Mesophilic and Thermophilic Cultures Used in Traditional Cheesemaking

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ABSTRACT  Most cheese varieties require acidification of milk by a select group of bacteria called starters. They ferment lactose to lactic acid and in so doing aid the cheesemaker in developing the desired texture as well as acidity of the cheese. However, while other microorganisms play the major role in flavor development of cheese, it is the starter that sets the stage for quality cheese manufacture. Starters were traditionally derived from the native microflora of the milk, but this practice is almost unheard of today. With the advent of better hygienic milking practices and industrialized cheesemaking, there was a need for more uniformity and reliable sources of the starter culture. Today’s starters are produced by companies specializing in their production as well as in the development of new strains for cheesemakers. The choice of starter for the manufacture of a specific cheese is dictated by the cheesemaking protocol, but it is also governed by the need to produce cheese with desired physical attributes. The properties of the starter that make it possible to do so help drive innovation in developing new potential choices in starter cultures. Indeed, the demands for predictable and reliable rates and extent of acidification of milk for cheesemaking and flavor development are as key for successful cheesemaking today with artisanal cheesemakers as they are for larger, more industrial-scale cheesemakers.

The art of cheesemaking is strongly rooted in the cheesemaker’s ability to control the growth and metabolism of microorganisms. Whether these microorganisms are already present in the raw milk as “natural” contaminants, are added to the milk, or are nurtured in ripening or curing rooms, the skilled cheesemaker knows that the quality and safety of cheese are due to controlling the growth and metabolism of microorganisms. Milk quality in terms of both chemical composition and microbiological populations is dependent upon the producer, but the milk accepted for cheesemaking is the choice of the cheesemaker. Most of the defects observed with cheese in terms of undesirable flavors or physical characteristics have their origin in microbial growth. However, there are two important aspects to be considered, growth of undesirable microorganisms and excessive or limited growth of desirable microorganisms. The former is largely addressed through strict hygienic practices on the farm and at the cheese factory or curing facilities. The latter is a critical step controlled by the cheesemaker during cheesemaking and by the affineur, who controls the curing or aging of the cheese. A quote from Cheddar Cheese Making by Decker (1) sums it up perfectly: “Nearly all the trouble we have in cheese making is due to the action of definite living vegetative cells that have the power of manufacturing certain decomposition products; on the other hand, we could not produce fine cheese without the presence of certain forms of bacteria that are able to change milk, producing the fine desired flavors.”

At its very essence, cheesemaking involves the fermentation of lactose with a few processing steps to control cheese composition, followed by controlled decomposition called ripening. The fermentation of milk sugar, lactose, to lactic acid is performed by a group of bacterial cultures collectively called “starters.” They are...
also called “lactic cultures” because they produce lactic acid. The rate and extent of lactic acid development during cheesemaking are critical to the success of cheesemaking and are fortunately things the cheesemaker can control through proper starter management. This review discusses the role that these special bacteria play in cheesemaking and how the cheesemaker selects and uses them.

**STARTERS**

**The Origins of Artisanal Cultures**

Starters are lactic acid bacteria, a group of bacteria that rely on sugar fermentation for energy with the concomitant formation of lactic acid, hence the name “lactic acid bacteria.” The evolutionary niche of these bacteria is organically rich soils and vegetation. Spontaneous or natural fermentation by lactic acid bacteria is essential for sauerkraut and pickle fermentation and includes species of bacteria similar to those important to dairy fermentations. The ability to ferment lactose is not ubiquitous in the bacterial world and requires specific genes. These genes are not part of the chromosome of the bacteria. They are on separate extrachromosomal entities called plasmids (2). Lactose is found only in milk. This means that lactose fermentation was not a requirement for the survival of starters in their natural environment but was an attribute that, once acquired, gave starters an advantage over other bacteria when it came to exploiting milk as a food source. The ability to ferment lactose and survive under the acidic conditions it creates gives lactic acid bacteria an advantage in an environment where other microorganisms may be inhibited. Such is it with starter bacteria. Although starters are almost always used to make cheese, today there are a few artisanal cheesemakers who rely simply on the high numbers of bacteria already present in the raw milk for acid development.

Such was it everywhere prior to commercial production of starter cultures for the cheese industry (late 19th century). At some point it was noticed that cheese quality could be improved if the cheesemaker added a previously fermented batch of milk. Thus began the development of natural or artisanal starters. It was common practice to select raw milk with the best taste and smell to ferment overnight at ambient temperature and then to use it as the inoculum (or starter) for the day’s cheesemaking. That is, milk was allowed to ferment by the bacteria already present in the milk. Of course the milk contained many types of bacteria as well as many bacteria, far more than are typically seen today. Bacterial counts in the millions per milliliter of milk were the rule of the day. Among these bacteria were lactic acid bacteria, perhaps picked up by the animals feeding on vegetation. Along with lactic acid bacteria there were many other types of bacteria, some beneficial to flavor development and others not. Included were potentially pathogenic bacteria. Since rapid or extensive cooling of milk prior to cheesemaking was not commonly done, lactic acid bacteria would become well established in the milk even prior to cheesemaking. Fermentation eventually created an acid environment that would selectively favor the lactic acid bacteria in the starter. Potential pathogens were unable to flourish. Successive transfers of fermented milks into fresh or heated milks and whey would further aid in establishing the dominance of lactic acid bacteria.

It was highly recommended that a new batch of starter be developed every day, and the use of previously inoculated cheese milk or whey as the source of starter was discouraged, as they were often of dubious quality. The technologists at the time recognized that by the end of cheesemaking many potentially good, bad, or indifferent germs could be in the whey and would simply be carried from batch to batch. To avoid a potential “bad” starter, it was recommended to start over each day. It is very unlikely that these starters were free of undesirable bacteria. Even though acid conditions could be used to slow the growth of some bacteria, they would not have killed them altogether. Incubation of raw milk at room temperature or higher allows the growth of coliforms, enteric bacteria, *Bacillus* spp., *Clostridium* spp., and even pathogenic bacteria. It was somewhat lucky that artisanal cultures were developed that made a good cheese. Those fortunate cheesemakers who found the secret stayed in business.

Although lactic acid bacteria may have been the dominant bacteria in raw milk many, many years ago, they are not so today. That honor falls to *Pseudomonas* spp., which tend to be the most common bacteria in raw milk. This is to be expected since they are probably among the most common bacteria in nature and are psychrotrophic. Thus, with the rapid cooling practiced today, they grow well at refrigeration temperatures, at which the lactic acid bacteria do not. Indeed, in today’s milk, high levels of lactic acid bacteria (>1,000 per ml) in raw milk indicate that the milk was not cooled sufficiently and rapidly after milking. The concept of developing a daily culture worked because lactic acid bacteria were present in sufficient numbers to rapidly ferment the raw milk. This was to be expected since sufficient and rapid cooling of milk was not standard practice at that
time. Raw milk bacterial counts were very high by today’s standards, over 10 million per ml of milk according to some reports. One report indicated numbers over 33 million to be common. By comparison, total bacteria in rapidly cooled raw milk are commonly less than 10,000 per ml. Most of these bacteria, however, are not lactic acid bacteria. It has been estimated that lactic acid bacteria in rapidly refrigerated raw milk account for less than 10% of the total bacteria found. The advantage of having low-bacterial-count milk and using purified starters is that after the addition of the starter, the total number of all bacteria would be approximately one million per ml of milk. To put all this in perspective, over 99% of all bacteria in inoculated milk is from the starter.

