The Good, the Bad, and the Ugly: Tales of Mold-Ripened Cheese

SISTER NOËLLA MARCELLINO, O.S.B.,1 and DAVID R. BENSON2
1Abbey of Regina Laudis, Bethlehem, CT 06751; 2Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269-3125

The history of cheese manufacture is a “natural history” in which animals, microorganisms, and the environment interact to yield human food. Part of the fascination with cheese, both scientifically and culturally, stems from its ability to assume amazingly diverse flavors as a result of seemingly small details in preparation. In this review, we trace the roots of cheesemaking and its development by a variety of human cultures over centuries. Traditional cheesemakers observed empirically that certain environments and processes produced the best cheeses, unwittingly selecting for microorganisms with the best biochemical properties for developing desirable aromas and textures. The focus of this review is on the role of fungi in cheese ripening, with a particular emphasis on the yeast-like fungus Geotrichum candidum. Conditions that encourage the growth of problematic fungi such as Mucor and Scopulariopsis as well as Arachnida (cheese mites), and how such contaminants might be avoided, are discussed. Bethlehem cheese, a pressed, uncooked, semihard, Saint-Nectaire-type cheese manufactured in the United Sates without commercial strains of bacteria or fungi, was used as a model for the study of stable microbial succession during ripening in a natural environment. The appearance of fungi during a 60-day ripening period was documented using light and scanning electron microscopy, and it was shown to be remarkably reproducible and parallel to the course of ripening of authentic Saint-Nectaire cheese in the Auvergne region of France. Geotrichum candidum, Mucor, and Trichothecium roseum predominate the microbiotas of both cheese types. Geotrichum in particular was shown to have high diversity in different traditional cheese ripening environments, suggesting that traditional manufacturing techniques selected for particular fungi. This and other studies suggest that strain diversity arises in relation to the lore and history of the regions from which these types of cheeses arose.

The history of cheese manufacture is a “natural history” in which animals, microorganisms, and the environment interact to yield human food. Part of the fascination with cheese both scientifically and culturally stems from its ability to assume amazingly diverse flavors as a result of seemingly small details in preparation. These details have been discovered empirically and independently by a variety of human populations and, in many cases, have been propagated over hundreds of years.

Cheeses have been made probably as long as mammals have stood still long enough to be milked. In principle, cheese can be made from any type of mammalian milk. In practice, of course, traditional herding animals are far more effectively milked than, say, moose, although moose cheese can be found in Sweden today. Unfortunately, the price of moose cheese, not to mention the scarcity of cooperative moose, dooms such enterprises to a niche market. The more common mammals whose milk is used to produce cheese include cows, sheep, goats, and, to a lesser extent, bison, camels, horses, yaks, and llamas, depending on where one lives in the world.

Words associated with cheesemaking have equally ancient roots. The ancient Greeks called the wicker cheese basket used for draining whey from the curds a formas, which became forma to the Romans, from which the modern Italian formaggio and French fromage were derived (1). The English “cheese” is
derived from the West Germanic reconstruekt *kasius via the Old English cyse and Latin caseus. Their roots are in the proto-Indo-European reconstruekt *kwat-, to ferment or become sour.

Human survival has a link to fermentation, the process by which yeasts and bacteria degrade organic compounds to by-products such as lactic acid and alcohol and transform perishable foods into preserved products. In the case of cheese, the final fermented product is safe, durable, and, due to a reduced volume, transportable, a feature upon which nomadic cultures depend (2). Historically, cheese was often reserved for use as a source of protein and fat during periods of low milk production due to lactation cycles of animals (3) or changes in climatic conditions (4).

Cheesemaking emerged as a by-product of dairying. Domesticated animals played a role in Neolithic (~4000 BCE) farming communities worldwide, but there has been much conjecture as to whether they were used only as sources of meat or were also exploited for “secondary products” such as milk (5–7). Clear evidence of cheesemaking is found in southern Mesopotamia, an area long recognized as the “cradle of civilization,” where great centers of religion and culture emerged in the late fourth millennium BCE (8). Mesopotamia is the Greek word for “between the rivers,” and it was here along the banks of the Tigris and the Euphrates in the Fertile Crescent that many believe that dairying progressed to actual cheesemaking (9), as evidenced by depictions of milking and processing of milk products (10). Eighteen to twenty varieties of cheese have been documented in the ancient Near East, including flavored, sweetened, sharp, crushed, and white (11).

Evidence for dairying in other ancient cultures has been found in temperate Europe and in Mediterranean regions, in the form of pottery. Shards of ceramic sieves have been found at Linear Pottery sites distributed across temperate Europe from the Ukraine to France and from Hungary to the Baltic Sea (12, 13). The sieves were probably used to drain whey from curds and suggest that dairying took place in temperate Europe as early as ca. 5400 BCE, the radiocarbon date for the Linear Pottery culture in Hungary (14). More direct evidence for prehistoric dairying has been obtained through the field of biomolecular archaeology, which has allowed scientists to move beyond speculation based only on reconstructed pottery vessels. Copley et al. (5) analyzed fat residues from pottery from prehistoric sites using fatty acid composition, double-bond positions, triacylglycerol distribution, and δ13 enrichment values to evaluate the origins of the fats in relationship to domesticated animals. Of the 237 pot shards analyzed, 22% yielded dairy fat, indicating that dairying was indeed an important aspect of farming in the British Iron Age. In other work, Craig et al. (15, 16) characterized lipids and proteins adsorbed to late Bronze Age and Iron Age ceramics from the island of South Uist in the Outer Hebrides. The authors used gas chromatography-mass spectrometry and gas chromatography combustion isotope ratio mass spectrometry to identify the source of lipids on the pottery. Twenty-five of 39 shards tested showed adsorbed lipids with a high abundance of saturated fatty acids, especially 18:0, indicating that the lipids were of animal origin. The ratio of δ13C18:0 to δ13C16:0 values further indicated that the animals were ruminants. They also developed an immunological detection method, the digestion-and-capture immunosay, to extract and characterize the 2,500-year-old proteins adsorbed to the ceramics. Their finding of bovine α-casein adsorbed to pottery shards was correlated with the large number of neonatal cattle remains at the site, evidence that calves were culled to sustain a dairy economy. Maintaining lactating cattle with only a few calves present in a herd implies that the cows were milked and a large milk supply was transformed into dairy products that could be stored and preserved. The authors note the example of bog butter that has been preserved and found in other sites where there is also evidence of calf culling. Bog butter, a whitish, grey waxy substance dating back to 400 BCE to 500 CE, has been found in bogs in Ireland and Scotland. During the Iron Age, butter was preserved in two-piece wooden kegs buried in the anaerobic and cool conditions of the bogs (17). Cronin et al. (18) analyzed the fatty acid content of prehistoric bog butter in comparison to present-day Irish fresh butter using gas-liquid chromatography and gas chromatography-mass spectrometry. They found that fatty acids are present in modern butter in the form of triglycerides, while free fatty acids, in particular free long-chain saturated fatty acids, predominated in the bog butter due to hydrolysis. Proteolysis in the butters was also studied using size exclusion high-performance liquid chromatography and ion-exchange chromatography. Not surprisingly, the bog butter consisted primarily of small peptides (<0.5 kDa) and free amino acids with little or no intact proteins, in comparison to modern butter, in which caseins and whey proteins, ranging in size from 14 to 30 kDa, remain. The authors concluded that proteolysis in the bog butter was probably due to microbial activity in the bog environment.

In ancient Greece and Rome, cheese was an essential and popular food. Fresh milk was never imbibed; in fact,
the barbarians were called, somewhat derisively, “milk-drinkers” (19). The Scythians, the horse-riding tribes of the Eurasian steppes, were called “mare-milkers” by Homer (ca. 1184 BCE) and consumed fermented mare’s milk and cheese called hippake (20). Because of the abundance of olive oil in the Mediterranean, butter was not used. The Greek geographer Strabo (66 BCE–25 CE) viewed the mountain people of the Iberian Peninsula with distaste because they lived on goat meat and acorn bread, drank beer rather than wine, and used butter instead of olive oil (21). Varro (116–28 BCE), in his treatise on Roman farm management, wrote, “Cheese made of cow’s milk is the most agreeable to the taste, but the most difficult to digest: next, that of ewe’s milk, while the least agreeable in taste, but the most easily digested, is that of goat’s milk” (22). Columella (4 BCE–ca. 70 CE) wrote of all aspects of cheesemaking in his work on agriculture, Rei Rusticae (23). The principles he enunciated in the 1st century could be applied today. He described whey as the “acid liquid” which should be pressed out of the cheese as quickly as possible (23). Today, we know that excess whey that contains lactose in pressed cheeses can cause “postacidification” of the curd, resulting in bitterness (24). Homer (ca. 1184 BCE) was familiar with aspects of cheesemaking, as evidenced by the detail with which he describes the scene in the cave of the cyclops Polyphemos in his epic poem the Odyssey. Upon entering the cave, Odysseus notices vessels “swimming with whey” and “willow baskets that were heavy with cheese” (25) which he and his men eat while awaiting their host. However, the cyclops, a “giant dairy farmer” (26), is also “...a savage monster who despises the laws of hospitality to the point that he devours guests in his own home, if indeed one can call his cave a home” (27). While his prisoners cower, the cyclops performs his dairy chores twice a day: he milks his sheep, adds fig juice to form a curd (fig juice contains the protease ficin, which acts to coagulate the milk), and drains the curd in willow baskets. Odysseus and his surviving men eventually escape by blinding the cyclops and riding out of the cave underneath the bellies of his rams.

Given the coincidence between dairying, cheesemaking, and civilization, it is not surprising that Kamber and Terzi regard cheese as “one of the primary symbols of mankind’s passage into civilization” (28), and techniques for its production, unique to each culture, were handed down from generation to generation as a cultural inheritance (29). In modern times, the establishment of the European Economic Community has prompted a new self-awareness of each country’s regional products in Europe that reflect the cultural and environmental characteristics of particular locales (30). In the developed world, there has been a trend towards decentralizing food production and a renewed interest in local foods that are identified with particular rural areas and ethnicity, to revitalize agriculture in economically depressed or depopulated regions (31).

Legislation to guarantee the territorial authenticity and vintage of a product first arose in France, where, in 1411, King Charles VI granted the people of Roquefort-sur-Soulzon a monopoly on Roquefort-style cheese and in 1666 the court of Toulouse applied a judicial text to Roquefort cheese, making it illegal for other villages to assert that they produced the cheese. These declarations simply recognized a cheese type that had been produced since at least Roman times. Subsequent legislation created the appellation d’origine contrôlée (AOC) to protect traditional products that arose within the boundaries of a rural community and its specific environmental context (32). Many European artisanal cheeses are now marked with the seal of the protected designation of origin (PDO), a denomination established by the European Union to recognize and protect agricultural products “whose quality or characteristics are essentially or exclusively due to a particular geographical environment with its inherent natural and human factors and the production, processing and preparation of which take place in the defined geographical area” (33). As early as 500 CE, Cassiodorus (480–575 CE), Praetorian Praefect of the Ostrogothic king Theodoric, evoked terroir when he described the banquets in Rome which included cheeses made from the milk of herds grazing in the lush pastures and forests on Mount Sila in southern Italy:

...and we praised the wines of Bruttii and the cheese of the district around Mount Sila. The cheese, which retains in its pores the milk which has been collected there, recalls by its taste the fragrant herbs upon which the cattle have fed; by its texture it reminds us of the softness of oil, from which it differs in colour by its snowy whiteness. Having been carefully pressed into a wide cask and hardened therein, it retains permanently the beautiful round shape which has thus been given to it.

