends Food Sci. Technol.* 108:71–83
3. Bachmann H, Molenaar D, Branco Dos Santos F, Teusink B. 2017. Experimental evolution and the adjustment of metabolic strategies in lactic acid bacteria. *FEMS Microbiol. Rev.* 41(Suppl. 1):S201–19
4. Bachmann H, Starrenburg MJC, Molenaar D, Kleerebezem M, van Hylckama Vlieg JET. 2012. Microbial domestication signatures of *Lactococcus lactis* can be reproduced by experimental evolution. *Genome Res.* 22(1):115–24
5. Beresford T, Williams A. 2004. The microbiology of cheese ripening. In *Cheese: Chemistry, Physics and Microbiology*, Vol. 1: *General Aspects*, ed. PF Fox, PLH McSweeney, TM Cogan, TP Guinee, pp. 287–317. London: Academic
6. Bertuzzi AS, Walsh AM, Sheehan JJ, Cotter PD, Crispie F, et al. 2018. Omics-based insights into flavor development and microbial succession within surface-ripened cheese. *mSystems* 3(1):e00211-17
7. Biango-Daniels MN, Wolfe BE. 2021. American artisan cheese quality and spoilage: a survey of cheesemakers' concerns and needs. *J. Dairy Sci.* 104(5):6283–94
8. Blasche S, Kim Y, Mars RAT, Machado D, Maansson M, et al. 2021. Metabolic cooperation and spatiotemporal niche partitioning in a kefir microbial community. *Nat. Microbiol.* 6:196–208
9. Bodinaku I, Shaffer J, Connors AB, Steenwyk JL, Biango-Daniels MN, et al. 2019. Rapid phenotypic and metabolomic domestication of wild penicillium molds on cheese. *mBio* 10(5):e02445-19
10. Bokulich NA, Amiranashvili L, Chitchyan K, Ghazanchyan N, Darbinyan K, et al. 2015. Microbial biogeography of the transnational fermented milk matsoni. *Food Microbiol.* 50:12–19
11. Bokulich NA, Bergsveinson J, Ziola B, Mills DA. 2015. Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. *eLife* 4:e04634
12. Bokulich NA, Lewis ZT, Boundy-Mills K, Mills DA. 2016. A new perspective on microbial landscapes within food production. *Curr. Opin. Biotechnol.* 37:182–89
13. Bokulich NA, Mills DA. 2012. Next-generation approaches to the microbial ecology of food fermentations. *BMB Rep.* 45(7):377–89
14. Bokulich NA, Mills DA. 2013. Facility-specific "house" microbiome drives microbial landscapes of artisan cheesemaking plants. *Appl. Environ. Microbiol.* 79(17):5214–23
15. Bokulich NA, Ohta M, Lee M, Mills DA. 2014. Indigenous bacteria and fungi drive traditional kimoto sake fermentations. *Appl. Environ. Microbiol.* 80(17):5522–29
16. Bokulich NA, Thorngate JH, Richardson PM, Mills DA. 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *PNAS* 111(1):E139–48
17. Castledine M, Padfield D, Buckling A. 2020. Experimental (co)evolution in a multi-species microbial community results in local maladaptation. *Ecol. Lett.* 23(11):1673–81
18. Chang C-Y, Vila JCC, Bender M, Li R, Mankowski MC, et al. 2021. Engineering complex communities by directed evolution. *Nat. Ecol. Evol.* 5(7):1011–23
19. Chang H-W, Kim K-H, Nam Y-D, Roh SW, Kim M-S, et al. 2008. Analysis of yeast and archaeal population dynamics in kimchi using denaturing gradient gel electrophoresis. *Int. J. Food Microbiol.* 126(1–2):159–66
20. Clark CJ, Poulsen JR, Levey DJ, Osenberg CW. 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. *Am. Nat.* 170(1):128–42
21. Cosetta CM, Kfoury N, Robbat A, Wolfe BE. 2020. Fungal volatiles mediate cheese rind microbiome assembly. *Environ. Microbiol.* 22(11):4745–60