However, those bacterial species that make up the other 1% of the total are extremely important to the quality of the cheese, as they are necessary for desired flavor development. On the other hand, they could also be the cause of both flavor and textural problems. The concept of successful cheese ripening is to create conditions during ripening (affinage) in which the desired microorganisms dominate. That is accomplished in many cheeses by the addition of large numbers of molds (Camembert, blue, and bandaged Cheddar) or yeasts and bacteria (washed-rind cheeses) and selected conditions of temperature and humidity. For other cheeses, such as aged Cheddar and Parmesan, naturally occurring lactic acid bacteria (nonstarter lactic acid bacteria [NSLAB]) eventually dominate even from such humble beginnings as comprising less than 1% of the original microflora. Over the years, it has become common practice to add microorganisms to the milk to supplement the naturally occurring lactic acid bacteria and enhance the development of flavor in cheese. These microorganisms are called adjuncts. Many of these are presently isolated from artisanal cultures or fully ripened cheese. Some are used as starters in one cheese and as adjuncts in another. Common adjuncts include Lactobacillus helveticus, Lactobacillus casei, and Leuconostoc mesenteroides subsp. cremoris, and Geotrichum candidum. The science and use of adjunct microorganisms are in their infancy and have become major areas of interest to the cheese industry.

Ultimately, developments in Europe in the late 1800s (particularly in Italy, France, and Denmark) would change the way artisanal starters were propagated. In Italy, artisanal cultures were being developed by exploiting the high temperatures of incubation and using boiled whey as the growth medium to select for thermostiles. In Denmark, cheesemakers were using lower temperatures to remove the undesirable bacteria and boiled milk as the growth medium. In both cases the inoculum was obtained from a proven artisanal culture, one that produced a quality cheese with no defects. The process of using a proven culture to reinoculate milk for the next day’s cheesemaking was called back-slopping. Back-slopping probably produced as many starters with a very diverse group of lactic acid bacteria as there were cheesemakers. Trading or borrowing quality starters between cheesemakers became a common practice.

Danish researchers began to isolate single strains of lactic acid bacteria from artisanal cultures to be used in buttermaking. A major shift in the production of starters and in cheesemaking began in the 1890s, when purified cultures (single strains) were developed and shown to produce cheeses of better and more consistent quality. The commercial production and development of starter cultures spread rapidly. At first, the cultures were grown in milk with buffers to prevent overripening. These were then shipped to the cheese plant. Later, the cultures were freeze-dried and sent to the factory. In either case, the cheesemaker propagated the culture at home, transferring it in increasing volumes to eventually obtain enough starter to inoculate the cheese milk.

However, by the late 1890s, certain circumstances were beginning to coalesce and drastically alter the dairy industry. It was recognized that pasteurization of milk for cheesemaking greatly lowered the incidence of illness associated with consumption of raw milk and greatly reduced some spoilage issues in cheese. Among the latter was the gassy, fruity defect caused by coliforms. Another development occurred about this time that continues to this day, the formal training of cheesemakers. Cheesemaking was (and is) a gift handed down from one generation of cheesemaker to another. Newly created dairy schools furthered that education and introduced the application of new tools that dairy technologists were developing for cheesemaking. Dairy technologists from the United States were also visiting dairy schools and factories in other countries, particularly Denmark. The correlation between cheese quality and milk quality was well known to some, but it was now reaching an audience eager to make quality cheese. The need for quality milk to make a quality cheese hit home and was even being promoted by progressive milk producers who realized that their livelihoods depended upon the success of the cheesemaker. It was no longer just about fat and protein content of the milk: it was also about bacteriological quality.

Pasteurization of milk for cheesemaking also meant that cheesemakers needed a source of starter. Better-quality milk meant that the previous methods for developing starters would not work. Naturally occurring
contaminating lactic acid bacteria were not present in sufficient quantities in pasteurized milk. The timing was perfect for commercially available cultures. Artisanal cultures would pay big dividends to the commercial starter companies, as they were the source of the starter cultures. Today, artisanal cultures are being winnowed as sources for potential phage-resistant strains and strains with a more diverse metabolic activity that may be exploited as ripening progenitors.

THE LACTIC ACID BACTERIA

In the dairy industry, starters are used primarily to ferment lactose, but other lactic acid bacteria are deliberately added to milk to produce flavor components or carbon dioxide (Table 1). Although they are able to ferment lactose, they are not used for that purpose and thus are called secondary starters. Many technologists do not make the distinction between primary and secondary starters and use the term starters to identify any bacteria (and yeasts and molds) deliberately added to milk regardless of the purpose. Some strains of primary starters are also used as secondary starters and are called

TABLE 1 Combined classification basis for lactic acid bacteria used as starters or secondary cultures used for flavor development

<table>
<thead>
<tr>
<th>Lactic acid bacteria</th>
<th>Classification basis</th>
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<tbody>
<tr>
<td><strong>Homofermentative mesophilic cocci used as primary starters</strong></td>
<td></td>
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<tr>
<td>Lactococcus lactis subsp. lactis</td>
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<tr>
<td>Lactococcus lactis subsp. cremoris</td>
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<tr>
<td><strong>Homofermentative mesophilic coccus used as secondary culture</strong></td>
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</tr>
<tr>
<td>Lactococcus lactis subsp. lactis bv. diacetylactis (also reported as Cit+ Lactococcus lactis)</td>
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<tr>
<td><strong>Heterofermentative mesophilic coccus used as secondary culture</strong></td>
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</tr>
<tr>
<td>Leuconostoc mesenteroides subsp. cremoris (poor acid producer, metabolizes citric acid)</td>
<td></td>
</tr>
<tr>
<td><strong>Homofermentative thermophilic cocci used as primary starters</strong></td>
<td></td>
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<tr>
<td>Streptococcus thermophilus</td>
<td></td>
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<tr>
<td>Enterococcus faecium or Enterococcus faecalis (only in some artisanal cultures)</td>
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<tr>
<td><strong>Heterofermentative mesophilic rod used as secondary culture</strong></td>
<td></td>
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<tr>
<td>Propionibacterium freudenreichii subsp. shermanii</td>
<td></td>
</tr>
<tr>
<td><strong>Homofermentative thermophilic rods used as primary starters</strong></td>
<td></td>
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<tr>
<td>Lactobacillus delbrueckii subsp. bulgaricus</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus delbrueckii subsp. lactis</td>
<td></td>
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<tr>
<td>Lactobacillus helveticus</td>
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<tr>
<td><strong>Obligate heterofermentative thermophilic rods generally a nuisance as secondary cultures (produce gassy cheese)</strong></td>
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<tr>
<td>Lactobacillus fermentum</td>
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<tr>
<td>Lactobacillus brevis</td>
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<tr>
<td><strong>Facultative heterofermentative lactobacilli (rods) commonly found in cheese (NSLAB) but not used as starters</strong></td>
<td></td>
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<tr>
<td>Lactobacillus casei</td>
<td></td>
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<tr>
<td>Lactobacillus plantarum</td>
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<tr>
<td>Lactobacillus curvatus</td>
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adjunct cultures, albeit in concentrations below those used as primary starters.

Of the lactic acid bacteria, only five species are relied on as primary acid producers in cheese: Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, and Lactobacillus helveticus. These species are not generally used interchangeably but are used for specific cheeses for reasons sometimes strongly related to tradition or, in other cases, specifically for flavor and rate and extent of acid development, often dictated by salt and temperature sensitivities related to manufacturing practices.

If the manufacturing protocol calls for cook temperatures over 39°C, thermophiles are used. Mesophiles are used in the manufacture of cheeses employing lower cook temperatures. Temperatures exceeding 39°C are sometimes used to slow acid development by the mesophiles, but they are not killed until temperatures exceed 45°C. Mixtures of Streptococcus thermophilus and mesophiles are used by many manufacturers using temperatures exceeding 39°C. Streptococcus thermophilus is used to speed up acid development during the cook step, and the mesophiles ensure that acid development will continue after the curd cools following whey separation and salt application.