Variae Epistolae, Book XII (34)

The concept of a geography of taste (31) has now taken hold on both sides of the Atlantic. Reacting to the globalized food market and suspicious of industrial food processing techniques, consumers increasingly demand to know the source of their food and are seeking a more direct link to the producers (35). Hence, “sustainable
agriculture,” which includes the concept of reversing the trend of disappearing farmland, is on the rise.

CHEESE RIPENING

Cheese is milk that has grown up...it is preeminently the food of man—the older it grows the more manly it becomes, and in the last stages of senility it almost requires a room to itself.

The Epicure’s Companion, Edward Bunyan (1872–1939) (36)

Throughout the centuries, a blend of empirical observation and isolated cultural development has helped sculpt the diversity of cheeses we know today. Flavor diversity is created by the biochemical activities of microorganisms degrading the curd and providing flavors and aromas absent from the initially bland young cheese (37, 38, 39). Pasteurizing the milk used for cheesemaking allows for more uniformity of the finished product and improves food safety but removes much of the indigenous microbiota of milk that imparts distinctive flavors (40). The large-scale industrialization of cheesemaking has caused a loss of traditional techniques and ripening venues, including natural caves where diverse populations of fungi and bacteria have developed sometimes over centuries. In response, some microbiologists have been isolating and characterizing the members of microbial populations originating in traditional cheeses unique to specific geographical regions (39, 40, 41, 42).

In one such project, 4,379 isolates of wild-type lactic acid bacteria were characterized from 35 products, including 24 artisanal cheeses made from cow, sheep, goat, and buffalo milk using traditional techniques in southern Europe. Not surprisingly, tremendous variability in acid and exopolysaccharide production, proteolytic activity, and bacteriocins of the isolates was discovered. A conclusion was that “each cheese was an ecosystem” in itself (43). Cheesemakers for centuries have exploited such extraordinary diversity in the cheese cave or cellar where a cheese is aged.

Even with this diversity, cheese microorganisms tend to follow a similar script during ripening. Three primary biochemical processes take place: fermentation of milk sugar (lactose) to lactic acid; hydrolysis of lipids to fatty acids; and breakdown of casein to peptides, amino acids, and ammonia. Amino acids and fatty acid derivatives are precursors to flavor compounds. Their metabolism plays a key role in determining the aroma of a cheese (44). These processes lead to the desirable, and sometimes undesirable, sensory qualities of flavor, texture, and appearance (45), and they are mainly due to the enzymes of the bacteria and fungi growing in and on the cheese that use various components of milk for their own nutritional requirements.

The normal microorganisms involved in cheese ripening include lactic acid bacteria naturally present in the milk and/or added in commercial starter cultures. Lactic acid bacteria, the live cultures with which most people who eat yogurt are familiar, quickly catabolize lactose to lactic acid and lesser amounts of acetic acid in the critical step of lowering the pH of the curd during cheese production. Other microorganisms play a more or less important role in ripening depending on the type of cheese. They include nonstarter indigenous lactic acid bacteria, other bacteria such as the aerobic surface-dwelling Brevibacterium linens, yeasts, and filamentous fungi (46). The microbial populations that develop on the surface of cheese can be quite complex depending on where they are ripened and how they were prepared. Some cheeses have traditionally been aged in jars and earthenware vessels buried in sand and soil (28), sealed pits called fosse dug in volcanic rock (47, 48), and/or in caves—each environment bringing the cheese in proximity to soil and diverse types of microorganisms (49, 50). Members of soil fungal genera such as Alternaria, Chrysosporium, Geotrichum, Mucor, and Penicillium are commonly found on cheese ripened in cave environments (51, 52).

Scott observed that “Fermentation organisms are ambient in the environment, whether humans make use of them or not” (53). And indeed humans have. If a cheesemaker observed empirically that certain conditions produced the good cheeses, he or she tried to reproduce or maintain the environment that by default selected for strains with the best biochemical properties. Affineur Pierre Androuët says it this way: “Our predecessors thought with reason that the natural agents in the environment conditioned the personality of cheeses and marked them with the indelible sign of vintage and territory” (54).

THE STRAIN YOU LOVE MAY BE YOUR OWN

A case study for how novice cheesemakers can come to terms with the cheesemaking process, indigenous microorganisms, and the terroir of a region may be told through the experiences of one of us (N.M.), who established a small artisanal cheesemaking facility at the Benedictine Abbey of Regina Laudis in Litchfield County, CT.

When one thinks of dairying in New England, Connecticut is not the first state to come to mind.
Connecticut’s farmland is disappearing faster than that of any other state; approximately 9,000 acres of farmland are lost to development every year.

As an aside, we note that the loss of farmland is at odds with Connecticut history. Contributing to the westward expansion of agriculture in the United States, “The Connecticut Yankee brought a cheese hoop with him and wherever he went made cheese” (55). The Statistical Account of the Town of Bethlehem for 1812 by the Reverend Azil Backus reports that “there are commonly sent to market 7,000 lb. of butter and 30,000 lb. of cheese” (56). The first cheese factory in America was built by Lewis Mills Norton in 1844 in Goshen, another town in Litchfield County known for its lush farmland and pastures. The “pineapple cheese” produced there earned its name from the pineapple-shaped wooden mold into which Norton pressed a soft Cheddar-type cheese (57). By the middle of the 19th century, Litchfield County was making nearly 3 million pounds of cheese a year. Pineapple cheese was first transported to the port at New Haven for distribution, but by 1884 the demand for the cheese was so great that the plant was eventually moved to Attica, NY, for better access to the railroads. From this period, cheesemaking in Connecticut began its decline.

Cheesemaking returned in earnest at Regina Laudis in 1977 when a third-generation cheesemaker from Cézallier in the Auvergne, France, came as a visitor. She brought with her the traditional methods of making a Saint-Nectaire-type cheese that she had learned from her grandmother. After 2 years and many disasters, a consistently good cheese was developed, which the abbey named Bethlehem cheese in deference to the AOC designation of Saint-Nectaire. Bethlehem cheese is a pressed, uncooked, semihard, mold-ripened cheese made from the fresh milk of the abbey’s Dutch Belted cows, a heritage breed whose milk, high in protein, fat, and total solids, is excellent for cheesemaking. The milk is not pasteurized, and commercial cultures of starter or fungi are not added during production or ripening.

Traditionally, the cheeses ripen on straw mats for 60 days in the cellar of a house—the “cave.” During this time, the appearance of fungi growing naturally on the cheese rinds is remarkably reproducible and predictable and parallels the course of ripening of authentic Saint-Nectaire cheese in the Auvergne (58).

Fungal genera from Bethlehem cheese and raw milk Saint-Nectaire cheeses from the Auvergne region of France were found to be similar and included Geotrichum candidum, Mucor, and Trichothecium roseum. The conclusion was that similar means of preparation lead to similar microbial successions during ripening. Bethlehem cheese thus was used as a model for the study of stable microbial succession during ripening in a natural environment (58).

Microbial succession on the ripening cheese over 60 days was monitored by scanning electron microscopy and light microscopy; the latter used stained paraffin sections to view cross-sections of the rind at various stages (58). When the cheese is removed from the cheese press, it has a rubbery consistency, very little flavor, and no discernible rind; indeed, no surface microorganisms were visible by scanning electron microscopy. In the curd, however, pockets of gram-positive cocci and yeasts had developed, indicating that bacterial growth and fermentation begin within the curd soon after formation. The morphology of all cells within an individual pocket was the same, suggesting derivation from single cells.

At 48 h, the cheese surface is covered with a lawn of budding yeast (Fig. 1A). The number of yeast CFU on the nascent rind increases at least 40-fold by day 4 and stabilizes from day 10 to the end of ripening. The number of lactic acid bacteria on the rind and in the curd remains fairly constant throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind. In many cheeses, this decacidification by yeast prepares the surface for the growth of acid-sensitive aerobic bacteria such as Brevibacterium linens (59). Strains of Streptococcus cremoris are the most abundant lactic acid bacteria within Bethlehem cheese, probably derived from the raw milk used in production. In contrast to the case with the rind, yeast counts remain low and fairly stable within the curd throughout ripening. The sharp increase of yeasts by day 4 is probably due to their ability to use some of the lactic acid produced by the lactic acid bacteria, leading to an increase in the pH of the rind.

By day 6 of ripening, the surface of Bethlehem cheese is covered by the whitish-gray Mucor, identified by its typical sporangiospores in spherical, black sporangia. Fig. 1B shows Mucor sporangia and hyphae on the surface of the cheese. By day 9, the sporangiospores release from the sporangia and the cheese takes on a grayish-brown velvety coat composed of mycelia and spores from Mucor entwined with Geotrichum candidum and drying of the cheese curd surface. G. candidum begins to dominate around day 4 of ripening and covers the cheese with a white powdery coat. G. candidum plays an important role in the ripening of soft cheeses such as Camembert.
and semihard cheeses such as Saint-Nectaire and Reblochon. Its lipases and proteases release fatty acids and amino acids and peptides involved in flavor development, and its aminopeptidases reduce bitterness imparted by low-molecular-weight peptides in cheese (62). While the growth of Mucor is typical on traditional Saint-Nectaire and Tomme de Savoie cheeses (38, 63), it can be a disaster on pâte molle-type cheeses such as Brie and Camembert.

By day 60, patches of pink mold can be seen highlighting the grayish coat left from the growth of Mucor. This pink fungus has been termed the “flower of the molds” and is valued by makers of Saint-Nectaire cheese. Microscopically, the two-celled conidiopores that develop from the tip of the vertical conidiophore are diagnostic of Trichothecium roseum (Pers.) Link 1809. Identification T. roseum is confirmed by isolation of the fungus and observation of conidiospore development as occurring in short chains in basipetal succession from the conidiophore using light microscopy (64). The contribution of T. roseum to the taste of cheeses like Bethlehem or Saint-Nectaire has not been studied, although the fungus is known for its lipolytic activity (65).

By day 59 of ripening, a more complex microbial community has settled in and stabilized on the surface of the cheeses, including filamentous fungi and coryneform bacteria that are first evident by day 20 of ripening. Species of Brevibacterium and Arthrobacter spp. can be isolated from Bethlehem cheese based on cell morphology and colony color. Fig. 1C shows the distinctive V shape of coryneform bacteria that have developed beneath the fungal debris on the cheese surface. Brevibacterium linens, the bacterium that predominates in the mixed culture on the surface of smear-ripened cheeses such as Époisses, Mont d’Or, Limburger, and Taleggio, gives cheeses a characteristic reddish-orange color (66). B. linens produces volatile sulfur compounds from the degradation of L-methionine, which contributes to the unique, sometimes pungent aroma of this class of cheeses and, by the way, to foot odor (67).

The ripening of Bethlehem cheese is similar in progression to that of other mold-ripened cheeses, with some of the same organisms involved, including the lactic acid bacteria, Geotrichum candidum, and various yeasts. The difference in taste and consistency is due to the differences in microbial communities, which, in turn, are selected for by differences in handling of the curd as it is being prepared. The selection often occurs at the level of salting of the cheese. Inhibition of G. candidum can occur at 1.0 to 2.0% salt and of Mucor at 2.0 to 3.0%. Penicillium species are the most salt tolerant of
the three genera, growing well at 5.0% salt. Saint-Nectaire-style cheeses are dry salted, and after 24 h in the cheese press, the residual salt is rinsed off, allowing for the growth of *Geotrichum candidum*, followed by *Mucor*. In the production of the Spanish blue-veined cheese Cabrales, during which no starter or fungal cultures are added, the drained cheeses are covered with a layer of coarse salt which is not washed off and is kept at room temperature for 10 to 15 days. The cheeses are subsequently placed in natural caves, where the salt content and high humidity in the environment encourage the growth of *Penicillium roqueforti* (68). Hence, the cheesemaker directs the microbial population to different outcomes.

