21

The production of smear cheeses

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21.1 Introduction: smear-ripened cheese varieties

A small proportion of the cheese varieties sold worldwide have a surface covered by a layer of yeasts and bacteria, e.g. Tilsit, Limburg, Romadour, Chaumes, and the acid-curd 'Harzer', or 'Handkäse' (Table 21.1). These aerobic microorganisms have a strong impact on the appearance, flavour and texture development of the cheeses, which usually leads to shorter ripening periods of several weeks rather than months. Smear cheeses are generally known for their intense sulphurous and ammoniacal smell (Reps, 1993).

Figure 21.1 illustrates several smear cheese varieties: left rear, soft Chaumes cheese with a bright orange surface which is tightly covered with a thin paper layer partly removed on this picture; left front, mature acid curd cheese (no quarg core) with a translucent light brown colour of rind and core and with a rubbery texture; right rear, semi-soft Tilsit cheese with a light reddish-brown surface and the typical irregular holes (size of cut area *ca.* 13 × 13 cm); right front, soft Limburg cheese with typical white streaks (*G. candidum*) on an orange background (yellow-pigmented *A. nicotianae* and/or *M. gubbeenense* predominant).

Apart from the influence of the physical and chemical parameters of the cheese milk, starter and non-starter lactic acid bacteria, these secondary cheese cultures contribute significantly to the complexity of cheese manufacture. Maintaining a high level of hygiene as well as a profound knowledge of the needs of a typical surface flora are essential during ripening because the cheese surfaces are exposed to an unsterile environment. Undesirable contaminating bacteria or moulds will grow immediately if the balance of the cheese microflora is disturbed (Bockelmann, 1999).

Table 21.1 Surface-ripened cheese varieties (adapted from Chapman and Sharpe, 1990, and Kammerlehner, 1993)

Cheese variety	Origin	Ripening period (months)
Soft		
Limburger	Belgium	<1
Romadour	Germany	<1
Chaumes	France	<1
Semi-soft		
Münster	France	1
Brick	USA	1–2
Monterey	USA	1–2
Saint Paulin	France	1–2
Taleggio	Italy	2
Havarti	Denmark	1–3
Tilsit	Germany	1–5
Bel Paesa	Italy	4–5
Hard		
Danbo	Denmark	1–2
Gruyère	France	4–12
Acid-curd		
Harzer Roller	Germany	<1

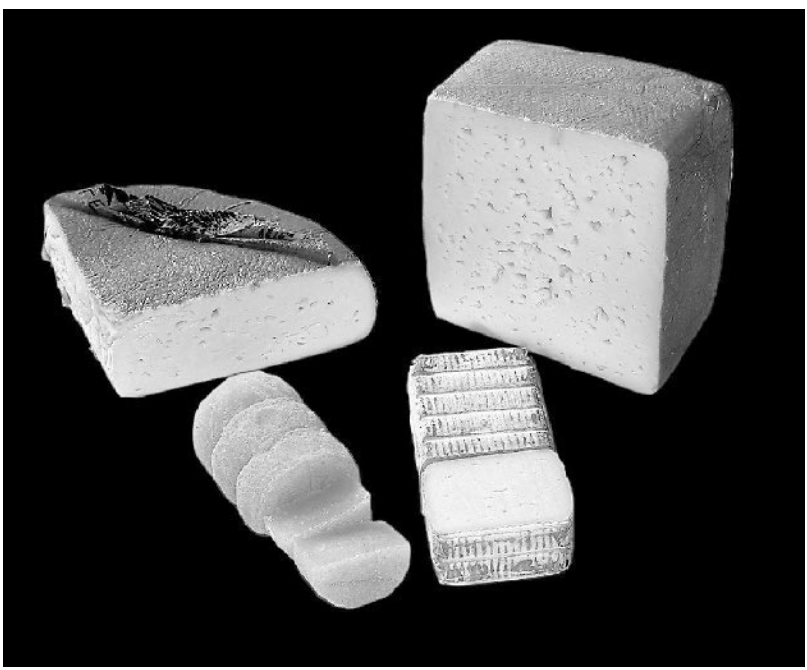

Fig. 21.1 Size and appearance of several smear cheese varieties.