Streptococcus thermophilus strains are generally very sensitive to salt and cool temperatures (<30°C). Rod cultures (Lactobacillus helveticus and Lactobacillus delbrueckii) are rarely used alone as the primary acid producer in cheese, as they are generally too slow in acid production by themselves. Consequently, Lactobacillus species are always used in conjunction with Streptococcus thermophilus or Lactococcus spp. Streptococcus thermophilus, Lactobacillus helveticus, and Lactobacillus delbrueckii subsp. bulgaricus are protocooperative (3). That is, they can each grow quite well on their own but do much better (with faster acid development and more proteolysis) when grown together. Streptococcus thermophilus produces formic acid from lactose metabolism, which, in turn, stimulates growth of the Lactobacillus. Lactobacilli, in turn, are more proteolytic and provide a pool of amino acids and peptides that enables the streptococci to grow faster. Regardless of the Lactobacillus strain used, Streptococcus thermophilus is responsible for most of the acid development (there are sufficient peptides for growth) during cheesemaking. Recently, strains of Streptococcus thermophilus have been used that are very capable of rapid acidification on their own, and they have been replacing the rod-coccus blends that were used in the past.
Some strains of *Lactobacillus helveticus* are used as adjunct cultures in a variety of cheeses, such as Gouda, Cheddar, and Parmesan, and strains of *Lactobacillus casei* and *Lactobacillus rhamnosus* have been used in Cheddar. As adjunct cultures, they are not relied on to produce acid but are used because of their ability to produce or promote development of desirable flavors. *Lactobacillus* spp. are much more proteolytic (able to break down protein into smaller pieces called peptides or amino acids) than are the *Lactococcus* spp. and *Streptococcus thermophilus*.

Not all lactic acid bacteria are suitable as primary or secondary starters in the manufacture of fermented dairy foods. Those that are have been selected on the basis of their ability to rapidly ferment lactose, have the ability to grow in milk, and produce some positive attribute to the fermented dairy product (flavor and texture or body). Lactic acid bacteria that are not used as starters but are common contaminants in dairy products are called NSLAB and can play major roles in the development of both desirable and undesirable flavors in cheese and fermented milks. Several species of NSLAB have been isolated from cheeses, cultured in pure form, and used commercially as ripening facilitators and as probiotics.

Taxonomists have separated bacteria into classes, genera, species, and sometimes subspecies based on several different criteria, including DNA analysis, metabolism of selected compounds, cell morphology, and other physical attributes. In the dairy industry, the lactic acid bacteria used as starters are referred to in accordance to their industrial application, such as the optimum temperature of acid development or production of products other than lactic acid when lactose is fermented.

**INDUSTRIAL CLASSIFICATION BASED ON CELL MORPHOLOGY**

There are two basic shapes for starters: rods (bacillus) and cocci (Fig. 1). Rods are longer than they are wide, so under a microscope, they appear as sticks or dashes. Cocci are round or oval. At times the distinction is very difficult under microscopic examination, as oval shapes may appear as short rods and vice versa. Both occur as single cells or pairs, but most commonly, they are connected end to end as short chains. The length of the chains is somewhat characteristic of the species. In the jargon of the industry, starters are often called just rods or cocci without reference to the species, as that distinction is often inferred in context to a specific cheese.

**INDUSTRIAL CLASSIFICATION BASED ON OPTIMUM TEMPERATURE OF FERMENTATION**

Traditional microbiologists use the following terms to indicate the general (slightly arbitrary) optimum temperature for growth of bacteria: psychrophiles (15 to 20°C), mesophiles (30 to 37°C), thermophiles (50 to 60°C), and extreme thermophiles (up to 122°C). However, there is a big difference between optimum growth temperatures and the lowest temperatures at which bacteria can grow. Psychrotrophic bacteria can grow at temperatures as low as 0 to 15°C but may be mesophilic (mesophiles) when it comes to their optimum growth range. Psychrotrophs are very important spoilage bacteria in dairy foods under refrigeration. Yeast and molds are common psychrotrophic microorganisms that can cause spoilage of fermented dairy products because of their ability to grow at refrigeration temperatures (4 to 8°C) and under the acidic conditions of these products.

Starters are most commonly separated on the basis of the optimum temperature at which they ferment lactose. Often this temperature is indicative of the temperature at which the curds (and whey) are scalded or cooked. Two classifications used are mesophiles and thermophiles. Dairy microbiologists use a slightly different range of temperatures when they refer to bacteria as being mesophilic or thermophilic. Psychrophiles are not used as starters, but optimum temperatures of growth for mesophilic starters are ~20 to 32°C, while for thermophilic starters, they are ~37 to 45°C. Mesophiles can still

**FIGURE 1** Electron micrograph of *Lactobacillus helveticus* (rods) and *Streptococcus thermophilus* (cocci). Courtesy of William McManus and Donald McMahon, Western Dairy Center, Utah State University. doi:10.1128/microbiolspec.CM-0004-2012.1
ferment lactose at temperatures as low as 10°C and as high as 40°C. Thermophiles can still ferment lactose at temperatures as low as 20°C and as high as 50°C. However, it is important to note that the optimum growth temperature is below the maximum temperature at which the bacteria will still grow and even much lower than the temperature at which they will begin to die. It is also important to recognize that the temperatures pertaining to any attribute of bacteria are given as a range due to natural variability in individual species and strains. Consequently, some mesophilic starters can grow at 40°C, while others do not, and some survive but do not grow at 45°C. Mesophilic strains may require prolonged times (20 to 30 min) at elevated temperature (56°C) to be completely killed. Many thermophiles can even survive slightly higher temperatures and times than are used to fulfill minimum pasteurization requirements. Bacteria that can survive pasteurization are capable of forming biofilms in the cooling section of a pasteurizer. Biofilms can become the major source of bacterial contamination in pasteurized milk, especially if the equipment has been run for extended periods (>8 h) and is not cleaned and sanitized effectively. *Streptococcus thermophilus*, a common contaminant in raw milk, has been known to reach sufficiently high numbers (>100,000 CFU/ml) by the end of a production day to interfere with the rate of acid development. A major source of gas formers in cheese (especially *Lactobacillus* spp.) is biofilms in the pasteurizer.

### CLASSIFICATION OF CULTURES BASED ON FERMENTATION

There are two types of fermentations, and they are based on the end products of lactose metabolism: homofermentation and heterofermentation. Under optimal and ideal conditions (initial conditions in milk and cheese), homofermentative starters convert lactose to more than 90% lactic acid and a small amount to acetic acid, while heterofermentative bacteria convert lactose to around 50% lactic acid and 40% or more other products such as ethanol, acetaldehyde, acetic acid, and carbon dioxide. Under stress conditions, i.e., salted curd, lower pH, anaerobic conditions, and lower-than-optimal temperatures, homofermentative bacteria demonstrate a slight shift in the end product of fermentation from lactic acid to acetic acid. This does not appear to alter any characteristics of concern to cheese quality.

The difference in heterofermentation and homofermentation is due to the presence of specific enzymes (thus pathways or processes) used for lactose fermentation. In addition, mesophiles and thermophiles ferment lactose differently due to unique mechanisms of bringing lactose into the cell. Lactose does not just passively diffuse into the cell. Lactose is a disaccharide composed of one molecule each of glucose and galactose. In the process of actively transporting lactose into the cell, mesophiles phosphorylate (add a phosphate group to) the lactose molecule (galactose moiety), and that requires the expenditure of energy and phosphate which the cell needs to retrieve later (4). In the homofermentative pathway used by mesophiles, recycling of phosphate requires that both glucose and galactose moieties be converted into lactic acid. Thermophiles bring lactose into the cell in a different manner that does not phosphorylate lactose (called a transporter system). Consequently, these bacteria do not have to ferment the galactose. Instead, the bacteria have a process by which the galactose moiety is pushed out of the cell and lactose is brought in. The homofermentation of lactose by mesophiles produces four lactic acid molecules per lactose molecule. Homofermentation of lactose by thermophiles produces two lactic acid molecules, with the release of galactose into the milk or cheese. A few strains of *Streptococcus thermophilus* can ferment the galactose molecule to two lactic acid molecules. This occurs only after most, if not all, of the lactose has been fermented. In actual practice, this scenario can occur only if the cheese is kept warm enough for metabolism by the bacteria. This may not occur in mozzarella or provolone (pasta filata cheeses) because these cheeses are cooled rapidly after the hot-water stretching process and both lactose and galactose remain in the cheese unfermented.