**FRENCH LESSONS IN BIODIVERSITY**

The fact that the same types of fungi are found on traditional Saint-Nectaire from France and Bethlehem cheese from the United States indicates that the critical microorganisms are present naturally in the environment and that the traditional methods of preparation and ripening determine the finished product. At the same time, the genetic and biochemical diversity of *Geotrichum* populations in the milk and curd and on cheeses ripened in traditional caves of France in six major cheesemaking regions was found to be immense (38). Saint-Nectaire, Reblochon, Mont d’Or, and Tamié were looked at with regard to strain diversity in relation to the lore and history of the regions from which they arose. The conclusion from that study indicated that a tremendous amount of biochemical and genetic diversity existed within the *Geotrichum candidum* clade. Further, such diversity can partly be explained by the empirical selection of cheesemaking techniques that favored the development of the “best” strains in local contexts. A corollary is that manufacturing practices aimed at producing a uniform cheese type would necessarily lead to the loss of specific local strains.

**Geotrichum candidum: The Good**

The loss of diversity has implications for cheesemakers. Among “the good, the bad, and the ugly” fungi, *Geotrichum candidum* stands as one of the best and thus receives particular attention in this review, but it should be noted that other microbial groups would paint a similar picture.

*G. candidum* contributes to the ripening of nearly all surface mold-ripened cheeses: *pâte molle* such as Brie and Camembert, semihard cheeses which include Saint-Nectaire, Tomme de Savoie, and Reblochon, and the class of smear cheeses to which Livarot, Limburger, and Taleggio belong (69, 70). Pottier et al. (71) evaluated the importance of *G. candidum* to the French dairy industry, calculating that *G. candidum* was present in about 600,000 tons of French cheese, one-third of total production. They concluded that in 1 year, French consumers ate close to 8 kg (18 lb) per person of cheese containing *G. candidum*. Fifteen years ago, *G. candidum* may not have been commonly discussed in the United States, yet now at the annual American Cheese Society conference, it is not unusual to hear artisanal cheesemakers comparing their strains of *G. candidum*, or “geo.” Both wild-type strains and commercial cultures affect the appearance and aroma of cheeses. In catalogues, starter strains are presented according to characteristics that might interest the cheesemaker: colony color, morphology (“yeasty” or “moldy”), biochemical properties (having known proteolytic or lipolytic activity), and capacity for neutralizing the curd, which affects the subsequent microbial ecology of the cheese.

Growth of *G. candidum* is generally observed on the cheese surface around the third day of ripening. In some cheeses, such as Saint-Marcellin, the main role of *G. candidum* is in the presentation of the finished product: the organism covers the cheese surface with a uniform white velvety layer, sometimes called a *jolie robe* (pretty dress) (72). On other cheeses, *G. candidum* is not obvious at the end of the ripening period. On ripened Camembert or Brie, *G. candidum* is covered by a white layer of *Penicillium camemberti* which first appears around day 6 of ripening (73, 74). *G. candidum* is the only filamentous fungus on Reblochon and, mixed with *Brevibacterium linens*, creates an orange-white crust (67, 75). In contrast, *G. candidum* is part of the more complex microflora on the surfaces of Saint-Nectaire and Tomme de Savoie (62). Figure 2A shows a layer of *G. candidum* on the rind of a 10-day-old Tomme de Savoie that will be covered by a gray layer of *Mucor* later in ripening.

Morphologically, *G. candidum* is described as a “yeast-like fungus” because it appears microscopically with both single-cell stages and filaments (72). Figure 2B shows a light micrograph of a *G. candidum* strain isolated from a 4-day-old Bethlehem cheese. Two cell types can be seen: arthrospores and short septate hyphae. During the process of asexual reproduction, the hyphae fragment and this process, called disarticulation, produces thallic-arthric conidia, called arthrospores (76) —the cylindrical cells seen interdispersed among the hyphae in Fig. 2B. The scanning electron micrograph in
FIGURE 2 The yeast-like fungus *Geotrichum candidum*. (A) *G. candidum* on the rind of a 10-day-old Tomme de Savoie. (B) Light micrograph of arthrospores and septate hyphae of a wild-type *G. candidum* strain isolated from a 4-day-old Bethlehem cheese. Bar, 10 μm. (C) Scanning electron micrograph of the surface of a Reblochon ripening shelf showing cylindrical *G. candidum* arthrospores and bacteria. Bar, 5 μm. Reproduced with permission from reference 77 (micrograph by Romain Briandet, UMR-UBHM, Institut National de la Recherche Agronomique et Ecole Nationale Supérieure des Industries Agricoles et Alimentaires, Massy, France). doi:10.1128/microbiolspec.CM-0005-2012.f2.

**Fig. 2C** (77) shows *G. candidum* arthrospores, roughly 10 μm in length, on the surface of a wooden Reblochon ripening shelf.

Yeast-like strains are generally characterized by cream-colored colonies, very similar to yeast colonies on agar medium. On a microscopic level, these strains produce many arthrospores and very few vegetative hyphae (62). They generally have an optimal growth temperature of 22 to 25°C, show acidifying activity, and are weakly proteolytic (78, 79). More filamentous strains are characterized by white colonies, more or less “furry” or felt-like, which have vegetative hyphae predominating with very little sporulation (62). These fungus-like strains grow best at 25 to 30°C, have alkalizing activity, and are highly proteolytic.

Classification of *G. candidum* may not necessarily interest cheesemakers, yet the commercial strain biotype may strongly influence the texture, cohesiveness, and thickness of the rind. The taxonomy of *Geotrichum* has a complex history and is still evolving. About 100 synonyms have been used for the same taxon since 1809, when Link designated the genus as *Geotrichum*, with *G. candidum* as the only species (79). Previous names, such as *Oidium lactis*, assigned in 1850 when it was isolated from milk by Fresenius, reveal a historical connection to milk and dairy products (80). Traditional French cheesemakers to this day sometimes refer to *G. candidum* as “*Oidium*.” The microorganism was reclassified as *Oospora lactis* in 1880 and finally placed in the genus *Geotrichum*. *G. candidum* was included in Barnett’s first and second editions of *Yeasts: Characteristics and Identification* and classified as a “fungal-like yeast” (81, 82).

Until the 1990s, fungal and yeast classification was based on observable (phenotypic) characteristics such as morphology, physiology, and biochemical properties (83). Together with its distinctive morphology, growth on D-xylose and lack of growth on cellobiose and maltose as sole sources of carbon are used in identifying *Geotrichum* to the species level (72). Despite its ability to live on cheese, *G. candidum* cannot use lactose as a carbon source, whereas assimilation of lactic acid is variable (62, 84). Phenotypic variability presents a difficult task for identifying each new strain (85).

Over the past 10 years, advances in molecular methods for identifying and typing strains have led to revisions in classification. *G. candidum* had been classified as the imperfect form, or anamorph, of the yeast *Galactomyces geotrichum* in 1986 by de Hoog et al. (86). However, taxonomic revisions of filamentous fungi that reproduce by arthric conidiogenesis, based on
internal transcribed spacer ribosomal DNA sequences and nuclear DNA (nDNA)/DNA reassociation data, led to renaming of the teleomorph (perfect form) Galactomyces candidus [87, 88]. Isolates of the anamorph genus Geotrichum were identified to the species level by using randomly amplified polymorphic DNA-PCR (RAPD-PCR) [89]. In our investigation into the biodiversity of G. candidum in French cheese, we differentiated strains within species using RAPD-PCR [38]. Other approaches have used chromosomal pulsed-field gel electrophoresis to correlate genetic data with phenotypic characteristics such as morphology [90]. The latter approach showed that the fungus-like strains and those with intermediate morphology had larger genomes than yeast-like strains. A standardized protocol has been proposed for identifying G. candidum that allows identification and comparison between laboratories [83]. It uses PCR to distinguish between species of Geotrichum and then differentiates strains within the species using RAPD-PCR in conjunction with random amplified microsatellite PCR. The GATA4 primer used in random amplified microsatellite PCR allowed strains to be grouped according to the ecological niche from which they were isolated, such as food, plants, the environment, and human sources. The protocol can be used to trace strains to their provenance and facilitates tracking of strains throughout cheese production and ripening.

G. candidum strains not only are part of the microbiota of raw milk but also are found in environments as diverse as soil, barley, grass, and silage. The optimum pH for growth is around 5.5, but they can grow across pH values of 3.5 to 9.0 [91]. G. candidum strains are generally acid-tolerant microorganisms that can create costly problems for citrus fruit and tomato industries [92, 93]. The organism is part of the natural microbiota of barley and is used as an inoculum in malt production to improve quality as well as to inhibit the growth of Fusarium species. The latter cause the defect of “gushing” in beer and can produce mycotoxins [94]. G. candidum possesses an array of enzymes with great potential for biotechnological applications. Its lactate oxidase has been used to determine lactate levels implicated in heart disease [95]. In toxic waste management, G. candidum is used to decolorize azo dyes in industrial waste water [96], and its peroxidases have shown high efficiency in decolorizing and biodegrading olive mill wastewater, a pollutant generated by olive oil extraction [97]. In 2001, G. candidum gained notoriety as the “fungus that eats CDs” when its enzymes bored holes in the aluminum and polycarbonate layers of compact discs and corrupted the information stored in them [98].

G. candidum is not an agent of food poisoning and is generally regarded as safe. No food-borne disease has been linked to the consumption of products containing G. candidum, and food technologists handling the microorganism are not at risk [71]. However, some G. candidum strains can act as opportunistic pathogens, causing superficial or internal mycosis known as geotrichosis [90, 99].

Lipolytic enzymes contributed by milk, starter bacteria, and fungi help develop texture and release free fatty acids, precursors to flavor compounds in many cheeses [100]. From a biochemical standpoint, G. candidum’s lipases are unique and contribute to flavor and texture development in several classes of cheese [101, 102]. One isofrom of G. candidum’s lipases exhibits a preference for cis (Δ-9) unsaturated fatty acids in triglyceride esters—fatty acids such as oleic or linoleic acid with a double bond at the ninth carbon position. Fresno et al. [103] attribute the high concentration of oleic acid in Armada goat cheese to the fact that G. candidum predominates on the cheese during the first 2 months of ripening. In addition, the lipase has been used for biotransformations in the oil industry, such as the synthesis of (S)-atenol and (R)-propanolol through lipase-catalyzed hydrolysis of the intermediate O-acetyl esters, and in pharmaceutical production for the stereoinversions of arylethanols [104, 105].

Of the three main biochemical activities involved in cheese ripening—proteolysis, glycolysis, and lipolysis—proteolysis is considered the most complex and important [106]. While proteolysis is a key to flavor development, it is also responsible for bitterness, a common defect in cheese [107]. Mold-ripened cheeses are especially prone to bitterness [108], as are cheeses with low salt concentrations. High salt concentrations in cheese may promote the aggregation of large peptides, making them less accessible to degradation by proteinases into bitter peptides [109].