In the present chapter special emphasis will be put on new developments in surface starter technology, a long neglected area. Contamination of smear cheeses with *Listeria monocytogenes* is still reported periodically, sometimes leading to food poisoning incidents and, consequently, to large economic losses for cheese manufacturers (Rudolf and Scherer, 2001). Most of the data presented on novel starters were obtained by studies on semi-soft Tilsit-like cheese (an EU-funded project). Some new results on new surface starters for soft cheeses and acid-curd cheeses that are presented here were obtained in two current projects funded by the German Federal Ministry of Economics and Labour (AiF projects).

21.2 Production and ripening

The annual production of cheese in the European Union exceeded 6 million tons in 1998. Probably due to their intense sulphurous smell, smear cheeses are not mass produced like Gouda or Cheddar cheese. Smear cheeses are traditionally produced on a small scale in a farmhouse environment in many European countries. Production is more industrialised in countries such as Denmark, France, Germany and The Netherlands; however, most cheese companies are still small or medium-sized enterprises.

Smear-ripened cheeses can be produced from any kind of rennet curd (Table 21.2). They can be divided into (semi-)soft (moisture 45–55%), semi-hard (moisture 45–50%) and hard cheeses (moisture 35–45%). Some well-known cheese varieties are listed in Table 21.1. In general, cheeses are salted by brining in $\geq$18% sodium chloride. Small soft cheeses such as Limburg and Romadour are brined for 1.5–4 h, the larger semi-soft and hard cheeses for approximately 24 h. An alternative traditional manual method is wiping small cheeses with cloths soaked in brines. A newer salting method is dry salting, where cheeses are sprayed with dry salt vapours, a method established for small soft smear cheeses.

After salting, the cheeses are smeared (brushed, wiped or sprayed) with salt water containing suitable yeasts and bacteria. Thus, apart from the influence of starter and non-starter lactic acid bacteria, cheese ripening is influenced by metabolic activities of the surface microflora (Bockelmann, 2002a). Typical ripening times are 2 weeks for the soft Limburg/Romadour varieties (200–500 g), 1–6 months for semi-soft Tilsit-type cheeses (2.5–3.5 kg), and 6–12 months for hard cheeses like Gruyère (>2.5 kg).

Quite different from all rennet-type cheeses are the ‘sour milk’ (acid-curd) cheeses which have a long tradition in Germany. They are produced from low-fat quarg (>30% dry mass), which is produced with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Salting of acid-curd cheeses is performed by mixing quarg with ripening salts (NaCl, $NaHCO_3$, $CaCO_3$) to obtain the appropriate salinity and pH. Ripening of acid-curd cheese is usually restricted to 1–3 days in the factory, progressing during transport and cold storage in food markets over the shelf-life of 37–45 days after packaging (Table 21.2, Engel and Roesch, 1995).

Table 21.2 Ripening patterns of the major groups of smear-ripened cheeses; 'mature' cheese indicates condition at the time of packaging (data taken from various textbooks on dairy technology).

Semi-hard cheese	Semi-soft cheese	Acid-curd cheese
Pressed rennet curd, pH 5.6	Pressed rennet curd, pH 5.0	Quarq pH 3.7–4.1
Brining, 24 h, pH 5.1	Brining or dry salting, 1–4 h, pH 5.0–5.1	Mechanical mixing of curd/salt/'culture cheese' pressing, pH 5.2
	Low-temperature sweating, 20–22ºC, >95% r.h.	High-temperature sweating, 30–33ºC, >95% r.h.
First smearing (addition of starters)	**First spraying** (addition of starters)	**First spraying** (addition of starters)
Ripening, 13–15ºC, 95–98% r.h.	Ripening, 13–15ºC, 95–98% r.h.	Ripening, 13–15ºC, 95–98% r.h., 1–3 days
Repeated smearing, ~6 times during 6 weeks	Repeated smearing, ~5 times during 2 weeks	
		Mature cheese (>2 days)
	Mature cheese (>2 weeks)	Extended ripening, 2 weeks for 'culture' cheese
Mature cheese (>4 weeks)		