In direct salted cheeses that are made with thermophiles, not only are the cheeses cooled before sugar metabolism is completed but also the addition of sufficient salt inhibits lactose metabolism. This may occur even if mesophiles are also part of the culture. The residual sugars can be fermented by other bacteria, or the sugars (particularly the galactose molecule) can react with lysine (an amino acid) to produce brown pigments during aging or upon heating (Fig. 2). This chemical process is called nonenzymatic browning or Maillard browning. This reaction is enhanced with lower water activity (drier and higher-salt cheese) and warm storage temperatures (>7°C), but it can also occur under refrigeration conditions (<7°C).

Currently, no strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* are known to ferment residual galactose. All *Lactobacillus helveticus* strains are capable of fermenting residual galactose, and this attribute is used to differentiate it from *Lactobacillus delbrueckii* strains. Consequently, it is used in combination with
Streptococcus thermophilus when complete sugar fermentation is desired. However, the cheese still needs to be kept warm (~37 to 40°C) long enough (generally >4 h) for that to occur. Excessive salting of the cheese inhibits the fermentation.

Heterofermentative bacteria are not used as primary starters (i.e., they are not used as the chief acid producers), but they are used almost exclusively as the secondary flora (i.e., they are used for flavor development and texture [they produce “eyes” or holes in cheese]). They ferment one molecule of lactose to two lactic acid molecules plus two acetic acid or two ethanol molecules and one molecule of carbon dioxide. The ethanol molecules may react with mono- and diglycerides to produce fruity flavors in cheese. Any residual lactose can be fermented by heterofermentative bacteria, and this may result in “gassy” or “slitty” cheese.

Lactobacillus (rods) strains of importance to the manufacture of Swiss and Baby Swiss cheeses to produce carbon dioxide, which results in the formation of the desired eyes. Props, as they are often called, ferment lactic acid to form acetic and propionic acids and carbon dioxide. They are stimulated by the amino acids and peptides formed by the proteolytic activity of the rod cultures added, especially Lactobacillus helveticus and Lactobacillus lactis.

NONFERMENTATIVE CHARACTERISTICS

Although the main contribution of the starter is the development of acid and, in some cases, citric acid metabolism, the starter also has other metabolic activities that can result in very distinctive sensory attributes of cheese. Starter cultures are often used in cheese specifically because of their proteolytic characteristics. Without proteolysis, flavor development is greatly curtailed. In addition, the proteolytic activity of the strain is directly proportional to its ability to grow rapidly in milk.

The heterofermentative Propionibacterium freudenreichii subsp. shermanii is added to milk in the manufacture of Swiss and Baby Swiss cheeses to produce carbon dioxide, which results in the formation of the desired eyes. Props, as they are often called, ferment lactic acid to form acetic and propionic acids and carbon dioxide. They are stimulated by the amino acids and peptides formed by the proteolytic activity of the rod cultures added, especially Lactobacillus helveticus and Lactobacillus lactis.

The facultative heterofermentative nonstarter (i.e., they are used as the secondary flora) of the proteolytic enzymes and fermentation pathways when conditions are less ideal: low sugar content, anaerobic conditions, and cold temperatures. The facultative heterofermentative lactobacilli are the dominant NSLAB found in most cheeses. Some strains are also capable of metabolizing the naturally occurring citric acid in cheese. This metabolism results in the formation of diacetyl, acetic acid, and carbon dioxide. Consequently, if there is sufficient citric acid present in the cheese, enough gas could be evolved to also produce slitty cheese or huffed (blown) packages of cheese. The metabolism is accelerated by temperatures above 10°C. These bacteria are often used as adjuncts in Cheddar cheese to enhance flavor development, and they are added as probiotics. Lactococcus lactis subsp. lactis bv. diacetylactis and Leuconostoc mesenteroides subsp. cremoris used in Gouda, Edam, and Havarti are deliberately added to metabolize citric acid to form eyes or expand mechanical openings.

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**FIGURE 2** Influence of sugar content on color of baked mozzarella. When cheese dries, the protein burns, causing the blisters to be a darker color. The presence of residual galactose (left side) darkens the blister color. doi:10.1128/microbiolspec.CM-0004-2012.f2

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it very suitable when proteolysis is not welcome in cheese, e.g., fresh mozzarella, stabilized Camembert, and Muenster. Compared to yeasts and molds, starter bacteria are very weakly proteolytic. The *Lactobacillus* species tend to be the most proteolytic of starter strains and have a vast arsenal of secondary proteolytic enzymes called peptidases that are responsible for hydrolyzing small peptides, including those responsible for bitter flavors. Also as a direct result of peptidase activity, they produce a variety of flavor components. It is for these reasons that *Lactobacillus* species are the flavor-producing adjuncts most often used to enhance or accelerate flavor development. Finding the most appropriate strain of lactobacillus to produce the desired impact on flavor in different cheeses has become a major pursuit in the culture industry.

All starter strains are poorly lipolytic. That is, they are not very effective at hydrolyzing triglycerides (fat) in cheese. Their metabolism of fat will never result in sufficient fat hydrolysis to cause cheese to become rancid. Some strains of *Lactobacillus helveticus* have a strong esterase activity which may result in the formation of desirable fruity notes in cheese, particularly in Parmesan. Esterase catalyzes the release of fatty acids from a monoo- or diglyceride in the serum of cheese rather than from an intact triglyceride.

**INDUSTRIAL CLASSIFICATION BASED ON STRAIN COMPOSITION**

In the beginning of cheesemaking, all cultures were artisanal or derived from spontaneous fermentations of milk. The types of lactic acid bacteria varied tremendously and must have included many different species, including some species of very undesirable bacteria. Today, artisanal cultures are still being used in some countries. Care must be taken in maintaining artisanal cultures. They are now grown under strict guidelines with respect to temperature and inoculation rates (from a previous culture) to maintain the types and balance of desirable species. Slight modification in growth temperature and inoculum rates can cause major shifts in dominance of species, with undesirable consequences. For example, in mesophilic artisanal cultures containing desirable gas producers, *Lactococcus lactis* subsp. *lactis* bv. diacetylactis and *Leuconostoc mesenteroides*, lower temperatures (18 versus 20°C) and higher inoculum rates (2% versus 0.5%) favor *Leuconostoc mesenteroides*. Excessive *Lactococcus lactis* subsp. *lactis* bv. diacetylactis results in rapid gas production from citric acid metabolism and excessive numbers of eyes, or openings in the cheese. In addition, if citric acid is depleted too fast (before the lactose fermentation is completed), then the main flavor component derived from citric acid metabolism, diacetyl, will be further metabolized by *Lactococcus* and lost. By their very nature, artisanal cultures vary between cheese factories using them, and the composition and dominance of strains fluctuate over time. They contain both coccis and rods, both homofermentative and heterofermentative strains, but usually a dominance of homofermentative lactic acid bacteria. The dominance of mesophiles or thermophiles is of course based on growth temperature of the culture. Mesophilic artisanal cultures are grown in steamed or boiled skim milk, while thermophiles are generally grown in steamed or boiled whey.

Commercial production of starters began with the realization that cheese of better and more consistent quality could be produced with purified strains of lactic acid bacteria. Culture companies have chosen to separate cultures on the basis of strain composition and include designations such as defined-strain and undefined or artisanal cultures. Defined-strain cultures come as single or multiple strains and are well characterized as to the exact strain(s) contained in them. As the desire for specific metabolic characteristics increases, these cultures are being carefully scrutinized for advantageous strains. Undefined-strain or artisanal cultures are not well characterized as to strain identity but may be dominantly mesophilic or thermophilic.