Aminopeptidases of G. candidum strains have been correlated with reduced bitterness in Camembert through the breakdown of low-molecular-weight peptides released by the proteinases of Penicillium camemberti [74]. The synergistic effects of the two fungi on protein catabolism in Camembert are summarized in Fig. 3. Proteolysis in cheese begins when the proteinases (chymosin) in coagulant, milk (plasmin), and starter cultures hydrolyze casein to high-molecular-weight peptides (Fig. 3A). The high-molecular-weight peptides are then catabolized to low-molecular-weight peptides by proteinases of starter
bacteria and secondary microorganisms (110, 111). G. candidum presumably does not have to hydrolyze caseins into peptides, as these can be provided by the proteolytic activity of other organisms. Boutrou et al. (112) have pointed out that although G. candidum is capable of using casein and peptone, in cheese its role is mainly peptidolytic rather than proteolytic. Bitterness in Camembert emerges at this step when the proteinases of P. camemberti degrade high-molecular-weight peptides to low-molecular-weight peptides (Fig. 3B) (113). The αs1- and β-caseins have hydrophobic regions that, when released by proteinases, increase bitterness (106, 114, 115).

The size of peptides (molecular mass < 6,000 Da), the nature of the N-terminal amino acids, and the higher mean hydrophobicity of amino acids in a peptide chain are factors implicated in bitter taste (109, 116). Peptides containing proline have been associated with bitterness in cheese, as have peptides with basic amino acids such as lysine and arginine in the N-terminal position (117, 118, 119). In one study, a synthetic peptide with arginine in the N-terminal position was ~250 times more bitter than caffeine (116). A high content of the hydrophobic amino acid leucine in a peptide also increases bitterness (120). Amino peptidases, in cleaving the N-terminal amino acids of a hydrophobic peptide, can reduce bitterness (121, 122). To that end, amino peptidases from Aeromonas caviae and the fungi Rhizopus oryzae and Aspergillus sojae have been used to debitter protein hydrolysates (120, 123). The released free amino acids are precursors to flavor compounds, and their catabolism is significant in mold-ripened cheeses (114). Through reactions such as deamination, transamination, and decarboxylation, the amino acids are converted to alcohols, carbonyls, or short-chain hydrocarbons (Fig. 3D) (106). Deamination of glutamate, aspartate, leucine, phenylalanine, and methionine by G. candidum has been reported (124). The breakdown of sulfur-containing amino acids such as methionine by G. candidum produces desirable garlic or cabbage aroma notes characteristic of some pâte molle and smear cheeses (125, 126). Molimard (127) correlated the cabbage aroma of traditional Camembert with the concentration of isovaleric acid, a derivative of leucine via Strecker degradation, which has also been found in Swiss cheese (128). Jollivet et al. grouped eight G. candidum strains according to their production of methyl ketones, secondary alcohols, phenol, phenylethanol, and dimethyl disulfide, when grown in milk and cheese media (129). All strains produced the compound 2-methylpropan-3-ol, an alcohol previously not identified in cheese.

We studied the extracellular amino peptidases of 13 wild-type G. candidum strains isolated from Camembert, Mont d’Or, Reblochon, Saint-Nectaire, Coulommiers, and Bethlehem cheeses chosen as representatives of major groups in our RAPD-PCR study. We found that differences in the enzyme activity (the hydrolysis of p-nitroanilide derivatives of lysine, arginine, leucine, methionine, and alanine) correlated with the complexity of microbial populations characteristic of these types of cheeses (unpublished data). Three strains isolated from Saint-Nectaire and 1 from Bethlehem cheese clearly had the highest activity of the 13 isolates tested. The strain with the highest activity of the 13 tested in the release of leucine, alanine, and methionine was the only
American strain, isolated from a 45-day-old Bethlehem cheese. The activity of strains isolated from cheese with complex fungal populations was in sharp contrast to that of the 4 strains isolated from Reblochon from two different cheesemaking facilities. Reblochon has a less complex microbiota than Saint-Nectaire. The gray covering seen on Saint-Nectaire and Bethlehem cheeses is due to the zygomycete Mucor, strains of which are known to be highly proteolytic. Indeed, the acid proteinases produced by Mucor pusillus and Mucor miehei which cleave the Phe
superscript 105–Met
superscript 106
peptide bond of κ-casein have been used commercially for microbial and genetically engineered rennet for years due to the shortage of calf rennet (130). Saint-Nectaire strains may thus be selected for by the accumulation of peptides released by Mucor proteinases that are, in turn, metabolized by the aminopeptidase of G. candidum. In contrast, a strain that was isolated from Saint-Nectaire curd before Mucor had developed showed little activity.

Besides having a less complex fungal population, Reblochon is dominated by the bacterium Brevibacterium linens, which is highly proteolytic, possessing both proteinases and aminopeptidases, and has been used as an adjunct in cheesemaking to accelerate ripening (106, 131). The pathways of methionine catabolism by B. linens have been investigated because the by-product, methanethiol, gives Reblochon and other surface-ripened cheeses their characteristic flavor (132, 133). Since B. linens has aminopeptidases, strains of G. candidum with high aminopeptidase activity may not be selected for in Reblochon.

Not all strains of G. candidum are equal. Some cause defects in cheese described as greasiness, bitterness, and a strong yeasty flavor (134). On some cheese rinds, the proteolytic activity of G. candidum causes a wrinkly appearance that is referred to as peau de crapaud, or “toad skin,” by French cheesemakers. This texture is desirable on some cheeses, such as goat cheeses; but in excess causes a slippery rind. When the cheese is turned during ripening, the skin can separate from the cheese surface, thus ruining the integrity of the rind. To stop excess growth, the cheesemaker may lower the temperature and/or humidity of the production area and cave. Due to G. candidum’s sensitivity to salt (inhibition can occur at 1.0 to 2.0% salt), using a higher concentration of salt or changing the salting method from brining to dry salting may resolve the problem of excessive growth (108). Attention must also be paid to the amount of inoculum. Le Bars-Bailly et al. (108) note that one spore of G. candidum per kilogram of curd introduced during cheese production can lead to a population of 10⁸ arthrospores per gram of cheese after 21 days of ripening at 10°C. The threshold for a yeasty taste is between 4 × 10³ and 10⁴ G. candidum spores per gram of curd.

The neutralization of the cheese surface by G. candidum and by other yeasts prepares the cheese surface for acid-sensitive bacteria, such as Brevibacterium linens, which follow in the later stages of ripening (67). B. linens grows best at a neutral pH of 7.0 (135). In experimental Camembert-type cheeses inoculated with G. candidum, the pH of the rind reached 7.0 at the end of ripening, whereas the pH of control cheeses remained at about 4.8 (112).

The increase in pH is generally attributed to the catabolism of lactic acid produced by starter bacteria to carbon dioxide (CO₂) and H₂O (72, 136). In our diversity studies, the four wild-type G. candidum strains isolated from Reblochon were all positive for assimilation of lactate as the sole source of carbon. The four producers used yogurt as the source of starter culture, perhaps selecting for strains capable of using lactate as a source of carbon.

Release of ammonia also plays a role in neutralizing the cheese environment. Studies of the growth kinetics of G. candidum on peptone-based medium with and without lactate showed that G. candidum preferred peptones as a carbon source, even when lactate was present in the medium. Since lactate consumption was significant only at the end of growth, it was concluded that peptone was metabolized for cellular biosynthesis and lactate for cell maintenance (137, 138). An increase in pH in the curd then results from both the assimilation of lactate as a carbon and energy source and the release of ammonia through the metabolism of amino acids. Ammonia is a significant part of the aroma compounds of Camembert (124). In the ripening of soft cheese, ammonia in the atmosphere can reduce Penicillium growth, thus lowering the level of proteolysis and bitterness (84).

The role played by G. candidum in controlling pathogens and contaminants in cheese continues to be explored. The safety of raw milk mold-ripened soft cheeses has been called into question due to the vulnerability of these cheese types to contamination, particularly by Listeria monocytogenes, due to a high moisture content and high pH of the rind (139). It has long been assumed that pathogens will not survive in these cheeses after a 60-day ripening period at a temperature of ≥1.67°C (>35°F). However, recent studies and epidemiologic data have shown that neither a 60-day ripening period nor, surprisingly, pasteurization ensures the safety of this class of cheeses (140). Dieuleveux et al.
(141) have shown that *G. candidum* can inhibit the growth of *L. monocytogenes*. The inhibitors were identified as d-3-phenyllactic acid and d-3-indollactic acid; these compounds were stable over a wide pH range and could be heated at 120°C for 20 min. d-3-Phenyllactic acid altered the cell wall of an antibiotic-resistant strain of *L. monocytogenes*, producing depressions and holes that were visualized with scanning electron microscopy (142, 241). *G. candidum* also inhibits the growth of the zygomycete *Mucor*, whose aerial hyphae (cat hair) are responsible for the cheese defect *poil de chat* (cat hair). The inhibition is strain and culture dependent (143). *G. candidum* may inhibit the sporulation of certain strains of the mycotoxin producer *Aspergillus flavus* (144) and undesirable species of *Penicillium* such as *P. commune* and *P. caseifulvum*, which can overtake fungal starters (145). More research is needed on the potential of *G. candidum* for microbial competition.

The results of our own studies of *G. candidum* suggest that cheese technology and the microbial ecology of a cheese rind select for populations of *G. candidum* strains with different biochemical properties. The variables in cheese technology and ripening—salting, humidity, temperature, and materials in the cave such as wood or rye straw—selected for strains with unique characteristics. The fact that a strain from Bethelhem cheese, a Saint-Nectaire-type cheese made in the United States, would have biochemical properties similar to those of strains from Saint-Nectaire in Auvergne, France, tends to confirm the hypothesis that technology breeds the strains.

**The Price of Innumerable Failures: The Ugly**

One will never know the patience and groping it took to put into place the recipes for the fabrication of cheeses. It is the principal merit of the humble farmers and silent monks to have succeeded at the price of innumerable failures to refine and perfect the assortment of cheeses of which we are justifiably very proud.

*Les Fromages, Pierre Androuët* (54)

Ask anyone who has tried making cheese and he or she will probably tell a tale of “innumerable failures” before and sometimes after success is achieved. The natural succession of microorganisms (and sometimes fauna) on cheese during ripening has its benefits and risks. A novice affineur may wonder if the pile of dust he saw on a cheese or shelf an hour earlier really had moved to another location. This observation would mark his first encounter with cheese mites, the microscopic Arachnida *Acarus farris* and *Tyrophagus neiswanderi* (146).

![FIGURE 4 Challenges for the cheesemaker. (A) Scanning electron micrograph of a cheese mite. Left unchecked, cheese mites can lead to a 25% reduction in the weight of a ripened cheese. Photograph by William R. McManus, generously provided and used with permission of William R. McManus and Donald J. McMahon, Utah State University. (B) Spoilage of a cheese by contamination and invasion of the rind by the fungal genus *Scopulariopsis*. (C) Scanning electron micrograph of the zygomycete *Mucor* showing sporangia among collapsed hyphae. When the fragile outer membrane, the peridium (arrowhead), of the sporangium breaks open, hundreds to thousands of spores can be released, contaminating a cheese cave overnight. Bar, 10 μm. doi:10.1128/microbiolspec.CM-0005-2012.f4.](image-url)
(Fig. 4A). In the Auvergne, cheesemakers call mites *artisans*, and in Haute-Savoie, they call them *cirons*. Cheesemakers may notice that mites are abundant after a humid season, following heavy fungal growth on the rind. They have asked, “Are the mites eating the cheese or the fungi?” *Tyrophagus* mites isolated from the Spanish mold-ripened cheese Cabrales are indeed fungivores (147). *Tyrophagus putrescentiae* commonly infest foods with high fat and protein contents (148), which would certainly include cheese. The compounds cis- and trans-octa-1,5-dien-3-ols produced by *Trichothecium roseum* have been found to be strong attractants for the mite (149). There is a relationship between mites and fungi from which both may benefit. Fungal spores can be transported to new substrates, and mites can promote a compensatory growth of fungal mycelium as new surfaces are created for the aerobic fungi (150). The mites’ choice of species of fungi upon which to feed depends upon their capacity to digest the fungus. Cell walls of fungal mycelium are composed of chitin and chitosan, but in experiments on the digestibility of fungi by various mite species, chitin passed through the digestive tracts of mites undigested. Trehalose in the mycelium, however, was digested by the mites, indicating that the mites possess the digestive enzyme trehalase (150). Of the mite species tested, trehalase activity was highest in the *Tyrophagus*.