21.2.1 Traditional ripening

Bacterial smear-ripened cheeses have a long tradition. Without knowledge of the bacterial nature of the surface flora, a large variety of smear cheeses was produced long before the year 1900 (Fox, 1993). When cheeses produced from raw milk – an important source of surface microorganisms – are exposed to air with a high relative humidity (>95%) they naturally tend to develop a smear layer on the surface, typically consisting of yeasts and bacteria. More than a century ago, Laxa (1899) already isolated and described yeasts and yellow-pigmented bacteria from the surface of smear cheeses.

Surface-ripening of smear cheeses begins with the growth of yeasts (e.g. *Debaryomyces hansenii*) which utilise lactate and increase the surface pH of the cheese (Busse, 1989; Eliskases-Lechner and Ginzinger, 1995b; Reps, 1993). When the pH increases above 6, *Brevibacterium linens*, other coryneform bacteria and staphylococci (micrococci) begin to grow and eventually cover the whole surface of the cheese (Fig. 21.2; Eliskases-Lechner and Ginzinger, 1995a; Bockelmann *et al.*, 1997c). Figure 21.2(a) shows typical 'coryneform bacteria', i.e. Gram-positive, aerobic, non-motile, irregularly shaped rods (club- or V-shaped) belonging to *Corynebacterium*, *Brevibacterium*, *Arthrobacter* and *Microbacterium*. The fissures seen in some cells indicate the post-fission

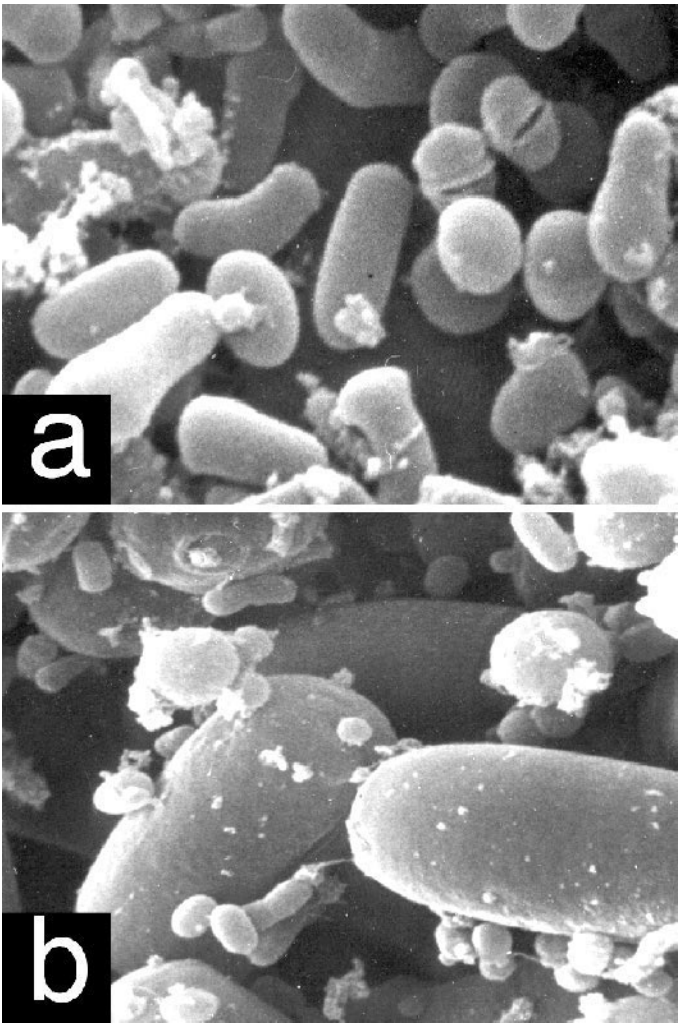

Fig. 21.2 Scanning electron micrograph of the surface of Romadour cheese (courtesy of H. Neve, Institute of Microbiology, Federal Dairy Research Centre, Kiel, Germany).