**WHAT DETERMINES WHICH CULTURES TO USE FOR SPECIFIC CHEESE TYPES?**

Cheesemakers have chosen starters largely based on tradition and the manufacture of cheese with distinctive flavor characteristics. “What starter is needed?” is usually the first question asked when cheesemakers venture out to make a cheese that they have not made before. From a published record or word of mouth, they notice that a given starter is used and they stick with it. Part of the tradition lies with the manufacturing schedule, i.e., the temperature at which the curd and whey are typically heated. Thus, thermophiles are used when the cook temperatures are above 39°C, and mesophiles are used with lower cook temperatures. Cottage cheese is an exception. Cook temperatures in cottage cheese manufacture are very high in order to kill the mesophilic starter to prevent postacidification after the cream dressing is added.

But how were the cook temperatures chosen? Again, tradition has much to do with this. It is generally assumed that higher cook temperatures result in cheese with lower
moisture. This is not necessarily correct. Many other factors play a role in how much moisture is lost during cheesemaking and are not covered here. As far as the contribution of the starter, the amount of acid developed before and after the coagulum is cut is a major determinant of how much moisture is lost. Cheesemakers can adjust other manufacturing parameters to obtain the desired moisture levels in cheese. Could the cheesemaker not just raise and lower the cook temperature in accordance with their choice of starters rather than have the cook temperature dictate the starter? The quick answer is yes. But there is more to it than that. Primary starter cultures and the precise strains and blends of bacteria in them are also chosen for their ability to produce acid at a prescribed rate and extent and also to produce certain desirable flavor characteristics in the cheese. Switching between mesophiles and thermophiles or even strains of each changes these attributes.

Particular strains are also chosen for their sensitivity to salt or temperature or their ability to break down casein in a prescribed manner. Proteolysis is a major contributor to flavor development in cheese. However, the role of the starter may eventually be overshadowed by other microorganisms as the cheese ripens. In terms of flavor development, the amount of acid developed by the starter (and pH obtained), their initial peptidase activity, and the potential to produce intermediate flavor precursors are critical for the overall flavor development in cheese. The starters vary considerably in these regards, but detailed molecular studies are limiting as to the exact mechanisms of flavor development.

**USING STARTERS IN THE CHEESE FACTORY**

Starter cultures, with very few exceptions, are purchased by the cheesemaker from a company with vast experience in culture technology. Even companies that require specialized (self-discovered) strains call upon these “starter companies” to produce and maintain their individual strains. Consequently, the older methods of self-propagation are rarely practiced and so are not covered here. Cultures are sold in a few forms: frozen bulk and freeze-dried both for use as bulk starter inoculants and designed for direct inoculation to the cheese milk. In addition, cultures are also described as defined single or multiple strains or undefined mixed-strain cultures.

In the case of defined single or multiple strains, cultures are of known composition and the strain(s) is well documented as to attributes such as phage resistance, proteolytic activity, and rate of acidification. These are overall the most commonly used starters. Even artisanal cheesemakers are more apt to blend defined strains for their specifications for both acid and flavor development than rely on the more undefined cultures.

In the case of undefined strains, the culture composition is unknown and may vary between batches. Artisanal cultures are undefined mixed strains.

How much starter to use depends on the strain(s) used, how it was grown (pH controlled or not), and the desired rate and extent of acid development. Cheesemakers need to be cognizant that cheesemaking recipes and the amount of starter suggested are often based on a culture grown in milk or whey in which the pH was not adjusted during propagation. As a result, it was standard practice many years ago to use 1 to 2% of the weight of milk as starter inoculants. Today, with pH-controlled bulk starters, that amount is greatly reduced.

**BULK STARTER**

In the industry jargon, “bulk culture” can refer to the inoculant or the medium that is to be inoculated, but most often the term refers to a starter culture that has been propagated in sterilized milk (usually rehydrated skim milk), whey, or a specially formulated medium. The propagated bulk culture is then added to the milk for cheesemaking. The amount of bulk culture to use for cheesemaking depends upon the culture, how it was grown, and the cheese to be manufactured. Precautions are necessary to produce a bulk culture that will have a consistent and predictable rate of acid development in the vat. It was recognized early on that with artisanal cultures, excessive acid development could harm the culture. It was recommended that the starter not be too thick prior to using it. Thick milk, i.e., milk having the consistency of yogurt, was a symptom of excessive acid development (low pH) and exhibited free whey at the surface. Even though lactic acid bacteria can survive low pH, mesophilic strains generally are sensitive to prolonged exposure at low pH (<4.8), while thermophiles are tolerant of a pH much lower than that (pH 4.4). Exposures at low pH may result in injury to the bacteria, followed by eventual death. The result is an extended lag phase before rapid acid development occurs. The length of the lag phase may vary depending upon the strain and degree of injury.

In artisanal cultures or mixed-strain cultures, difference in acid tolerance between strains could alter strain balance and thus the cultures’ characteristics, including flavor development. To prevent acid injury, cheesemakers learned to “cut” (stir and cool) the starter at a
high pH (~5.5). The pH would still be above pH 5 by the
time the culture cooled to a temperature at which the
starter would no longer produce acid. It took much care
by and experience of the cheesemaker to coordinate in-
oculation rate, propagation time, and cooling rate of the
bulk culture. It became common practice to add a neu-
tralizer (ammonium hydroxide) if the pH dropped too
low. Today, cheesemakers continuously monitor the pH
of the bulk starter as it is propagated and automatically
inject neutralizer when the pH falls below a set value
(generally 6.0, but it can be as low as pH 5). An alter-
native is to manually add neutralizer after the pH drops
below ~5.2 to adjust the pH to just above pH 6.0, allow
the pH to drop again to ~5.5, and then cool the culture
until use (Fig. 3). A consequence of pH control growth is
twofold compared to non-pH-controlled growth. First,
the starter numbers are higher (Fig. 4), and second, the
starter is more active. There is no lag in acid production
as there is often with bulk cultures grown without pH
control (Fig. 5).

Defined-strain cultures grown in this manner have
been known to maintain desired activity for several days
after cooling. The number of cycles of pH drop and neutralizer addition is generally two or three, but one-time neutralization is also used in small-scale cheese plants and is done 1 or 2 h before the starter is used. Additionally, bulk medium has been developed where the neutralizer (or buffer) is already in the medium. The neutralizer (trimagnesium phosphate) is somewhat insoluble; however, when the pH falls below 5.5, it dissolves and neutralizes the lactic acid.

Some cheesemakers have begun to use non-pH-controlled bulk starters as a means to rapidly acidify cheese milk in addition to using direct-vat set (DVS) or direct-vat inoculum (DVI) cultures. This is done in lieu of direct acid addition, a process often needed when high-protein milks are used for cheesemaking.

In practice, undefined or artisanal cultures are generally not grown with pH control via neutralization but are monitored to prevent the culture from being exposed to low pH for extended times. Because some strains are more acid tolerant than others, there is the potential with pH control that less acid-tolerant strains will grow to higher levels than desired, while there is a potential for a decrease in numbers of more acid-tolerant strains. Buffered media or neutralization methods result in increased numbers of bacteria in the starter, so a lower starter volume is generally needed to inoculate the cheese milk. The starter volume can be decreased by as much as 50 to 75%. There are at least two benefits to the cheesemaker to use bulk starter, especially if it has been neutralized: the starter is highly active, and the lag phase between starter addition and pH drop is short. The downside to using bulk medium is the isolated or designated space required and the cost of equipment, skilled labor, and medium. In addition, care must be taken to prevent phage infection.