The presence of mites affects the appearance of the rind and certainly the taste of the cheese. According to Sánchez-Ramos et al., “Mites feed and reproduce on cheese surfaces producing an accumulation of dead mites, faeces, exuvia, eggs and bits of food, which appear as a light brown dust that can reach a depth of 2 cm or more in heavy infestations” (146). This may not appeal to everyone’s tastes, yet *Tyrophagus casei* mites are deliberately introduced into the German cheese Altenburger (151). The very hard cheese Mimolette from Lille, France, which can be aged for 2 years, develops its distinctive flavor with the help of mites. Yet on the whole mites are a nuisance, especially for Cheddar makers, and their removal is time-consuming, expensive, and often frustrating.

At 25°C and a relative humidity of 80 to 90%, one generation of fungivore mites takes 10 days. Adult mites can live for about 2 to 5 months, and *Acarus farris*, the predominant species in Cabrales cheese, can reach a population density of 260 mobile mites/cm². It should come as no surprise, then, that these *artisans* carving and eating their way through the cheese surface, if left unchecked in a cave, can be responsible for a loss of up to 25% by weight of the final product (146).

Not only do cheese mites cause economic loss, but also their presence can cause enteritis, diarrhea, and damage to the urinary tract (148). They are the source of allergens implicated in asthma, rhinitis, and atopic dermatitis. The storage mites *Acarus siro*, *A. farris*, and *Tyrophagus putrescentiae* can cause occupational allergies for farmers and cheesemakers (152), and the affineur who spends long hours without a mask in a mite-infested cave is vulnerable to a condition known as “cheesemaker’s lung” caused by inhaling mites and their dust (153). Mites can be the vectors of microorganisms (154), and some have even suggested that hay mites may be a self-sustaining reservoir for the prion which causes bovine spongiform encephalopathy, or mad-cow disease (155).

Control of mites continues to be a challenge for cheesemakers. Generally, conditions in a cave which are best for cheese ripening, with temperatures ranging from 10 to 15°C and relative humidity near 90%, are also favorable for mite populations (156). In the past, methyl bromide fumigation and organophosphate insecticides were used to control mites, but these have been banned in consideration of food safety (156, 157). Many affineurs still find that mechanically removing mites from cheese by brushing or vacuuming is the best and safest method. Other measures have been tried, such as washing cheeses with hydrogen peroxide after vacuuming and introducing ozone into the cave for a few hours a day (158). The Bleu Mont Dairy Company in Wisconsin has used diatomaceous earth (DE) as a successful material for mite control. DE does not leave harmful residues, nor is it toxic to mammals (159). DE injures mites through a physical mode of action, rather than chemical. The mite’s protective cuticle is damaged through abrasion and adsorption of the cuticular waxes to the DE, which leads to dehydration of the insect’s body (160).

Other approaches to control include the use of modified-atmosphere (MA) technology that alters the natural ratio of the atmospheric gases, oxygen (O₂), nitrogen (N₂), and CO₂ (157). Mobile stages of larvae, nymphs, and adults are controlled quickly, whereas preventing egg hatching is more difficult. One study reported 100% mortality in the egg stage using 99.5% CO₂ at 85% relative humidity after 6 days of exposure, while the mobile stages of the mites were killed in 1 day under the same conditions. Preventing egg hatching should therefore be the goal of any technology. Monoterpenes, including eucalyptol, showed acaridical activity against mobile stages of *Tyrophagus putrescentiae* through vapor action but adversely affected the flavor and odor of the treated cheese, so this approach was
abandoned (161). Other approaches include altered ripening temperature and humidity levels; a lower ripening temperature used with Cabrales cheese reduced mite density (156).

Problems with fauna aside, some fungi are considered contaminants that lead to defects in appearance and flavor (162) and, on occasion, the loss of entire batches of cheese. The American mycologist Charles Thom described the filamentous fungus *Scopulariopsis brevicaulis* growing on Camembert in 1930 while working for the U.S. Department of Agriculture at the Agricultural Experiment Station in Storrs, CT. He described how *S. brevicaulis* “attacks Camembert cheese, overgrowing the Camembert mold, producing an ammoniacal odor and imparting an ammoniacal taste to the cheese” (163). In 1951 Keilling et al. described what they called a “cheese canker” in Emmental, Gruyère, Edam, and Gouda, which “manifests itself during ripening and storage through the appearance of little holes dug into the rind of the cheese, containing a white, yellow, or brown floury substance.” They attributed the canker to yeast and a fungus, *Penicillium brevicaulis*, a previous name for *Scopulariopsis brevicaulis* (164). Unlike most fungi, which prefer an acidic medium for growth, *Scopulariopsis* grows best in alkaline and high-nitrogen substrates, with an optimum growth pH of 9.0 (163). *Scopulariopsis* reportedly is more apt to grow on pasteurized rather than raw milk Camembert and Carré de l’Est, because the lactic acid bacterium population in the pasteurized version is less complex, leading to a weaker fermentation and lower acidity (165). *Scopulariopsis* spp. can contaminate many classes of cheese, with *Scopulariopsis fusca* predominating, followed by *S. brevicaulis* (108, 134). Samples of the aged pâte molle cheese Pont-l’Évêque collected in four different departments of Normandy showed contamination by the fungus, as did aged artisanal goat cheeses and pressed hard cheeses such as raclette and Gruyère (108, 134). *S. fusca* appears as powdery brown or mauve spots on a cheese rind and is a common contaminant along with *Penicillium* species (108, 134). Artisanal cheesemakers recognize only too well the “cankers” in the rind and the strong smell of ammonia produced by *Scopulariopsis* in their caves. The holes in the rind created by *Scopulariopsis* allow it and other fungi to penetrate into the curd. To add insult to injury, Keilling et al. warned that “mites invade the cavities which they extend considerably deeper” (164). Many cheesemakers have observed that *Scopulariopsis*, besides destroying the integrity of the rind as shown in Fig. 4B, creates defects in flavor such as bitterness and a musty taste.

*Scopulariopsis* species are commonly found associated with soil, plants, and animal dwellings (166, 167). They are considered anamorphs of the fungal class *Microascaceae* (240) and microscopically can be confused with *Penicillium* species. The conidia of *Scopulariopsis* are oval and form in chains, with a truncated base attached by a ring to a vial-shaped conidiophore (165). The conidiospores often have rough surfaces and are hyaline. Though not food-borne pathogens, five species have been associated with human diseases, and they can be serious emerging pathogens for immunocompromised individuals (167, 168, 169).

The fungus possesses an array of enzymes that increase its capacity to grow and flourish within the environment of a ripened cheese rind. It is highly proteolytic: its keratinases, which break down keratins, the principal proteins in hooves, claws, and feathers, are being studied for use in the field of bioremediation (170). Its mode of invasion of a substrate is specialized boring hyphae that penetrate fibers perpendicularly to the surface (171). Considering that the fungus can penetrate the spines of hedgehogs and cattle hair, it should not be surprising that *Scopulariopsis* species bore their way through cheese rinds. Since it prefers an alkaline environment, from an ecological standpoint it follows that *Scopulariopsis* would grow late in the ripening process. On a ripened cheese surface, yeasts and fungi raise the pH through lactate metabolism and ammonia production via proteolysis (44). Bothast et al. noted that *S. brevicaulis* breaks down more protein than it uses for synthesis when insufficient carbohydrate is present in the substrate (163), which is probably the case in ripened cheeses, which contain only trace amounts of residual carbohydrate (172). The fungus then liberates nitrogen as ammonia from the excess and utilizes carbon for energy production (163). Atmospheric conditions in a cheese cave, including concentrations of CO₂, oxygen, and ammonia, determine microbial growth and enzyme activity (173). In their study of atmospheric conditions during ripening of Camembert, Leclercq-Perlat et al. found that at high levels of CO₂ and low levels of oxygen, the yeast-like fungus *Geotrichum candidum* grew well, whereas *Penicillium roqueforti* was negatively affected (174). It would seem that an efficient ventilation system in a cave which would allow for adjustments in atmospheric conditions, particularly removal of ammonia, would play a role in controlling *Scopulariopsis*. A low-tech natural solution used by some cheesemakers to slow down *Scopulariopsis* growth is to wipe the cheese surface and shelves with vinegar.

Among its other enzymes, *S. brevicaulis* has an extracellular chitin deacetylase, which enables it to use
chitin as a sole source of carbon (175). This enzyme may enable *S. brevicaulis* to feed on fungi of preceding populations on the cheese rind, using chitin contained in their cell walls. Other enzymes of *Scopulariopsis*, such as xylanases, are of interest to the paper and pulp industry for their bleaching capacity and the breakdown of lignin (176). *Scopulariopsis* is often carried into the ripening environment on wrapping paper, so such paper should be stored in a separate area (134, 177).

*Scopulariopsis* species are halotolerant, halophilic, and xerophilic; they grow well in salty foods such as cured fish and smoked bacon (178), in environments of high salt concentration (179), and in salt-based media (180). During cheese ripening, water evaporates from the surface, thus lowering the water activity of the rind. Fox et al. report that after 10 days of growth in nutrient broth of pH 6.6 at 25°C, *Scopulariopsis fusca* grew at 14% of its maximum development at a salt concentration of 20% (water activity, 0.880). At the same time, *Penicillium candidum* grew at 1.1% and *Mucor mucido* and *Geotrichum candidum* were completely inhibited (172). Keilling et al. suggested that on Comté and Gruyère-type cheeses, meticulous wiping and care of the rind are critical during the first 15 days of ripening, when the cheese rind is still acidic and the desired morge, or smear, is still developing on the surface. When the morge does not develop quickly enough, patches of *S. brevicaulis* can appear (164). The members of the microbiota comprising the morge, including *Brevibacterium linens*, are salt tolerant and from the 15th to 30th day of ripening reach a maximal growth of 10^9 to 10^10 microorganisms per cm^3 on the rind (181). Adjusting salt concentrations and/or care of the rind may inhibit undesirable fungi such as *Scopulariopsis* and select for desirable species to compete for nutrients on the cheese surface.

Considering what a widespread problem *Scopulariopsis* is for the cheese industry and the lack of dairy-related research data currently available, cheesemakers would benefit from investigations into modes of its inhibition. In a recent investigation into antifungal activity by bacteria, eight strains of lactobacilli isolated from vegetables and one strain of *Propionibacterium jensenii* of dairy origin strongly inhibited the growth of *Scopulariopsis brevicaulis* (182). It is of interest to note that while many classes of fungi were inhibited, *Geotrichum candidum* was not, which is good news for cheesemakers. Testing of fermentative bacteria that exhibit antimicrobial activity while not affecting sensorial quality within a cheese environment could be of interest.