22. Cosetta CM, Wolfe BE. 2019. Causes and consequences of biotic interactions within microbiomes. *Curr. Opin. Microbiol.* 50:35–41
23. D'Amico DJ, Donnelly CW. 2017. Growth and survival of microbial pathogens in cheese. In *Cheese*, ed. PLH McSweeney, PF Fox, PD Cotter, DW Everett, pp. 573–94. London: Academic
24. De Vuyst L, Leroy F. 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J. Mol. Microbiol. Biotechnol.* 13(4):194–99

25. De Vuyst L, Van Kerrebroeck S, Harth H, Huys G, Daniel H-M, Weckx S. 2014. Microbial ecology of sourdough fermentations: diverse or uniform? *Food Microbiol*. 37:11–29
26. De Vuyst L, Vrancken G, Ravyts F, Rimaux T, Weckx S. 2009. Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiol*. 26(7):666–75
27. Di Cagno R, Coda R, De Angelis M, Gobbetti M. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol*. 33(1):1–10
28. Dobson A, O'Sullivan O, Cotter PD, Ross P, Hill C. 2011. High-throughput sequence-based analysis of the bacterial composition of kefir and an associated kefir grain. *FEMS Microbiol. Lett.* 320(1):56–62
29. Dunn RR, Wilson J, Nichols LM, Gavin MC. 2021. Toward a global ecology of fermented foods. *Curr. Anthropol.* 62(S24):S220–32
30. Einson JE, Rani A, You X, Rodriguez AA, Randell CL, et al. 2018. A vegetable fermentation facility hosts distinct microbiomes reflecting the production environment. *Appl. Environ. Microbiol.* 84(22):e01680-18
31. Ercolini D, Pontonio E, De Filippis F, Minervini F, La Storia A, et al. 2013. Microbial ecology dynamics during rye and wheat sourdough preparation. *Appl. Environ. Microbiol.* 79(24):7827–36
32. Falardeau J, Keeney K, Trmčić A, Kitts D, Wang S. 2019. Farm-to-fork profiling of bacterial communities associated with an artisan cheese production facility. *Food Microbiol*. 83:48–58
33. Gallone B, Steensels J, Prahl T, Soriaga L, Saels V, et al. 2016. Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. *Cell* 166(6):1397–410.e16
34. Gänzle M. 2022. The periodic table of fermented foods: limitations and opportunities. *Appl. Microbiol. Biotechnol.* 106(8):2815–26
35. Gänzle M, Ripari V. 2016. Composition and function of sourdough microbiota: from ecological theory to bread quality. *Int. J. Food Microbiol.* 239:19–25
36. Gänzle MG, Loponen J, Gobbetti M. 2008. Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality. *Trends Food Sci. Technol.* 19(10):513–21
37. Gayevskiy V, Goddard MR. 2012. Geographic delineations of yeast communities and populations associated with vines and wines in New Zealand. *ISME J.* 6(7):1281–90
38. Gibbons JG, Rinker DC. 2015. The genomics of microbial domestication in the fermented food environment. *Curr. Opin. Genet. Dev.* 35:1–8
39. Gibbons JG, Salichos L, Slot JC, Rinker DC, McGary KL, et al. 2012. The evolutionary imprint of domestication on genome variation and function of the filamentous fungus *Aspergillus oryzae*. *Curr. Biol.* 22(15):1403–9
40. Harrison K, Curtin C. 2021. Microbial composition of SCOBY starter cultures used by commercial kombucha brewers in North America. *Microorganisms* 9(5):1060
41. Hittinger CT, Steele JL, Ryder DS. 2018. Diverse yeasts for diverse fermented beverages and foods. *Curr. Opin. Biotechnol.* 49:199–206