**DVS AND DVI**

DVS and DVI are highly concentrated cultures ($10^{11}$ to $10^{13}$ CFU/ml of culture) that are added directly to the cheese milk without further propagation by the cheesemaker. They generally come frozen in a can or as frozen or freeze-dried pellets in large bags. If they come in a can, they are not to be thawed and refrozen after a specified amount has been removed. Because of this, the frozen pellets are becoming more popular, especially with secondary or adjunct cultures. The advantages of using DVS or DVI cultures are that potential phage infection during preparation of a bulk culture is eliminated and there is no need for a starter room and specially trained personnel to handle bulk culture propagation. Phage can still infect the culture, but only after it is added to the cheese vat. Consequently, strict adherence to sanitation in the cheesemaking room is still essential. A disadvantage of using DVS or DVI cultures is that their use may extend the lag times before pH drop occurs compared to that in bulk cultures. The rate and extent of acid development are critical control
points for calcium loss from casein. If the pH is too high when the coagulum is cut, too much calcium will remain bound to the casein. The cheese could become too rubbery, the curds may not knit together properly, and the cheese may not flow or stretch when heated. The recent use of high-solid (higher-casein) milks has exacerbated the problem. To overcome this problem with DVS and DVI cultures, cheesemakers use preacidification rather than wait for the starter to lower the pH. Preacidification is the direct addition of acid (usually lactic or acetic acid) or carbon dioxide injection to lower the pH of the milk (usually to ~6.3 to 6.4) prior to the addition of rennet. However, the normal amount of starter must still be used. Another means to prevent the lag period is to add the amount of DVS or DVI culture that would be used as the inoculum to the cheese milk to a small amount of pasteurized milk at 28 to 30°C an hour or more before it is added to the vat. Many variations on this theme are being used. Improvements to DVI and DVS cultures have also been made to shorten or prevent the lag phase.

An advantage for the cheesemaker to using these concentrated forms of starter is the elimination of a “starter” room. Potential disadvantages include the need for a low-temperature freezer (−18°C) and potential adjustments (initially) to the manufacturing process. Newer freeze-dried versions are replacing the frozen concentrates and require only refrigeration. Frozen concentrates are initially very slow to produce acid, with later rapid acid development. Thus, changes may have to be made in manufacturing to account for this.

**ARTISANAL CULTURES**

Propagation of undefined-strain starters at the cheese plant requires special attention to details of time, temperature, pH, and time at that pH to prevent shifts in strain balance which could result in major changes in desired cheese quality. When propagating undefined cultures, it is advisable to begin with an original, or “mother,” culture to initiate propagation of a new batch of starter. Although back-slopping of an artisanal culture is a time-honored tradition, shifts in strain balance can occur. The mother culture (original culture from which all others are derived) must also be transferred in sterile milk on a routine schedule, as holding these cultures under refrigeration for extended periods could result in strain imbalance or even loss of certain strains. Strict adherence to growth parameters such as temperature and pH and even inoculum levels must be maintained to ensure strain balance. Although most artisanal cultures by definition are “home” grown, they are being produced commercially as frozen bulk set cultures or freeze-dried DVS or DVI cultures. It is extremely important that the cheesemaker not try to carry these cultures on their own once they get them from the company, as strain numbers and types could be adversely impacted. One artisanal commercially available culture was found to contain over 20 strains of bacteria. Individual artisanal cultures can contain a myriad of lactic acid bacteria, both cocci and rods, both mesophiles and thermophiles, and both homofermentative and heterofermentative, but usually a dominance of homofermentative lactic acid bacteria.

**PROTECTION OF STARTER CULTURE ACTIVITY**

The ideal starter is predictable and dependable; i.e., the rate and extent of acid development are consistent between batches of cheese. In the larger production facilities, a premium is put on time; the cheesemaker cannot afford to have inconsistent acid development. At artisanal, or farmstead, cheese factories, the cheesemaker can “babysit” each vat and make needed changes to the manufacturing schedule as conditions might demand. There is very little time allotted for manufacturing changes in the larger, by-the-clock manufacturing facilities. When problems are encountered, the cheesemaking process must still go on albeit with the knowledge that the cheese will be of inferior quality. This has put severe pressure on the culture suppliers to deliver a dependable starter, which has fueled intense scrutiny by the culture suppliers to research strategies to combat the most important inhibitor to predictable starter activity. That distinction goes to bacteriophage, viruses that infect bacteria and can wipe out a sensitive culture in a very short time.

Starter cultures are also very sensitive to antibiotics, but the command to test every batch of milk for the presence of antibiotics, and better handling of milk on the farm from treated animals, has largely eliminated this as a threat. Another potential threat is the blending of cultures by the cheesemaker of which they have no knowledge. Companies that supply cultures to the cheesemakers have put the cultures they sell through rigorous testing for metabolic activities and compatibility of the strains with each other. Some strains of starter might produce bacteriocins (antibiotics) which can kill other bacteria, including some starter strains. To toss together strains or add strains to existing culture blends may lead to the inadvertent loss of strains that
were part of the original culture blend. Thus, the culture blend may not produce the same desired cheese characteristics as before.

Although it is good manufacturing practice to sanitize equipment prior to use, residual sanitizer can be detrimental to starter activity. Starters are particularly inhibited by quaternary ammonium sanitizers (quats) because they maintain their activity in milk. Chlorine- or iodine-based sanitizers are generally inactivated by the proteins in milk. These sanitizers are more likely to create oxidized flavors on the surface of cheese if residuals are not removed from surfaces in contact with cheese.

**BACTERIOPHAGE**

A bacteriophage (phage) attack is often seen as a reduction in both the rate and extent of acid development but not necessarily complete inhibition (dead vat), especially if more than one strain of bacterium is used in the starter. Phage attach to specific sites on the bacterial cell wall and inject their genetic material into the cell. Subsequently, the infected cells produce phage, and ultimately, the cell bursts, releasing many more potentially infectious phage. The bacterial cell of course dies. For every bacterial cell, as many as 10 to 300 phage may be released (6). A complete cycle of phage infection to burst could take as little 10 to 140 min. Consequently, a starter culture could be wiped out in a very short time.

Phage are found everywhere bacteria are found, so care in proper sanitation is critical. Phage can maintain the infectivity even years after a plant has removed a sensitive strain from its starter rotation. Other than sanitation, the most common measure taken to prevent phage infection is to rotate cultures. Starter rotation is based on the fact that one phage only infects one or a few closely related strains and cannot infect different species. The commercial starter companies have tested all of their strains against all known phage. Thus, they can isolate phage-resistant strains, but they can also identify which phage can potentially infect a particular strain of bacterium. Both concepts are used to develop a starter rotation plan. In a starter rotation, one strain of starter is used for a few vats (or days) and is followed by another strain that cannot be infected by the phage that can infect the first strain. In reality, there is a bank of such strains that can be used following each other without a phage infection. In this manner, the level of phage capable of infecting a particular strain is reduced. The starter companies have developed extensive charts for rotation of strains. Along with this program, whey is monitored for the level of phage, and if the level gets too high, the phage-sensitive strain in the rotation can be removed and replaced by another strain not sensitive to the same phage. A precaution is necessary: while it may seem that different starter companies produce different strains of starter, this may not be true, or a strain from one company may be sensitive to the same phage as that infecting a starter from another company.

In addition, rather than just one strain in the starter, two or more strains are used. Using more than one strain not infected by the same phage means that if a phage infects one strain, the other strain(s) can still generate acid. In practice one strain tends to dominate, so while acid development continues, the rate of acid development is reduced. Phage may not be killed by pasteurization. Since phage are in the whey, care must also be given to prevent aerosols from forming during whey processing as well as to separation between cheesemaking and whey processing areas. Cheesemakers who use bulk starters also use separate rooms for starter propagation and cheesemaking. Starter rooms also have filtered air and are under a positive air pressure so that the flow of air is outwards. In most large cheese plants, the employees who are responsible for the starter are never allowed in the rest of the plant, and of course no one else is allowed into the starter room.