The fungus that is probably the most common cause of what French cheesemakers call *accidents de fromages* (loosely translated as cheese disasters) is the zygomycete *Mucor*. As mentioned earlier, *Mucor* is part of the normal microbiota of Saint-Nectaire *fermier* (farmstead) cheese and Tomme de Savoie (58, 63). However, when *Mucor* appears on a *pâte molle* cheese, it can instill fear in the heart of a cheesemaker or affineur. A cheese such as Camembert, meant to be covered with a uniform white coat of *Penicillium camemberti*, can become covered with tufts of grayish-white hairs, a defect known as *poil de chat*. Spore germination is the critical step in the growth of food spoilage fungi because “a product is spoiled as soon as visible hyphae can be observed” (183). At the tip of each sporangiophore in *Mucor* is a sporangium that appears as a black or gray ball to the unaided eye (143). The electron micrograph of the surface of a 9-day-old Bethlehem cheese in Fig. 4C shows two sporangia among collapsed *Mucor* hyphae. One can see the fragile outer membrane, called a peridium, of the larger sporangium breaking apart, revealing the spores. One sporangium can liberate thousands of spores (108) that will spread over the cheese surface and eventually impart a grayish coat to the cheese. This may explain why *Mucor* has earned the name *la bête noire* (the black beast) by some traditional cheesemakers. It grows 5 to 10 times faster than *Penicillium* species (108), giving it a competitive advantage over *Penicillium camemberti*. When *Mucor racemosus* was grown in potato dextrose agar medium at 25°C, its hyphal extension rate, based on the measurement of colony radial growth, was 13.3 mm per day (183).

*Mucor* is ubiquitous in the environment and, because of its proteolytic and saccharolytic activities, is responsible for decomposing fruit, meat, and bakery products (184, 185). Though not a food-borne pathogen, *Mucor* can cause mucormycosis, an infection of the sinuses, brain, or lungs to which immunocompromised populations are most vulnerable (186). Zygomyces have rarely been reported to produce mycotoxins (183). *Mucor racemosus* is the most widespread cheese contaminant, but other *Mucor* species associated with cheese are *M. plumbeus*, *M. biemalis*, *M. globosus*, *M. fuscus*, and *M. mucido* (108). A characteristic that distinguishes *Mucor* from other genera of the zygomycetes is its capacity for dimorphism (187), allowing it to adjust its morphology to changing environmental conditions. In an anaerobic environment and in the presence of a fermentable sugar, this filamentous fungus can assume a yeast-like morphology (185). In 1876, Louis Pasteur described the dimorphic capability of *Mucor racemosus*, which was implicated in the fungal contamination of beer (184). Le Bars-Bailly et al. (108) noted that in a
cheesemaking facility, the filamentous morphology of *Mucor racemosus* predominates on the aerated surface of a cheese, whereas under a cheese, where less oxygen is available, cells may assume a yeast-like morphology.

Zygomycetes grow poorly under conditions of low water activity (183). With *Mucor*’s preference for high humidity, the dairy environment presents many opportunities for contamination to which cheesemakers must be alert. Its optimum growth conditions include a temperature of 20 to 25°C, a pH of 5.0 to 6.0, relative humidity of 90 to 95%, and water activity of >0.95 (188). The spores of *Mucor* are described as “myxospores” due to the tendency of their cell walls to absorb moisture, facilitating their adherence to various surfaces. The spores stick to clothes, cheese molds, utensils, and the cheesemaker’s hands, thrive in liquids such as whey and stagnant water, and are dispersed in moist air (188, 189). Once contamination has occurred, it is difficult to get under control, so prevention is the first goal.

During cheese production, newly formed cheeses are extremely vulnerable to contamination during the draining of whey. The high humidity in the draining room and the high moisture content of the undrained curd favor *Mucor* development. The temperature of the room ideal for drainage (24 to 27°C) is also ideal for germination of *Mucor* spores (189). Whey contains abundant lactose (about 70% of dry solids) (172), and through its β-galactosidase activity, *Mucor* is able to break down lactose into glucose and galactose (190). The presence of lactose in the whey allows for growth of the yeast-like form of *Mucor* in the anaerobic environment of the interior of the curd (189). Acidification of the curd in production enhances the expulsion of whey and reduces drainage time. If cheese is made with milk with a high fat content, good drainage may not occur (188, 189). The use of fresh milk for cheesemaking minimizes the time that psychotropic bacteria can break down the casein in milk, providing polypeptides as a substrate for *Mucor* (189). It is also recommended that draining cheeses be covered to avoid airborne spores. Tormo and Barral suggest that if *Mucor* growth is visible on cheese 1 to 2 days after the curd is placed in the forms, one should suspect that the milk used in production was the original source of contamination. When *Mucor* appears 3 to 4 days after draining and the entire cheese is covered with hyphae, then the curd, cheese forms, and utensils should be suspected; if growth appears on only one side of the cheese, the atmosphere in the room is the source (188).

*Mucor* can be controlled by fungi and bacteria involved in cheese production and ripening. The sporulation of *Mucor* can be partially inhibited by good growth of *G. candidum* on the rind of a *pâte molle* cheese during the early stages of ripening. This competitive inhibition is strain dependent for *G. candidum* (144, 191). Le Bars-Bailly et al. observed that since *Mucor* needs access to nutritive medium to grow, a previously implanted thick, uniform layer of *G. candidum* creates a physical barrier between *Mucor* and the cheese substrate (108). As mentioned earlier, tolerance to salt plays a large role in growth and survival in or on cheese. Cheeses of the *pâte molle* type, such as Camembert and Brie, generally are salted within the range of 1.5 to 2.0% (wt/wt). The cheesemaker may have to adjust the degree and method of salting if a good implantation of *G. candidum* does not occur. *Lactobacillus coryniformis* subsp. *coryniformis* Si3 has displayed a broad spectrum of antifungal activity against *Mucor hiemalis* as well as *Aspergillus* and *Penicillium* species (192), and *Brevibacterium linens* has shown antifungal activity via thiol production (143). Using modified atmospheres for preventing fungal growth and mycotoxin production to extend the shelf life of some kinds of food was evaluated (183). Taniwaki et al. found that the growth of *Mucor plumbeus* and other fungi could be reduced in atmospheres containing 20 to 40% CO2 with 1 to 5% O2 (193). Tormo and Barral report that *Mucor* is sensitive to ammonium and UV light (188).

*Penicillium roqueforti* and *Penicillium camemberti*, growing naturally or added as secondary starters, contribute to the flavor and unique characteristics of blue-veined and *pâte molle* cheeses, respectively (52, 91). However, when they or other species of *Penicillium* grow on the wrong type of cheese, they are considered contaminants that can cause great economic loss (108). *P. roqueforti* can contaminate Camembert and other *pâte molle* cheeses (194) as well as hard cheeses such as Emmental and Parmesan (195), and *P. caseifluvum* can cause yellow spots in blue-veined cheeses (196). Other *Penicillium* species—*P. commune*, *P. solitum*, *P. palitans*, and *P. crustosum*—are especially problematic because they are closely related genetically to *P. camemberti*. *P. camemberti*, the widely used starter in Camembert and Brie, is considered a domesticated species derived from *P. commune* (195). The taxon *P. commune* was first introduced by Thom in 1910 because it was the major source of cheese contamination in the United States (197). According to Lund et al. (198), it remains the most widespread spoilage fungus, causing discoloration and flavor defects in cheese. The authors have observed that one conidium of *P. commune* on a cheese can develop into a colony of 10,000 CFU in 4 days. Therefore, great care
must be taken to prevent the dispersal of contaminating conidia in the air and on hands, clothes, and packaging. Biocontrol of penicillia has also been noted. *G. candidum* can inhibit the growth of *P. roqueforti* in blue-cheese medium without NaCl, and some strains of *P. camemberti* inhibit the growth of *P. roqueforti, P. caseifuinum*, and *P. commune* (196).

**TALES OF MOLD-RIPENED CHEESE**

Difficulties arise when one attempts to describe linkages between natural and human history of cheesemaking in the context of its science. Perspectives range from the small farmer interested in *terroir*—that elusive property associated with the specificity of space, including climate, soil, and people—and the industrialist who wishes to control all aspects of a ripening process to control product consistency. An example of these differing perspectives plays out in the Auvergne region of France.

The Auvergne is a volcanic region in the Massif Central dominated by the Chaîne des Puys, a series of volcanic cones, domes, and craters west of Clermont-Ferrand (199). On the route from Montaigut-le-Blanc to Champeix in Puy-de-Dôme, the arched openings to the caves of Saint-Julien where wine was aged before the powdery mildew (*Oidium tuckeri*) and phylloxera epidemics of grapevines, can be seen from a distance. Some of those caves belong to the Bellonte family.

The elusive link of cheese to its environment is immediately apparent as one enters the Bellonte caves and sees hundreds of Saint-Nectaire cheeses laid out on rye straw on the floor. The owner, Alphonse Bellonte, speaks passionately about the patrimony of the Auvergne. He is fiercely attached to his land and cheese. The pride and spirit of resistance expressed in his words are common to the people of the Auvergne. The region is named for the Celtic tribe Arverni, best known for their chieftain Vercingétorix, who led his troops to victory over Julius Caesar's forces at Gergovia in 52 BCE (200). Although he was later defeated and taken in chains to Rome, he remains a symbol of heroism to the region (201).

Saint-Nectaire is an uncooked semihard surface mold-ripened cheese that has been made since at least the 17th century in two departments, southwestern Puy-de-Dôme and northern Cantal. It is a PDO cheese, and the 69 communes of origin are characterized by the geographical and geological diversity found in the Sancy Mountains, the plateaus of Cézallier and Artense, and the valleys known as Pays coupés (42, 202). The cheese was named after Henri de Senecterre (1573–1662), who is said to have introduced it to the court of King Louis XIV (203). The Bellonte family has made Saint-Nectaire for eight generations in the town of Farges, 3 km from Saint-Nectaire, a village located on the top of Mont Cornadore and renowned for its Romanesque church and ancient Roman thermal baths. Farges was built in the volcanic crater of the Massif du Sancy, the oldest mountain in the Auvergne, and is part of the Regional Park of the Auvergne Volcanoes.

The decline in population and number of farms in Farges since 1870 reflects Alphonse’s sentiment: “Modern agriculture has made French countryside a desert.” In 1870, there were 90 inhabitants and 22 farms; in 1945, 46 inhabitants and 10 farms; in 1995, 23 inhabitants and one 474-ha farm, that of the Bellonte family. During the summer, cows graze in mountain pastures. The herd is composed of Montbéliardes, a breed originating in the Jura region of France. In the past, milk of native breeds of the Auvergne, such as Ferrandaise and Salers, was required for making Saint-Nectaire with the AOC designation (204). The Salers, a red cow whose ancestors were depicted in cave art near the town of Salers in Cantal in the Haute-Auvergne, is considered one of the oldest of all European breeds (http://www.ansi.okstate.edu/breeds/cattle/salers/index.htm). This dual-purpose cow is well adapted to the rugged terrain and climate of the high mountains. These cows are often pictured on labels for Saint-Nectaire cheese (203), yet many farmers no longer milk them because a calf must be tethered to the mother during milking (205). This requirement makes for a fairly chaotic pastoral scene not necessarily practical for dairy operations today.

The Bellonte Montbéliarde cows are fed only hay and grain, never silage. Silage is not allowed in the production of most AOC cheeses since it may contaminate milk with spores from *Clostridium* species, particularly *C. tyrobutyricum* and *C. butyricum*. These strains produce carbon dioxide and hydrogen gases during the butyric acid fermentation that cause openings, splitting, and “blowing” defects in cheese, particularly in large cheeses such as the Swiss types (206, 207). Silage can also be a source of mycotoxin-producing fungi and the foodborne pathogen *Listeria monocytogenes* when silage has undergone deterioration aerobically (208, 209, 210).