snapping of cells typical of 'coryneform bacteria' (magnification 10 000×). Figure 21.2(b) shows cylindrical *Geotrichum candidum* and globular *Debaryomyces hansenii* yeasts coexisting with coryneform bacteria and cocci, most likely *Staphylococcus equorum* or *S. xylosus* (magnification 5000×). The sources of the typical surface microorganisms are cheese milk, cheese brines, the air of ripening rooms, ripening pads and the human skin. Since the introduction of pasteurisation, which has considerably improved food safety, the cheese milk flora has less influence on the surface microflora of cheeses (Holsinger *et al.*, 1997). From starter companies, some yeasts and bacteria can be obtained to mimic the endogenous raw milk microflora (see Chapter 3).

Correct handling and storage of smear cheeses during ripening is essential. Ripening temperatures range from 14ºC to 19ºC, and the humidity should be at least 95%. Excessive ventilation should be avoided. In addition, repeated turning of cheeses and surface treatment by repeated smearing (brushing) are most important for ripening (Kammerlehner, 1995).

The smear is applied to the cheese using a rotating brush, which is wetted when moving through the smear liquid placed at the bottom of the machine. For soft cheeses spraying machines are used instead. The smear liquid is made up in water or whey containing ~3% salt to mirror the salinity of the cheeses and is generally inoculated by commercially available surface starters (Bockelmann, 2002a). The recommendation is about 10^4 cfu/ml in the cheese milk, or 10^6 cfu/ml in the smear or 10^{11} cfu per 100 kg of cheese. However, cheese producers do not often rely on these cultures alone. Traditionally, mature cheeses are smeared (sprayed) before young cheeses in the same machine, which is called 'old–young' smearing because part of the 'old' flora from mature cheeses is retained in the smear liquid and thus is brushed (sprayed) subsequently onto the surface of green cheeses. For acid-curd cheeses a different old–young step is included with the same effect: a special batch of cheeses, ripened for 2 weeks instead of 2 days ('culture cheese'), is mixed with quarg and ripening salts (2–4% w/w) to initiate the ripening of the 'sour milk cheeses' (ripening 2 days, with further uncontrolled ripening in the packaged state during transport and marketing; Bockelmann, 2002b).

21.2.2 The surface microflora of commercial smear cheeses

Semi-soft cheeses

Due to their natural presence in cheese brines (Jaeger *et al.*, 2002), *D. hansenii* and cream or orange *S. equorum* are always found on semi-soft cheeses, with the highest cell counts in the first week of ripening. If commercial 'Micrococci-Preparations' (trade name) are used for smearing, *Staphylococcus xylosus* can also be detected on the cheese surface (Bockelmann and Hoppe-Seyler, 2001). A certain percentage of yellow-pigmented *Arthrobacter nicotianae* and *Microbacterium* sp. is detected, species with similar technological properties that can be reliably identified only by molecular methods (e.g. partial sequencing of the 16S rDNA or ARDRA; Hoppe-Seyler *et al.*, accepted for publication). Cream-coloured corynebacteria are most abundant in the surface flora. Unfortunately, classification of *Corynebacterium* spp. is difficult. A species frequently associated with smear cheeses is *C. casei* (Bockelmann, 1999). *Brevibacterium linens* is generally called the 'typical red smear bacterium' before all other species because of its bright orange pigments. It is one of the best studied cheese bacteria; the strong sulphur metabolism and the bacteriocins specific for *L. monocytogenes* have been studied in detail (Eppert *et al.*, 1997; Valdes-Stauber and Scherer, 1996). However, the cell counts of *B. linens* on the cheese surface are rather limited. Even with no *B. linens* detected on selective agars (< 0.1% of the microflora) cheeses can be of normal appearance and flavour (Bockelmann *et al.*, 1997c).