Artisanal cultures have been looked upon as sources of phage-insensitive strains because of the diversity of strains they contain and the high probability that they have at one time or another been exposed to many phage types. One out of a million cells in a population of an individual sensitive strain is resistant to phage infection. With time, it is probable that the artisanal undefined-strain culture has been infected with multiple phage types and that these strains have developed a certain level of immunity. Over time, it is conceivable that all artisanal cultures will be composed of multiple phage-resistant strains.

**PROPER STORAGE OF STARTERS**

In most commercial factories, frozen cultures are ordered in large quantities and are not held longer than a few weeks prior to use. In addition, cultures are kept frozen in very expensive freezers designed to maintain very cold temperatures (<−40ºC). In this manner, the activity of the culture upon use is consistent from batch to batch. Unfortunately, for very small cheesemaking operations, the scenario may be different if they use a home freezer. Loss of cell viability may occur during prolonged frozen storage (several months) due to the
higher temperatures (−17°C) and the likelihood of a lot of temperature fluctuation. The use of freeze-dried cultures has gained popularity with cheese factories because these cultures can be held at commercial refrigeration temperatures for several months without substantial loss of viability. In addition, packets of the freeze-dried cultures can be opened, the amount needed poured out, and the package resealed. However, once opened, the remaining culture should be used within a few days. Exposure to oxygen may damage the cells. Older cultures may also lose strain balance. This is especially important when a mix of acid producers and flavor producers is used as a starter. Bacteria differ in their ability to survive frozen or refrigerated storage and in their ability to recover from any damage that may have occurred. In the case of single-strain cultures, older frozen cultures may be salvaged by first putting the culture (in slight excess of what is needed in the cheese vat) in a small amount of milk for a few hours prior to inoculation into the cheese vat.

THE IMPORTANCE OF LACTOSE FERMENTATION

The origins of most undesirable characteristics in cheese have been found to be related to one or more of the following:

1. Milk quality
2. Rate and extent of acid development
3. Microbiological contamination
4. Temperature abuse during ripening or retail

Fermentation of lactose to lactic acid serves many purposes in the manufacture of cheese, and therefore, if the amount of acid produced is altered between batches of cheese, then characteristics or quality of the cheese may be changed. Indeed, when there are defects related to body, texture, and functional properties such as stretch and flow, they are generally fixed by changing the rate and extent of acid development at key points in the manufacturing process. There are three distinct inseparable features of fermentation: lactic acid is formed and as a consequence the pH of the milk or cheese is lowered, and eventually lactose is depleted. Upon release of lactic acid from the bacteria, a small number of lactic acid molecules dissociate (release a hydrogen molecule, often denoted as H⁺). The accumulation of H⁺ ions lowers the pH and causes many of the physical changes to milk or curd that occur during cheesemaking. These changes are due to the impact that pH has on enzyme activity and casein molecules. The influence of pH on the coagulation process, solubilization of calcium, and molecular interaction between casein molecules is covered in Cheese and Microbes (7).

The tolerance of the starter to salt is of major importance to the rate and extent of acid development. Together with temperature, it is the method used by cheesemakers to control starter activity. The extent of pH decrease in cheese is ultimately under the influence of starter activity, lactose content of the curd, initial colloidal calcium phosphate content of the milk, and rate of acidification. The last impacts the buffer capacity of curd, namely, the amount of colloidal calcium phosphate remaining in the curd after whey separation. The more acid develops in the milk or in the curd during syneresis, the more buffer is lost and consequently the lower the pH of the cheese will be. The amount of acid produced is determined by the amount of lactose in the milk and that remaining in the curd. Curd washing and whey dilution are two steps taken by cheesemakers to limit the amount of acid formed in the cheese. If such methods are not used, it is very likely that the pH of the cheese will fall below pH 4.9, well below the pH desired in most cheese varieties. The other method used to control acid development is to slow or inhibit the starter with the application of salt at a level high enough to do so. Salt tolerance of bacteria is based on the amount of salt in the water of cheese (percent salt in cheese/percent water in cheese). Like other characteristics such as the response to temperature and pH, salt tolerance is strain dependent. Lactobacillus spp., especially nonstarter species, are the most salt tolerant of the starter bacteria (>6% salt in moisture). Streptococcus thermophilus is the least tolerant of salt (<3% salt in moisture). Lactococcus species typically tolerate between 4.5 and 5% salt in moisture. In the past, many of the most often used Lactococcus starters were not very salt tolerant (>4.5% salt in moisture would inhibit them), but today, with the importance put on fast acid development, this has changed, and more salt-tolerant Lactococcus strains are being used. As a consequence of both rapid and excessive acid development, cheese will have a low pH and develop defects such as acid flavor, brittle body, free serum at the cheese surface (sweaty cheese), and early development of calcium lactate crystals in Cheddar cheese. In some cheeses, such as mold-ripened or washed-rind cheeses, the mold or secondary flora may be slow to develop at low pH (4.7).

Another distinction between cultures is based on the rate of acid development, i.e., fast and slow cultures. It is not clearly defined, since the rate of acid development is
related to the number of active bacteria in the starter and pretty much pertains to *Lactococcus* spp. The separation between fast and slow starters is somewhat arbitrarily based on the pH obtained with a 1% inoculum rate in skim milk after 18 h at 20°C. Fast starters drop the pH below pH 5, while slow starters do not. Fast cultures tend to be more salt tolerant than slow starters. Consequently, fast starters have a proclivity to produce acid cheese (low-pH cheese) and the defects associated with it, but they remove lactose very rapidly from curd during the initial storage of cheese. Slow starters have a tendency to result in cheese with residual lactose unless adequate time is allowed for fermentation before salt is applied. If sufficient numbers of heterofermentative bacteria are present and there is sufficient residual lactose and if the cheese is held at a warm enough temperature, enough gas can develop upon fermentation to cause splits or round holes in the cheese or cause cheese to puff up during storage. Slow starters also tend to be less prone to the development of bitterness and to die more rapidly during aging than fast starters. These characteristics have made the slow starters in demand for aged cheeses by some cheesemakers even though they may have to use more of the starter to achieve the desired rate of acidification during cheesemaking.

Lactic acid imparts its own unique flavor. Well-trained cheese judges can separate lactic acid taste from sour taste. This is useful in determining corrective measures when trying to eliminate the sour note. An acid cheese means that there is too much lactic acid flavor, but it can also refer to distinctive physical characteristics of cheese, such as short or brittle cheese that results from a low pH. A sour cheese implies that there are other acidic components, particularly high levels of acetic acid (vinegar). Lactic acid (or pH) also impacts the perception of other flavor components. Lactic acid has also has antimicrobial properties which, in turn, are determined both by the amount of acid and by the pH of the cheese. Indeed, a pH of >5.4 is often a red flag for potential pathogen growth in cheese.

Metabolic activity of the starter cultures produces a cheese environment conducive for desired flavor development, particularly through acidification but also through their death. When bacteria die, intracellular enzymes are released. These enzymes retain activity and play a vital role in the breakdown (hydrolysis) of casein. In addition, the dead cells are a source of nutrients for the next generation of NSLAB and other secondary microorganisms. Microorganisms feed on cell debris and through their feeding frenzy produce a myriad of chemicals that in part give cheese its flavor. Other flavor components are derived from lactose, citric acid, and fat and its constituents, the fatty acids, through both biochemical processes of microorganisms and the activity of enzymes naturally present in the milk and those that are deliberately added.

Recently, there has been a resurgence of interest in identifying the complex relationship that exists between the starter, the secondary flora, and the cheese environment and the impact that they can have on the development of flavor. Such interest is in part related to the demand for higher-quality lower-fat and lower-salt cheeses. The environment (i.e., salt, lactic acid, pH, and equilibrium between fat and moisture phases) is often very different in these cheeses from that in full-fat and higher-salt cheeses. Cultures that were once widely used in the industry but that lost favor in the last few decades due to their slower acid development have been identified as potential starters for lower-salt and lower-fat cheeses. A major concern with revitalizing the use of these cultures is that they were often prone to bacteriophage infection. Progress in the genetics of starter bacteria may make it possible to use these “forgotten” cultures.