The Bellonte farm makes Saint-Nectaire fermier cheese using raw milk produced only on their farm. The production of Saint-Nectaire laitier (industrial Saint-Nectaire) using pasteurized milk collected from many producers began in the region in 1964, and at the time it was feared that farmstead production would be threatened (211). However, Saint-Nectaire fermier is alive and well: of the 13,676...
metric tons of Saint-Nectaire produced in 2005, 6,194 metric tons were fermier and 7,482 metric tons were laitier (http://www.fromages-de-terroirs.com/fromage-detail.php?id_article=1634&lang=en). The Bellontes make 45 metric tons of Saint-Nectaire fermier annually.

The bulk of Saint-Nectaire produced by the Bellontes is aged in the caves of Saint-Julien that were dug from tuff (porous volcanic rock) in 1813; some also is aged in caves in Farges dating to medieval times (742–814 CE). The medieval caves were dug from stone, called pierre de Farges, that is very porous and retains moisture, thus providing an excellent natural environment for the growth of fungi. Until the 1890s, the caves of Saint-Julien were used to store wine, but by then the effects of phylloxera (Daktulosphaira vitifoliae), the grapevine louse that destroyed the vines of France, had reached the Auvergne. As Gale describes it, “...the entire wine-growing world was not only affected but transformed—a agriculturally, economically and socially—by the plague. Effects of the disaster rippled out from the vineyards into their embedding cultures, invoking such large-scale consequences as rural depopulation and massive emigration” (212). Until that time, the Auvergne had been the third-largest winemaking region of France, but it would never again regain that stature.

Many of the caves in the countryside of Saint-Julien are abandoned, but others are used to store root vegetables or ripen cheese. In compliance with regulations of the European Union, the cheeses are now ripened on plastic mats on wooden shelves and not directly on rye straw on the cave floor (213). In many Saint-Nectaire caves, the cheesemaker slips stalks of rye straw between the mats and shelves or into the plastic crates of aging cheeses. This modification may be seen as resisting the regulations, or as Vollet et al. suggest, the cheese is presented on a bed of rye straw as a marketing tool. The regulations, or as Vollet et al. suggest, the cheese needs to be milked a second time is called pinch a cow’s teat.” According to the peasants hand-milking a cow, the sound of a stream of milk hitting a pail is similar to ploutch, which developed into the verb blocher (http://projetbabel.org/forum/viewtopic.php?t=9246). Re-blocher means to “milk again,” and a cow which holds back her milk and needs to be milked a second time is called une vache reblochonniuse (219). In the 13th century, farmers were taxed according to the quantity of milk produced by their herds, so they began the practice of not milking the cows out completely on the day of the tax collector’s visit. After the tax collector departed, the cows were milked again. To protect the ruse, the small quantity of rich milk obtained from the second milking was quickly transformed into cheese. There is a play on words in Savoyard between reblocher (to milk again) and rablasse or rablache (a fraudulent action), and thus Reblochon came to be known as the cheese of fraud or deception (220). Fresh raw whole milk is still required for producing AOC Reblochon today, and the cheese is made immediately after milking, twice a day. Reblochon fermier (with a green identification label) is made on a farm with milk produced on that farm only, the cheesemakers of the Auvergne have observed for centuries that rye straw placed under ripening Saint-Nectaire seems to produce the best cheeses. From a microbiological standpoint, the use of rye straw is a factor in fungal succession on the cheese rind: namely, it encourages the growth of Trichothecium roseum, the “flower of the molds.” T. roseum is a saprophyte as well as a plant pathogen (215). The fungus is associated with wheat, especially during seasons of high precipitation (216), and is among the fungi that can use wheat gluten as a substrate in the fermentation of minchin, a Chinese cereal-based food (2). T. roseum can overwinter in soil or crop debris, while under conditions of high humidity, such as one would find in a cheese cave, it grows well and sporulates (217). Proteases of T. roseum may warrant investigation to clarify the role of T. roseum in the microbial ecology of cheese ripening.

Some French cheeses have emerged bearing a mix of natural and political history that includes universal truths about the struggles and ingenuity of small farms and partly explains the passion with which traditions are held. Reblochon, for example, is a cheese that originated in the 14th century in the Haute-Savoie region in the Chaîne des Aravis, a mountain range of the pre-Alps (218). It received its AOC appellation in 1958 and is among the first French cheeses to do so. The story behind its inception is often recounted with pride by the French. Reblochon is derived from blocher, which in Savoyard dialect literally means to “pinch a cow’s teat.” According to the peasants hand-milking a cow, the sound of a stream of milk hitting a pail is similar to ploutch, which developed into the verb blocher (http://projetbabel.org/forum/viewtopic.php?t=9246). Re-blocher means to “milk again,” and a cow which holds back her milk and needs to be milked a second time is called une vache reblochonniuse (219). In the 13th century, farmers were taxed according to the quantity of milk produced by their herds, so they began the practice of not milking the cows out completely on the day of the tax collector’s visit. After the tax collector departed, the cows were milked again. To protect the ruse, the small quantity of rich milk obtained from the second milking was quickly transformed into cheese. There is a play on words in Savoyard between reblocher (to milk again) and rablasse or rablache (a fraudulent action), and thus Reblochon came to be known as the cheese of fraud or deception (220). Fresh raw whole milk is still required for producing AOC Reblochon today, and the cheese is made immediately after milking, twice a day. Reblochon fermier (with a green identification label) is made on a farm with milk produced on that farm only,
whereas Reblochon laitier (with a red label) is made in a dairy from milk collected from many farms. In both cases, the cheese is made from milk of three breeds: Abondance and Tarentaise, both native to Haute-Savoie, and Montbéliarde. Reblochon is considered a semihard cheese, but Mariani (75) notes that it could be classified technologically as a transition between pâte molle (moist) cheese, to which it is similar at the beginning of ripening, and a pressed cheese at the end of ripening. It is washed twice during the 4- to 6-week ripening period, encouraging the growth of the coryneform Brevibacterium linens and Geotrichum candidum, which is the only filamentous fungus on Reblochon (221).

On Reblochon, Mucor is considered a contaminant. Bärtschi et al. (221) suggested that contamination of Reblochon with Mucor may be less common than in pâte molle types because the curd is pressed, thus shortening the draining period, when the cheeses are most vulnerable to contamination. Washing the rind during ripening may also reduce the risk. However, Reblochon is not immune to contamination, and some stories illustrate the vigor with which Mucor can foment disaster. One cheesemaker in Haute-Savoie recounted a time in the 1950s when on a Wednesday she returned from her cheese cellar and exclaimed to her husband, “How beautiful the Reblochons are!” The ripened Reblochons were ready to go to market on Saturday. But by Friday morning, the cheeses were covered with poil de chat (Mucor), and in the evening, they were completely covered with black sporangia. The dairy consultant from La Roche-sur-Foron did not find Mucor in their milk and concluded that such a drastic contamination could only be due to a mauvais sort—someone had put a spell on their cheese. He suggested that they contact the priest to perform an exorcism. The outcome of the exorcism is unknown. The competition between Mucor and Geotrichum can be unpredictable and may be one-sided in new caves that have not had time to establish appropriate microbial populations. For that reason, newly established Reblochon caves are sometimes watered down and seeded with Geotrichum candidum (75).

In the area of the Haut-Doubs in the region of Franche-Comté, the Sancey-Richard family has been making cheese since 1961. At 1,463 m of altitude, the Fromagerie du Mont d’Or of Métabief is located 10 km from the Swiss border at the base of Mont d’Or, a summit in the rugged Jura Mountain range. The Jura Mountains traverse the Franco-Swiss border north of the Alps between the Rhône and Rhine rivers and were named by the Celtic tribe the Sequani, who inhabited them in the 4th century BCE (222). The Celtic root jor, later Latinized to juria, means “forest,” which aptly describes these “forest mountains” dominated by forests of Norwegian spruce (223). It was here near the source of the Doubs River that the cheese which perhaps best expresses the landscape from which it arose, Mont d’Or, was created. There are two versions: the French-called Mont d’Or or Vacherin du Haut-Doubs, which is made from raw milk, and the Swiss version, Vacherin Mont d’Or, made from thermized milk. Mont d’Or is a seasonal cheese which according to French law can be made only from 15 August to 31 March (224). Cheesemonger and writer Patricia Michelson describes well the seasonal quality of the cheese: “The first true taste of autumn for me comes when the cheese table in my shop displays Vacherin Mont d’Or. Not for me the bland taste of early September cheeses: I prefer to wait until the end of October—or the weekend when we turn back the clocks in Britain—until the most seductive and sensual of cheeses is available” (225). The timing has its roots in the ancient rhythms of mountainous regions. In late spring, the fête de la transhumance or l’estive, the day the herds and flocks pass through the villages on their way to the higher elevations for the summer, is celebrated with music and dance (226). Traditionally, the herds came down the mountains just after 15 August. A cow decorated with a wreath of flowers on her head leading a herd down the mountain may be the cow that has given the most milk that summer. Once the cows returned to their winter quarters and were no longer grazing on lush pastures, they gave less milk, and thus, Mont d’Or, a small cheese in comparison to other celebrated cheeses of Franche-Comté such as Gruyère de Comté and Morbier, was created. It is often called the Christmas cheese because it is available during the holiday season, at which time it is in great demand throughout France.

Mont d’Or is a creamy croûte fleurie (flowered crust) cheese that must be made at an altitude of 700 to 1,400 m. At Métabief, the cheese is produced with the milk of local herds of Montbéliardes, the popular breed that has been exported from her native Franche-Comté to many regions in France. The cheese is made in copper vats, and Patrick Sancey-Richard explained that since milk contains very little copper, the vats encourage the growth of microorganisms that require copper for their growth.

This master cheesemaker’s observation about the use of traditional materials provides an example of the empirical knowledge of microbial ecology gleaned through centuries of hands-on experience, the complexity of which scientific research is only beginning to elucidate.
European cheesemakers have long considered copper vats essential to Emmental production. Sieber et al. speculate that in the evolution of humans’ use of metal, the extraction of copper from ores brought an end to the Stone Age 9,000 years ago. Copper is a prized metal because it is malleable and, second to silver, the best conductor of electricity and heat (227). Copper is required as a micronutrient to serve as a cofactor for metalloproteins and enzymes involved in microbial metabolism (228, 229). Cow milk contains very little copper (0.1 to 0.2 ppm) (228). However, the use of copper vats for cheesemaking brings the copper content of the finished cheese to 7.6 and 16.5 ppm due to the leaching of copper ions by the casein in milk (230). As copper vats have been replaced by stainless steel in the production of Emmental, the addition of CuSO₄ salt to the cheese milk has become standard practice. However, in organic cheesemaking operations, the supplementation with copper sulfate is not allowed (231). Similarly, the concentration of added copper must be finely tuned, as some authors have noted that supplementary copper can inhibit the growth of starter and adjunct cultures such as Streptococcus, Lactobacillus, and Propionibacterium species critical to Emmental production (230). Investigations into the inhibition of food-borne microorganisms by copper are promising. Mato Rodriguez and Alatossava (231) report that the use of copper vats in cheese production inhibits the germination, vegetative growth, and sporulation of strains of Clostridium tyrobutyricum, the bacterium known to cause the late-blowing defect in Swiss-type cheeses. Noyce et al. (232) found that food surfaces composed of a copper alloy inhibited the growth of the food-borne pathogen Escherichia coli O157:H7, whereas stainless steel and aluminum surfaces did not. When they inoculated seven different types of cast copper alloys with 10⁵ CFU of E. coli O157:H7, all of the cells were killed in 20 min or less at 22°C. Likewise, the food-borne pathogens Salmonella enterica and Campylobacter jejuni were inhibited by contact with copper surfaces, while surfaces comprised of stainless steel and a synthetic polymer showed no antibacterial activity (229). This research underscores the complexity of microbial inhibition and may cause us to reconsider “advances” in food safety such as the use of stainless steel in certain food production environments.