Table 21.3 Surface cell counts of several types of smear cheeses

Surface microflora	Tilsit-type cheeses	Chaumes	Limburg/ Romadour	Acid-curd cheese
Yeasts (cfu/cm^2)	10^3–10^6	10^4–10^7	10^4–10^7	10^7–10^8
Smear bacteria (cfu/cm^2)	10^8–>10^9	10^8–>10^9	10^8–<10^9	10^8–>10^9
Corynebacterium spp.	50–90%	50–70%	10–>60%	20–90%
Arthrobacter, *Microbacterium*	0.1–5%	1–30%	2–>50%	n.d. (<0.1%)
Brevibacterium linens	0.1–15%	<1%	<1–15%	0.1–50%
Halomonas spp.	—	—	30–40%[a]	—
Staphylococcus spp.	0.1–5%	0.1–5%	<1%	1–40%
Micrococcus spp.	—	0.1–20%	—	—
Enterococcus spp.	<10^3	<10^5	<10^4	<10^6
Pseudomonas spp.	<10^2	<10^2	<10^3	<10^3
Coliform bacteria (titre)	<10^2	<10^5	<10^3	<10^5
Gram-negative rods (VRBD)	<10^5	<10^6	<10^4	<10^6

[a] Typical for one factory only; detected in all samples from 2000 and 2001.

A certain low level of contaminating bacteria (enterococci, enterobacteria) can always be expected on the surface of smear cheeses (Gianotti, 1999). Summarised results are shown in Table 21.3, for which red smear cheeses were analysed routinely over several years (1995–2002) with more than 20 samples for each cheese variety. Generally, cream-coloured colonies belonged to the species *C. casei*, yellow orange to two species, *Arthrobacter nicotianae* and *Microbacterium* sp. and orange colonies of irregular rods to *Brevibacterium linens*. Staphylococci (cream and orange colonies) were generally classified as *Staphylococcus equorum*. Sometimes *S. xylosus* and *S. saprophyticus* were identified. The latter species was quite commonly found on the surface of acid-cured cheeses. Identification was performed by ARDRA and the restriction patterns were compared to type strains of the species (Hoppe-Seyler *et al.*, accepted for publication).

Soft cheeses

The surface microflora of soft cheeses shows distinct differences (Table 21.3). The yeast flora consists of two predominant yeasts, *D. hansenii* and *Geotrichum candidum*. Apart from numerous cream-coloured coryneform bacteria (*Corynebacterium* spp.) a high percentage of yellow coryneform *Arthrobacter nicotianae* and *Microbacterium* sp. is observed (Table 21.3). Although usually at 20–50% of the flora (Limburg, Romadour), the percentage can be near 100% of total cell counts in some factories. *Brevibacterium linens* and *Staphylococcus* spp. show normal low cell counts as observed for semi-soft cheeses. A typical finding for the French Chaumes cheese over several years was orange *Micrococcus* species, microscopically being large diplococci (Bockelmann and Hoppe-Seyler, 2001). The presence of a high percentage of salt-tolerant, motile rods (*Halomonas* sp.) was typical for a single cheese producer, being

always detected on cheeses over several years (Table 21.3). The degree of contamination with enterococci and enterobacteria is usually higher than for semi-soft cheeses.

Acid-curd cheeses

The different technology of production of acid-curd cheeses from quarg results in significant differences in the appearance, flavour and surface microflora (Table 21.3). A number of yeast species is found, *Kluyveromyces marxianus*, *Candida krusei*, *C. utilis*, *C. lipolytica* and *Trichosporon asahii* being frequently identified. Most common in many of Engel's analyses (Engel and Roesch, 1995) were *K. marxianus* and *C. krusei*. Since many *C. krusei* strains show a preference for lactic acid as C-source leading to fast deacidification of the acid curd, this species may be typical and important for smear development. *Trichosporon* species are unwanted contaminants because they are frequently related to human skin diseases in the scientific literature.

As for semi-soft cheeses, cream-coloured coryneform bacteria are predominant (Table 21.3). Yellow-pigmented *Arthrobacter* or *Microbacterium* species are rarely found in high cell numbers. The percentage of *B. linens* and *Staphylococcus* species of total cell counts is quite variable. A considerable amount of the *Staphylococus* counts can be caused by *S. saprophyticus*, an important contaminant of acid-curd cheeses (Bockelmann, 2002b).