42. Hutkins RW. 2008. *Microbiology and Technology of Fermented Foods*. New York: John Wiley
43. Hutkins RW. 2013. *Microbiology and Technology of Fermented Foods*. New York: John Wiley. 2nd ed.
44. Irlinger F, Layec S, Hélinck S, Dugat-Bony E. 2015. Cheese rind microbial communities: diversity, composition and origin. *FEMS Microbiol. Lett.* 362(2):1–11
45. Irlinger F, Mounier J. 2009. Microbial interactions in cheese: implications for cheese quality and safety. *Curr. Opin. Biotechnol.* 20(2):142–48
46. Jung JY, Lee SH, Jeon CO. 2014. Kimchi microflora: history, current status, and perspectives for industrial kimchi production. *Appl. Microbiol. Biotechnol.* 98(6):2385–93
47. Kable ME, Srisengfa Y, Laird M, Zaragoza J, McLeod J, et al. 2016. The core and seasonal microbiota of raw bovine milk in tanker trucks and the impact of transfer to a milk processing facility. *mBio* 7(4):e00836-16
48. Kamelamela N, Zalesne M, Morimoto J, Robbat A, Wolfe BE. 2018. Indigo- and indirubin-producing strains of *Proteus* and *Psychrobacter* are associated with purple rind defect in a surface-ripened cheese. *Food Microbiol*. 76:543–52
49. Kamimura BA, Cabral L, Noronha MF, Baptista RC, Nascimento HM, Sant'Ana AS. 2020. Amplicon sequencing reveals the bacterial diversity in milk, dairy premises and Serra da Canastra artisanal cheeses produced by three different farms. *Food Microbiol*. 89:103453

50. Kang SE, Kim MJ, Kim TW. 2019. Diversity and role of yeast on kimchi fermentation. *J. Korean Soc. Food Cult.* 34(2):201–7
51. Kastman EK, Kamelamela N, Norville JW, Cosetta CM, Dutton RJ, Wolfe BE. 2016. Biotic interactions shape the ecological distributions of *Staphylococcus* species. *mBio* 7(5):e01157-16. Erratum. 2017. *mBio* 8(2):e00329-17
52. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, et al. 2012. Architectural design influences the diversity and structure of the built environment microbiome. *ISME J.* 6(8):1469–79
53. Klaenhammer TR. 1988. Bacteriocins of lactic acid bacteria. *Biochimie* 70(3):337–49
54. Landis EA, Fogarty E, Edwards JC, Popa O, Eren AM, Wolfe BE. 2022. Microbial diversity and interaction specificity in kombucha tea fermentations. *mSystems* 7(3):e0015722
55. Landis EA, Oliverio AM, McKenney EA, Nichols LM, Kfoury N, et al. 2021. The diversity and function of sourdough starter microbiomes. *eLife* 10:e61644
56. Lawrence D, Fiegna F, Behrends V, Bundy JG, Phillimore AB, et al. 2012. Species interactions alter evolutionary responses to a novel environment. *PLOS Biol.* 10(5):e1001330
57. Lee SH, Whon TW, Roh SW, Jeon CO. 2020. Unraveling microbial fermentation features in kimchi: from classical to meta-omics approaches. *Appl. Microbiol. Biotechnol.* 104(18):7731–44
58. Leech J, Cabrera-Rubio R, Walsh AM, Macori G, Walsh CJ, et al. 2020. Fermented-food metagenomics reveals substrate-associated differences in taxonomy and health-associated and antibiotic resistance determinants. *mSystems* 5(6):e00522-20
59. Leite AMO, Miguel MAL, Peixoto RS, Rosado AS, Silva JT, Paschoalin VMF. 2013. Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. *Braz. J. Microbiol.* 44(2):341–49
60. Macori G, Cotter PD. 2018. Novel insights into the microbiology of fermented dairy foods. *Curr. Opin. Biotechnol.* 49:172–78
61. Marcellino N, Benson DR. 2013. The good, the bad, and the ugly: tales of mold-ripened cheese. *Microbiol. Spectr.* 1(1). **https://doi.org/10.1128/microbiolspec.CM-0005-12**
62. Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, et al. 2017. Health benefits of fermented foods: microbiota and beyond. *Curr. Opin. Biotechnol.* 44:94–102