**TEMPERATURE SENSITIVITY**

An important means to increase the rate of fermentation is to warm milk or curd to the optimal temperature for this process. This usually is at a temperature for optimal growth. At times the cheesemaker may need to decrease the rate of acid development. The most commonly used method is to increase the temperature slightly or dramatically decrease the temperature (Fig. 6 and 7). The latter may have negative consequences to cheese unless the curd is warmed again (usually not done). Cool curd may not knit properly, and demineralization due to acid development may not be sufficient for proper body development or subsequent melting of the cheese. At times the cheesemaker inadvertently cools the curd too soon. As a result, fermentation is curtailed, and there is residual lactose or galactose in the cheese that can be fermented later by heterofermentative contaminants. A gassy, slitty, or huffed cheese may result. When thermophiles are used, both residual lactose and galactose result with excessive cooling (<20°C). In traditional manufacture of Romano and Parmesan cheeses and where both *Streptococcus thermophilus* and *Lactobacillus helveticus* are used, the pressed curd is kept warm (>37°C) overnight to allow complete fermentation of both sugars. Without complete sugar fermentation, the cheese may brown during aging.
SALT SENSITIVITY OR SALT TOLERANCE

Salt in cheese is usually reported as percent sodium chloride (weight per weight), but to a microbiologist the important criteria relating to salt measurement are salt in moisture and water activity. Salt in moisture (S/M) is calculated by the following formula in the United States:

\[
\frac{\% \text{ salt in cheese}}{\% \text{ moisture in cheese}} \times 100 = S/M
\]


In Europe the following formula is used:

\[
\frac{\text{% salt in cheese}}{\text{% salt in cheese + % moisture in cheese}} \times 100 = S/M
\]

Water activity is largely due to salt and moisture content and is a measurement of the availability of water. Since salt ties up water, the more salt and less moisture in the cheese, the less water activity. Water activity requires special equipment for measurement. Parmesan cheeses tend to have one of the lowest water activities (0.92), while cottage cheese has the highest (0.98). Water activity is used more in context of the potential for pathogen growth, while salt in moisture is used mostly for the salt tolerance of desirable bacteria, although they are sometimes used interchangeably.

Starters vary considerably in their response to salt. A sufficient salt concentration (measured as salt in moisture) slows or stops acid development, but several factors contribute to the extent of inhibition. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains tend to be the most salt-sensitive starter cultures. Salt-in-moisture values exceeding ~3% stop fermentation. *Lactobacillus helveticus* strains are inhibited by ~5% salt in moisture. *Lactococcus lactis* subsp. *cremoris* strains tend to be more sensitive to salt (~5% salt in moisture) than *Lactococcus lactis* subsp. *lactis* strains, which are inhibited by slightly higher salt in moisture (~6%). Nonstarter lactobacillus species tend to be more salt tolerant than the starters, with some strains being able to grow at salt in moisture exceeding 8%. Addition of salt to lactobacillus selection agars (6% salt) is often used to differentiate nonstarter lactobacilli from starter lactobacilli. However, there is considerable variability in resistance to salt between all genera of starters and nonstarters. Salt in moisture above 6% generally retards the development of flavor unless more salt-tolerant secondary microorganisms are used for ripening, as in mold- or smear-ripened (washed-rind) cheeses. *Propionibacterium freudenreichii* subsp. *shermanii* strains show wide variability in salt sensitivity but, if combined with low pH (<5.4), are sensitive to salt-in-moisture values of >3%.

Salt is either applied directly to curd (direct salting), via brine immersion (brining), or rubbed on the surface of the cheese after it is taken out of the form (dry salting). Regardless of the means of application, there is some degree of time lag between when the salt is applied and when inhibition occurs. In direct salting, the time lag is decreased by applying salt to smaller pieces of curd. The rate of acidification (culture strain and amount of starter) must also be taken into account. If the time lag is too long and if the acidification too rapid, there is less, if any, inhibition. In the not-too-distant past, the rate of acidification used in cheesemaking was lower than is normally seen today. For example, salt was applied to Cheddar curd at a pH of ~5.4 and the drift of pH downwards continued to pH ~5.0 before it stopped due to salt inhibition. Less salt (1.4 to 1.7%) and lower moisture (37%) were used than are typical today (1.7 to 1.9% salt and ~38%). Today, with more rapid acidification, the salting pH of Cheddar is typically above 5.6, with the hope that the pH will not drift below pH 5.0 before the culture is inhibited. However, the pH of the curd may still eventually fall below 5.0. Indeed, excessively acid-tasting cheese is the most common fault of Cheddar cheese and is strongly correlated to the more rapid speed of acidification and decreased salt sensitivity of the starter. In the early 1980s, the incidence of calcium lactate crystals in Cheddar cheese was increasing. This increase was attributed by many cheese technologists to the changes in the starter cultures used, and this was confirmed by the culture companies. The demand for faster acid development by cheesemakers but without the required use of larger volumes of starter culture to accomplish this led the starter supply companies to develop starter cultures that could produce acid much faster than before. pH-controlled starter systems were now common. The cultures also tend to be the more salt-insensitive strains of *Lactococcus lactis* subsp. *lactis*. The rate of acidification and salt tolerance of the starter are thus important attributes of the starter to consider to control pH in cheese. Since the extent of acid development is due to the amount of lactose in the curd, removal of some lactose by curd rinsing is used to combat the potential for excessive acidity. Curd rinsing or washing (many cheese varieties including brick, Colby, and Havarti) and whey dilution (Gouda, Swiss, and Baby Swiss) are common techniques used by cheesemakers to remove lactose and prevent excessive acidification.

Brine salting or dry salting of blocks of cheese is generally not done to stop or slow starter activity, since by the time the cheeses are salted the starter has generally completed sugar fermentation or the cheese has been sufficiently cooled to preclude any more fermentation by the starter. However, in the case of pasta filata cheeses, the curd is cooled before brining and residual sugars at levels above 0.6% are common.

Salt sensitivity of starters is an extremely important issue in reduced-sodium cheeses. When salt is reduced, previously salt-sensitive starters and nonstarter bacteria can grow, and their metabolism may negatively impact
cheese quality. Unless the lactose content of the cheese is reduced, the cheese will become excessively acid (pH < 4.9), and there is also a propensity for the development of bitter flavor. In order to reduce lactose content, either the whey is diluted with water or the cheese curds are washed or rinsed. This may have negative consequences on flavor development for more traditionally acid cheeses such as Cheddar. The chemical environment within cheese is a critical parameter for determining survival and growth and metabolism of bacteria. Alterations of this environment such as increasing pH, lowering salt in moisture, and decreasing acid levels can shift the microbiological ecology and consequently the sensory attributes.

OTHER CRITERIA FOR STARTER CULTURES

The companies that supply starter cultures have exhaustively characterized their starter strains, often to a molecular level. This means that for a cheese company it is often up to them to ask their supplier for the given characteristics of the primary or secondary starter or that are needed for their specific cheese. Indeed, recently it has been the flavor-producing adjuncts that have received the most attention. Of particular interest to the starter company is to define their strains on the bases of rate of acidification, salt, pH, temperature sensitivity or tolerance, proteolytic activity (usually ability to utilize peptides rather than break down intact proteins), compatibility with other strains either detrimental (production of bacteriocins active against other starter strains) or beneficial (bacteriocins active against pathogens), phage resistance, and ability to produce selected flavor components under defined conditions in cheese such as low salt, low acidity, and low fat (8–10).

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REFERENCES

1. Decker JW. 1895. Cheddar Cheese Making, p 6. Published by the author, Madison, WI.