21.2.3 Current problems during ripening

It is still general practice to use the mature smear layer of aged cheeses for the treatment of young cheeses by performing old–young smearing. The associated hygienic problems are obvious: saprophytic or pathogenic bacteria as well as moulds can become part of the house microflora and can persist over long periods of time by this in-house contamination cycle. A certain undetected low level of contamination with enterobacteria, pathogens such as *Listeria monocytogenes* and other contaminants can be assumed for traditionally old–young smeared cheeses. For reasons largely unknown, pathogens sometimes grow to high cell numbers, only then posing a risk for consumers. Not the pathogenicity but the common (transferable) multiple antibiotic resistances are matters of concern when enterobacteria and enterococci are contaminating the cheese surface (Teuber, 1999; Teuber and Perreten, 2000). Therefore old–young smearing is more and more criticised and efforts have been initiated to establish alternative methods, i.e. functional defined surface starter cultures, to meet the continuously increasing hygienic demands of European guidelines and regulations.

21.3 Developing ripening cultures

Trade in the so-called secondary cheese cultures is small compared with that in lactic starters for cheese and fermented milks. Therefore culture development is

rather poor. Mainly, *Debaryomyces hansenii*, *Geotrichum candidum* and *B. linens* (*B. casei*) are used, and these are offered as smear cultures by all major starter companies (e.g. Chr. Hansen, Denmark; Danisco-Cultor, Germany; Degussa-Bioactives, Germany/France; Rhodia-food, France; Sacco srl, Italy). So-called 'Micrococci' preparations contain *Staphylococcus xylosus* or *S. carnosus*. One supplier offers *Microbacterium* sp. and mixed-strain combinations containing unspecified corynebacteria.

The range of cultures offered does not reflect the microflora of the different smear cheese varieties. The recommended doses for smear cultures are 5×10^{10} cfu per 1000 litres of cheese milk, or 10^6 cfu/ml in the smear liquid, or 10^{11} cfu per 100 kg cheese (data taken from starter suppliers' leaflets). However, cheese manufacturers seem to use lower concentrations for cost reasons (several German cheese manufacturers, personal communication). According to Bockelmann *et al.* (1997b) a defined surface culture should contain at least 10^7 cfu/ml to initiate smear development without using old–young smearing.

The functionality of some commercial culture strains seems to be questionable. In a Swiss study it was shown that the deacidification properties of commercially available yeasts were rather poor compared to natural isolates from various sources (Wyder and Puhan, 1999). *Brevibacterium linens* strains used in smear starters are often not found on mature cheese in significant numbers, without any negative effect on cheese quality (Bockelmann, 2002a). In one study in which 'Micrococci-preparations' (*S. xylosus*) were used to inoculate cheese brines, naturally occurring *S. equorum* were predominant in the brines after several weeks (Bockelmann and Hoppe-Seyler, 2001). Looking at the complex composition of a smear cheese microflora (Table 21.3), only a minor role of these commercial secondary cultures can be expected; the influence of an intact house microflora is still essential today. However, the use of fully functional smear cultures might be necessary for cheese factories in the future to meet the continuously increasing hygienic demands of European guidelines and regulations.

To define the requirements for a functional surface starter, essential components of the surface microflora have to be identified, and the role of the species detected has to be understood. The composition of the surface flora of commercial smear cheeses still depends on the specific house microflora of the cheese manufacturer. However, some general bacteriological similarities can be found for all smear cheese varieties. The bacterial surface flora consists mainly of so-called 'coryneform bacteria' which is not an accepted taxon but a useful descriptor for irregular, club- or V-shaped rods belonging to the genera of smear bacteria *Corynebacterium*, *Brevibacterium*, *Arthrobacter* and *Microbacterium* (Bockelmann, 1999).