63. Marco ML, Sanders ME, Gänzle M, Arrieta MC, Cotter PD, et al. 2021. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. *Nat. Rev. Gastroenterol. Hepatol.* 18(3):196–208
64. Marsh AJ, Hill C, Ross RP, Cotter PD. 2014. Fermented beverages with health-promoting potential: past and future perspectives. *Trends Food Sci. Technol.* 38(2):113–24
65. McGovern P, Jalabadze M, Batiuk S, Callahan MP, Smith KE, et al. 2017. Early Neolithic wine of Georgia in the South Caucasus. *PNAS* 114(48):E10309–18
66. Miller ER, Kearns PJ, Niccum BA, O'Mara Schwartz J, Ornstein A, Wolfe BE. 2019. Establishment limitation constrains the abundance of lactic acid bacteria in the Napa cabbage phyllosphere. *Appl. Environ. Microbiol.* 85(13):e00269-19
67. Miller ER, O'Mara Schwartz J, Cox G, Wolfe BE. 2020. A gnotobiotic system for studying microbiome assembly in the phyllosphere and in vegetable fermentation. *J. Vis. Exp.* 160:e61149
68. Monnet C, Landaud S, Bonnarme P, Swennen D. 2015. Growth and adaptation of microorganisms on the cheese surface. *FEMS Microbiol. Lett.* 362(1):1–9
69. Morrison-Whittle P, Goddard MR. 2018. From vineyard to winery: a source map of microbial diversity driving wine fermentation. *Environ. Microbiol.* 20(1):75–84
70. Mounier J, Goerges S, Gelsomino R, Vancanneyt M, Vandemeulebroecke K, et al. 2006. Sources of the adventitious microflora of a smear-ripened cheese. *J. Appl. Microbiol.* 101(3):668–81
71. Mounier J, Monnet C, Vallaeys T, Arditi R, Sarthou A-S, et al. 2008. Microbial interactions within a cheese microbial community. *Appl. Environ. Microbiol.* 74(1):172–81
72. Nascimento MS, Pena PO, Brum DM, Imazaki FT, Tucci MLS, Efraim P. 2013. Behavior of *Salmonella* during fermentation, drying and storage of cocoa beans. *Int. J. Food Microbiol.* 167(3):363–68
73. Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, et al. 2013. Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* 77(3):342–56

74. Niccum BA, Kastman EK, Kfoury N, Robbat A Jr., Wolfe BE. 2020. Strain-level diversity impacts cheese rind microbiome assembly and function. *mSystems* 5(3):e00149-20
75. Obafemi YD, Oranusi SU, Ajanaku KO, Akinduti PA, Leech J, Cotter PD. 2022. African fermented foods: overview, emerging benefits, and novel approaches to microbiome profiling. *npj Sci. Food* 6(1):15
76. Parente E, Cogan TM. 2004. Starter cultures: general aspects. *Cheese Chem. Phys. Microbiol.* 1:123–48
77. Pažin V, Jankuloski D, Kozačinski L, Dobranić V, Njari B, et al. 2018. Tracing of *Listeria monocytogenes* contamination routes in fermented sausage production chain by pulsed-field gel electrophoresis typing. *Foods* 7(12):198
78. Peñas E, Martinez-Villaluenga C, Frias J. 2017. Sauerkraut: production, composition, and health benefits. In *Fermented Foods in Health and Disease Prevention*, ed. J Frias, C Martinez-Villaluenga, E Peñas, pp. 557–76. Boston: Academic
79. Pierce EC, Dutton RJ. 2022. Putting microbial interactions back into community contexts. *Curr. Opin. Microbiol.* 65:56–63
80. Pierce EC, Morin M, Little JC, Liu RB, Tannous J, et al. 2021. Bacterial-fungal interactions revealed by genome-wide analysis of bacterial mutant fitness. *Nat. Microbiol.* 6(1):87–102
81. Primack RB, Miao SL. 1992. Dispersal can limit local plant distribution. *Conserv. Biol.* 6(4):513–19
82. Quigley L, O'Sullivan O, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD. 2011. Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. *Int. J. Food Microbiol.* 150(2–3):81–94