The use of natural materials can be required for producing some PDO cheeses. The 2006 regulations for Mont d’Or production stipulate that the cheese must be surrounded by a strip of spruce bark and placed in a spruce box (75). The inner layer of spruce bark is cut into strips, or sangles, by an artisan called a sanglier. The sangles are rolled into packets of 10 that are dried, soaked in salt water for 24 to 48 h, boiled for 2 h, and stored in salt water until use. After the cheese is removed from the mold, a sangle is placed around the fragile newly formed cheese to hold it together and secured with a pine toothpick. During ripening, Penicillium candidum grows on the bark, presumably because Penicillium is salt tolerant. The use of salt in preparing the bark may have selected for Penicillium in years gone by, although most producers now add a commercial strain. Early in ripening, G. candidum grows on the cheese surface. G. candidum and the coryneform bacteria B. linens and Arthrobacter create a whitish-orange crust characteristic of Mont d’Or (66). At 21 days of ripening, the cheese, still encircled by the strip of bark, is placed in the spruce box. The cheese is served in the box, and its creamy pale yellow interior is often eaten with a spoon. The aroma is dominated by the smoky flavor of terpenes, compounds contributed by the wood. Among the antimicrobial terpenes isolated from Mont d’Or are p-cymene, limonene, γ-terpinene, borneol, and terpineol (75).

Several types of cheeses owe their origins, continuance, or development to various abbeys in Europe. These include common varieties such as Munster (from the Latin Monasterium), Cheddar, Saint-Nectaire, and Maroilles. St. Benedict, the founder of Western monasticism (ca. 500 CE) describes the enclosure of the monastery as a “workshop” in his Rule for monks (233). His emphasis on manual labor as well as the stability and rhythm of monastic life created an environment for developing knowledge based on experimentation. An example is the creation of Maroilles cheese, which is attributed to the Benedictine Abbey of Maroilles in northern France in 960. A monument in the town bears the inscription “Stile created to commemorate the creation of Maroilles by the monks of the celebrated Abbey: 960–1960.” The cheese was first called craquegon but was known in the Middle Ages as “the marvel of Maroilles” (234).

The Order of Cistercians of the Strict Observance was founded in the 11th century and as the name implies was a reform movement within the Benedictine tradition. A subsequent even stricter reform movement of the Cistercian Order took place at the abbey La Grande Trappe in Normandy in 1664, and thus the monks were called Trappists. The Cistercians were known for their technological innovation and for transforming wilderness and malaria-ridden marshy land into farmland. The monks reintroduced water wheels to Europe, technology which had been abandoned by the Romans (235).
the sustenance of the monastic community, pilgrims, and surrounding population, the monks developed food technologies, including beer brewing and cheesemaking (236). Cheese was an important part of the monastic diet because according to Benedict’s Rule, eating the flesh of “quadrupeds” was forbidden except by the infirm (237). Since monastic meals were eaten in strict silence, the monks used sign language to communicate serving needs. The Code of Signs found in records from the abbey of St. Thomas the Martyr in Dublin, founded in 1177, provides us with evidence that there were cheesemakers at the monastic table:

Pro signo casei, utramque manum con junge per obliquum, quasi qui caseum premit.

[For the sign of cheese, join both hands obliquely, as one who presses cheese.] (238)

Tamié cheese is made at the Trappist Abbaye Notre-Dame de Tamié, founded in 1132 in Haute-Savoie in the Alps at 900 m. Since 1863, the monks have made the cheese we now know as Tamié, but presumably other varieties were made far into the past before the abandonment of the abbey during the French Revolution in 1792 (239). From the archives of 1600 we read that “someone lost the key to the cheese cave.”

Tamié cheese was trademarked in 1946 and is made and sold as a means of sustainability for the monastic community, yet its production involves a social obligation on the part of the monks. In 1132, the abbey was founded to provide food for the peasants of the area as well as travelers passing through the Alps from Geneva to the Little Saint Bernard Pass. During the 12th century, the monks helped the peasants clear land for pastures and roads and aided them in organizing agricultural granges for large-scale cheese production (239). The obligation to local farmers is continued today; the monks use milk from 11 local farms for the production of Tamié. Clary (239) has noted that the supplier-producer relationship between the monks and farmers is maintained through professional contracts yet is built upon hundreds of years of establishing trust. Many of these farmers are descendants of those who maintained the abbey property during and after the French Revolution, and some are descendants of the original farmers whose land was surrendered for the building of the original abbey.

Frère Nathanaël, the monk in charge of cheese production, attended dairy school and worked in the industry for 9 years before entering monastic life. He calls cheesemaking a “complex alchemy” and brings his professional and monastic training to cheesemaking. The monks keep their artisanal cheese “local” by using milk from breeds native to the Alps, the Tarentaise and Abondance, which, when not grazing in alpine pastures in the summer, eat feed from the region. Frère Nathanaël extends safeguarding the patrimony of the region to the microbiota. Like other master cheesemakers, Frère Nathanaël wants to make a consistently uniform and safe product yet does not want to lose the biodiversity of local microorganisms. Studies have shown that commercial cultures of starter bacteria such as Streptococcus thermophilus and Lactococcus lactis quickly outnumber the wild-type species initially present in raw milk (42). Thus, in collaboration with a commercial culture company, he isolated bacteria from hay and milk of the region and developed a collection of starter cultures that could be alternated in Tamié production in case the starters became infected by bacteriophage. Tamié thus is a cheese that stands at the crossroads of scientific and traditional methods of cheese fabrication.

Frère Nathanaël’s latest innovation involves the recycling of whey, a by-product of cheesemaking and a potential pollutant. Cheese production at the abbey produces 1,000 m³ of whey annually, and Frère Nathanaël developed and installed a methane-generating system that transforms the whey into enough energy to heat water for the domestic needs of the abbey and its dairy for a year. The Abbey of Tamié was awarded the Rhône-Alpes 2005 Energies of Today prize.

The cheese cave of the abbey, with its arched vaults and limestone walls, lies beneath the Monastic Choir. Standing in the cave, Frère Nathanaël shared the secret of knowing when a Tamié cheese is ready to be eaten. One gently taps the surface, and if there is no indentation, it is ready. One then smells it underneath. If “ça sent la vache” (it smells like the cow), it is good. In another context, he compared the smell to a less acceptable by-product of the cow. Everyone may not be at ease with Frère Nathanaël’s analogy, yet it reminds us of the earthiness associated with the origins of cheese. In comparing the language used to describe wine “consisting generally of associations with various fruit and flowers” to that of cheese, cheesemaker and philosopher Jim Stillwagon mused, “Cheese on the other hand is less discreet. If we address frankly what is evoked by cheese I think it becomes clear why so little is said—usually little more than sounds of approbation, and global adjectives: ‘Mmmmmm, good! Interesting! Fantastic!’ So what does cheese evoke? Damp dark cellars, molds, mildews and mushrooms galore, dirty laundry and high school locker rooms, digestive processes and visceral fermentations,
he-goats which do not remind of Chanel. ... In sum, cheese
reminds of dubious, even unsavory places, both in nature
and within our own organisms. And yet we love it
(personal communication).

ACKNOWLEDGMENT
The authors declare that they have no commercial af-
nouncements, consultancies, stock or equity interests, or patent-licensing
arrangements that could be considered to pose a conflict of in-
terest regarding the manuscript.

REFERENCES
1. Elkort M. 1991. The Secret Life of Food: A Feast of Food and Drink
8:57–59.
5. Copley MS, Berstan R, Dudd SN, Straker V, Payne S, Evershed RP.
2005. Dairying in antiquity. I. Evidence from absorbed lipid residues
6. Copley MS, Berstan R, Mukherjee AJ, Dudd SN, Straker V, Payne V,
Evershed RP. 2005. Dairying in antiquity. III. Evidence from absorbed
lipid residues dating to the British Neolithic. J Archaeol Sci
523–546.
Pattern of the Past: Studies in Honor of David Clarke. Cambridge Uni-
Horne L (ed), Treasures from the Royal Tombs of Ur. University of
Pennsylvania Museum, Philadelphia, PA.
Chemistry, Physics and Microbiology, 2nd ed. Chapman and Hall,
10. Edwards EE. 1949. Europe’s contribution to the American dairy
Leiden, The Netherlands.
Expedition 28:51–58.
13. Greenfield HJ. 1988. The origins of milk and wool production in the
Old World: a zooarchaeological perspective from the Central Balkans.
The identification of prehistoric dairying activities in the Western Isles of
Archaeol 8:25–42.
18. Cronin T, Downey L, Synnott C, McSweeney P, Kelly EP, Cahill M,
the Kitchen. Scribner’s, New York, NY.
University Press, Cambridge, MA.
5:6–79.
22. Harrison F. 1913. Roman Farm Management: The Treatises of Cato
Harvard University Press, Cambridge, MA.
24. Choisy C, Desmazaud M, Gripon JC, Lamberet G, Lenoir J,
In Eck A (ed), Cheesemaking, Science and Technology, 2nd ed.
Lavoisier, New York, NY.
Philol 81:195–212.
121:345–366.
regional traditional cheese varieties of East-Mediterranean countries:
30. Bérard L, Marchenay P. 1995. Lieux, temps et preuves: la construc-
31. Montanari A, Staniscia B. 2009. Culinary tourism as a tool for re-
33. Bérard L, Marchenay P. 2008. From Localized Products to Geographi-
cal Indications. Awareness and Action. Centre National de la
Recherche Scientifique, Bourg-en-Bresse, France.
34. Semple EC. 1922. The influence of geographic conditions upon ancient
knowledges and the reconnection of production and consumption: the
of Food Preserving Changed the World. Simon & Schuster, New York, NY.
37. Beresford TP, Fitzsimons N, Brennan NM, Cogan TM. 2001. Recent
Diversity of Geotrichum candidum strains isolated from traditional
cheesemaking fabrication in France. Appl Envir Microbiol 67:4752–
4759.
Fanni J. 2009. Bacterial diversity of Dar and goat cheeses in Southern Italy.
Papa A, Dragoni I. 2010. Genotypic and technological characterization of
Leuconostoc isolates to be used as adjunct starters in Manchego cheese
Bacterial diversity of Manchego cheese manufacture. Food Microbiol
27:85–93.
41. Montagna MT, Santacroce MP, Spilotros G, Napoli C, Minervini F,
and goat cheeses in Southern Italy. Mycopathologia 158:245–249.
communities in raw milk and cheese by culture-dependent and -indepen-
1891.
43. Cogan TM, Barbosa M, Beuvier E, Bianchi-Salvadori B, Coccenelli
44. Mounier J, Rea MC, O’Connor PM, Fitzgerald GF, Cogan TM.
2007. Growth characteristics of Brevibacterium, Corynebacterium,
and microbiological quality of Fossa cheese.


101. Marcellino and Benson


140. D’Amico DJ, Donnelly CW, Druat MJ. 2008. 60-day aging requirement does not ensure safety of surface-mold-ripened soft cheeses manufactured from raw or pasteurized milk when *Listeria monocytogenes* is introduced as a postprocessing contaminant. *J Food Prot* 71:1563–1571.


receiving liposomal amphotericin b and caspofungin for suspected aspergillosis. 


176. Malcon and Benson (Deuteromycota). 


The Good, the Bad, and the Ugly: Tales of Mold-Ripened Cheese


214. Bonnaud P. Auvergne.