23.3.1 Classification

The taxonomy of smear bacteria is still undergoing frequent changes. Depending on the classification system used, many authors give different names for isolates,

e.g. for staphylococci. Until the mid-1970s, all clump-building, Gram-positive and catalase-positive cocci which did not metabolise glucose under anaerobic conditions were grouped into the genus *Micrococcus*. In contrast to staphylococci (e.g. *S. aureus*), micrococci were considered food grade and were described as typical smear bacteria. Later, differences such as the molecular architecture of cell walls, sensitivity of staphylococci to lysostaphin and furazolidon, resistance against bacitracin, and differences of the GC content were used to distinguish between the two genera. Therefore, part of micrococci are now grouped in the genus *Staphylococcus* (for references see Bockelmann, 1999). But still, the term 'micrococci' is used as a trade name to acknowledge the non-pathogenic, food-grade status of cultures. Food-grade staphylococci are of general importance for smear cheeses, true micrococci were only found on French Chaumes cheeses (Bockelmann, 2002a; Table 21.3).

A reliable identification can only be obtained by a combination of methods such as biochemical identification (e.g. API system), partial sequencing of 16S rDNA, amplified ribosomal DNA restriction analysis (ARDRA), and perhaps other methods. With only limited data available the early studies of Bockelmann *et al.* (1997b) described the use of *A. nicotianae* CA12 in a defined five-strain smear culture. According to new results obtained by ARDRA (Hoppe-Seyler *et al.*, accepted for publication) this strain might belong to a new *Microbacterium* species. This study also showed that all yellow-pigmented bacteria with coryneform morphology isolated from the surface of smear cheeses ($n > 20$) could be divided into two species, *A. nicotianae* and perhaps *M. gubbeenense*.

However, further taxonomic changes may be possible. Brennan *et al.* (2002) described three new species isolated from Irish farmhouse smear cheeses, *Microbacterium gubbeenense*, *Corynebacterium moorparkense* and *Corynebacterium casei* which are closely related (homology of 16S rDNA sequences >98%) to the known species *M. barkeri*, *C. variabile* and *C. ammoniagenes*. Since the type strains of the three latter species were not isolated from smear cheeses but from raw domestic sewage (MB: DSM-20145), food (CV: DSM-20132) and infant faeces (CA: DSM 20306), it is possible that the species isolated and described in many papers on the microflora of smear cheese will have to be renamed.

Molecular classification is time-consuming and can only be used for a small number of isolates. To obtain a fast, rough picture of a smear microflora the very rich 'modified milk agar' can be used for plating (i.e. plate-count agar containing additional casein hydrolysate, vitamins, sodium chloride and skim milk powder, Hoppe-Seyler *et al.*, 2000). By colony morphology (pigmentation) and microscopy (irregular rods, clumping cocci), cream-coloured colonies indicate the presence of *C. casei* (and other species), while orange colonies are most likely caused by *B. linens* and *Staphylococcus* sp. (e.g. *S. equorum*). Staphylococcal colonies are quite typical (larger diameter) and easy to distinguish from brevibacteria by microscopy. Most likely, yellow colonies can be classified as *Arthrobacter nicotianae* or *Microbacterium* sp.

At the moment only molecular analysis is able to distinguish between these two genera.

21.3.2 Screening of yeasts and bacteria

A mixed-strain surface ripening culture consisting of several species has to meet the following criteria:

- Fast growth on the cheese surface (i.e. fast deacidification)
- Development of typical smell and taste
- Prevention of bacterial and fungal contaminations.

Bacteria and yeasts isolated from the surface of smear cheese varieties can be screened to some degree in pure or mixed culture in the shake liquid milk model described by Bockelmann *et al.* (1997b). The most important property for yeasts (*D. hansenii*) is probably deacidification which can be studied easily in the model. A slight yeasty smell was the only contribution of *D. hansenii* to the volatile aroma in pure or mixed cultures. *C. casei* present in very high cell numbers on smear cheeses and staphylococci showed a neutral aroma in the model. In pure culture, *B. linens* (BL) possessed a fishy, ammoniacal smell for which the species is known. The yellow-pigmented *M. gubbeenense* (MG) and *A. nicotianae* (AN) showed a distinct urine-like smell. However, in mixed culture (BL+MG or BL+AN) a more or less typical smear cheese aroma was produced. A similar aromatic profile was obtained with pure cultures of *B. linens* supplemented with methionine (Bockelmann, 1999). Mixed cultures grew to cell counts two magnitudes higher than the pure cultures of both species.