83. Raynaud T, Blouin M, Devers-Lamrani M, Garmyn D, Spor A. 2022. The central role of the interspecific interactions in the evolution of microbial communities. Preprint, bioRxiv. **https://www.biorxiv.org/content/10.1101/2022.01.17.476584v1**
84. Reese AT, Madden AA, Joossens M, Lacaze G, Dunn RR. 2020. Influences of ingredients and bakers on the bacteria and fungi in sourdough starters and bread. *mSphere* 5(1):e00950-19
85. Rezac S, Kok CR, Heermann M, Hutkins R. 2018. Fermented foods as a dietary source of live organisms. *Front. Microbiol.* 9:1785
86. Rizo J, Guillén D, Farrés A, Díaz-Ruiz G, Sánchez S, et al. 2020. Omics in traditional vegetable fermented foods and beverages. *Crit. Rev. Food Sci. Nutr.* 60(5):791–809
87. Rizzello CG, Cavoski I, Turk J, Ercolini D, Nionelli L, et al. 2015. Organic cultivation of *Triticum turgidum* subsp. *durum* is reflected in the flour-sourdough fermentation-bread axis. *Appl. Environ. Microbiol.* 81(9):3192–204
88. Rogalski E, Ehrmann MA, Vogel RF. 2021. Intraspecies diversity and genome-phenotype-associations in *Fructilactobacillus sanfranciscensis*. *Microbiol. Res.* 243:126625
89. Romulo A, Surya R. 2021. Tempe: A traditional fermented food of Indonesia and its health benefits. *Int. J. Gastronomy Food Sci.* 26:100413
90. Ropars J, Didiot E, Rodríguez de la Vega RC, Bennetot B, Coton M, et al. 2020. Domestication of the emblematic white cheese-making fungus *Penicillium camemberti* and its diversification into two varieties. *Curr. Biol.* 30(22):4441–53.e4
91. Rosa DD, Dias MMS, Grześkowiak ŁM, Reis SA, Conceição LL, Peluzio MCG. 2017. Milk kefir: nutritional, microbiological and health benefits. *Nutr. Res. Rev.* 30(1):82–96
92. Ross RP, Morgan S, Hill C. 2002. Preservation and fermentation: past, present and future. *Int. J. Food Microbiol.* 79(1–2):3–16
93. Roy B, Kala CP, Farooquee NA, Majila BS. 2004. Indigenous fermented food and beverages: a potential for economic development of the high altitude societies in Uttaranchal. *J. Hum. Ecol.* 15(1):45–49
94. Saak CC, Pierce EC, Dinh CB, Portik D, Hall R, et al. 2023. Longitudinal, multi-platform metagenomics yields a high-quality genomic catalog and guides an in vitro model for cheese communities. *Food Microbiol.* 8(1):e00701-22
95. Satora P, Skotniczny M, Strnad S, Ženišová K. 2020. Yeast microbiota during sauerkraut fermentation and its characteristics. *Int. J. Mol. Sci.* 21(24):9699
96. Scherlach K, Graupner K, Hertweck C. 2013. Molecular bacteria-fungi interactions: effects on environment, food, and medicine. *Annu. Rev. Microbiol.* 67:375–97

97. Sieuwerts S, de Bok FAM, Hugenholtz J, van Hylckama Vlieg JET. 2008. Unraveling microbial interactions in food fermentations: from classical to genomics approaches. *Appl. Environ. Microbiol.* 74(16):4997–5007
98. Siezen RJ, Tzeneva VA, Castioni A, Wels M, Phan HTK, et al. 2010. Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from various environmental niches. *Environ. Microbiol.* 12(3):758–73
99. Sloan WT, Nnaji CF, Lunn M, Curtis TP, Colloms SD, et al. 2021. Drift dynamics in microbial communities and the effective community size. *Environ. Microbiol.* 23(5):2473–83
100. Soininen J, McDonald R, Hillebrand H. 2007. The distance decay of similarity in ecological communities. *Ecography* 30(1):3–12
101. Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, et al. 2013. Quantifying community assembly processes and identifying features that impose them. *ISME J.* 7(11):2069–79
102. Steinkraus K, ed. 1995. *Handbook of Indigenous Fermented Foods.* New York: Marcel Dekker. 2nd ed.
103. Sun L, D'Amico DJ. 2021. Composition, succession, and source tracking of microbial communities throughout the traditional production of a farmstead cheese. *mSystems* 6(5):e0083021
104. Sundarraman D, Hay EA, Martins DM, Shields DS, Pettinari NL, Parthasarathy R. 2020. Higher-order interactions dampen pairwise competition in the zebrafish gut microbiome. *mBio* 11(5):e01667-20
105. Tamang JP. 2010. Diversity of fermented foods. In *Fermented Foods and Beverages of the World*, ed. JP Tamang, K Kailasapathy, chap. 2. Boca Raton, FL: CRC
106. Tamang JP, Cotter PD, Endo A, Han NS, Kort R, et al. 2020. Fermented foods in a global age: East meets West. *Compr. Rev. Food Sci. Food Saf.* 19(1):184–217
107. Tamang JP, Watanabe K, Holzapfel WH. 2016. Review: diversity of microorganisms in global fermented foods and beverages. *Front. Microbiol.* 7:377
108. Tofalo R, Fusco V, Böhnlein C, Kabisch J, Logrieco AF, et al. 2020. The life and times of yeasts in traditional food fermentations. *Crit. Rev. Food Sci. Nutr.* 60(18):3103–32
109. van Hijum SAFT, Vaughan EE, Vogel RF. 2013. Application of state-of-art sequencing technologies to indigenous food fermentations. *Curr. Opin. Biotechnol.* 24(2):178–86
110. Vellend M. 2010. Conceptual synthesis in community ecology. *Q. Rev. Biol.* 85(2):183–206
111. Wastyk HC, Fragiadakis GK, Perelman D, Dahan D, Merrill BD, et al. 2021. Gut-microbiota-targeted diets modulate human immune status. *Cell* 184(16):4137–53.e14
112. Winters M, Panayotides D, Bayrak M, Rémont G, Viejo CG, et al. 2019. Defined co-cultures of yeast and bacteria modify the aroma, crumb and sensory properties of bread. *J. Appl. Microbiol.* 127(3):778–93
113. Wolfe BE, Button JE, Santarelli M, Dutton RJ. 2014. Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell* 158(2):422–33
114. Wolfe BE, Dutton RJ. 2015. Fermented foods as experimentally tractable microbial ecosystems. *Cell* 161(1):49–55
115. Xue Z, Brooks JT, Quart Z, Stevens ET, Kable ME, et al. 2021. Microbiota assessments for the identification and confirmation of slit defect-causing bacteria in milk and cheddar cheese. *mSystems* 6(1):e01114-20
116. Yeluri Jonnala BR, McSweeney PLH, Sheehan JJ, Cotter PD. 2018. Sequencing of the cheese microbiome and its relevance to industry. *Front. Microbiol.* 9:1020
117. Yu AO, Leveau JHJ, Marco ML. 2020. Abundance, diversity and plant-specific adaptations of plant-associated lactic acid bacteria. *Environ. Microbiol. Rep.* 12(1):16–29
118. Zabat MA, Sano WH, Wurster JI, Cabral DJ, Belenky P. 2018. Microbial community analysis of sauerkraut fermentation reveals a stable and rapidly established community. *Foods* 7(5):77
119. Zhang Y, Kastman EK, Guasto JS, Wolfe BE. 2018. Fungal networks shape dynamics of bacterial dispersal and community assembly in cheese rind microbiomes. *Nat. Commun.* 9(1):336