The nature of colour development was also studied in the liquid milk model. Mixed cultures of *B. linens* and *M. gubbeenense* liberated extracellular red-brown colour typical of semi-soft smear cheeses like Tilsit. The orange cell-bound pigments of *B. linens* did not seem to be responsible, since the spectrum of the pigments was different (Bockelmann *et al.*, 1997a). The addition of casein hydrolysate to pure cultures of *M. gubbeenense* also led to the liberation of red-brown pigments. Thus the proteolytic properties of the highly proteolytic *B. linens* are probably important for colour development on cheese.

When *M. gubbeenense* was replaced by *A. nicotianae* in more recent studies, the same effects regarding aroma and colour development were observed. Colour development, however, seemed to be more pronounced when *A. nicotianae* was used (Bockelmann, unpublished results).

Small-scale cheese trials

For further screening of defined smear cultures a real cheese environment is necessary. A simple and fully functional setup for smallest-scale cheese trials was described by Bockelmann *et al.* (2000). A glass tank (e.g. a 50-litre aquarium), closed with a lid and equipped with a stainless steel grid for the cheeses, can be used for ripening. Sufficient humidity (>95%) can be provided either by a water reservoir at the bottom of the tank or by placing the tanks in

climatic rooms with appropriate humidity, which is the better solution. With this setup many surface cultures can be tested in separate units at the same time without the risk of cross-contamination, and the generally too strong ventilation of climatic chambers or rooms can be avoided. Cheese ripening with defined surface cultures as described below was always performed in these ripening cells.

21.3.3 Defined cultures for smear cheeses

Cultures for semi-soft cheeses

After completion of an EU project (CT98-4220) in 2001 the minimum requirements for defined surface smear cultures for semi-soft Tilsit-like cheeses could be formulated. The composition of the culture used by Bockelmann *et al.* (1997b) was slightly modified (*S. sciuri* was replaced by *S. equorum*). Several strains of all species have been used successfully for numerous laboratory-scale cheese trials (unpublished results). In the following, strain names are not given since the general importance of the species is assumed.

One yeast species (*D. hansenii*) and *S. equorum* are essential for ripening to start. Their highest cell counts were always observed during the first week of ripening. Initially, both species were added to the smear cocktail which was used for smearing. Later it was shown that these microorganisms could be inoculated into the cheese brines instead (see the subsection headed 'Brine microflora' below). Together, *M. gubbeenense* or *A. nicotianae* and *B. linens* are essential for the development of aroma and colour. As stated before, the contribution of *B. linens* to colour development seems to be the enzymatic properties and not their orange pigments. *Microbacterium gubbeenense* or *A. nicotianae* reach highest cell numbers in the second week of ripening, while the cell counts of *B. linens* increase slowly over weeks. All three species are usually found at 1–5% of total cell counts but are essential for ripening nonetheless. *Corynebacterium casei* belongs to the group of cream-coloured coryneform bacteria which are important for ripening owing to their fast growth. Corynebacteria are predominant in all stages of ripening and can build over 90% of the surface flora (Bockelmann, 2002a).

Defined surface cultures were used successfully if 10^7–10^8 cfu/ml were present in the smear. Smearing with cultures was performed once at start of ripening, then cheeses were turned every second day and brushed with 3% salt water after 5–7 days which was repeated in the second week when the smear distribution was not uniform. Deacidification was comparable to that in old–young smeared control cheeses (Fig. 21.3). In the figure, batch 1 (mean of four cheeses, open circles) was smeared with smear bacteria of cheese origin (two strains of *B. linens*, two strains of *A. nicotianae*, one strain of *C. casei*, one strain of *S. equorum*). For batch 2 (mean of four cheeses, open squares), DSM and ATCC type strains of the species *B. linens*, *A. nicotianae*, *B. casei* and *S. equorum* were used in the five-species starter. The concentration in the smear liquid was $>10^8$ cfu/ml for each species. For both batches, the yeast

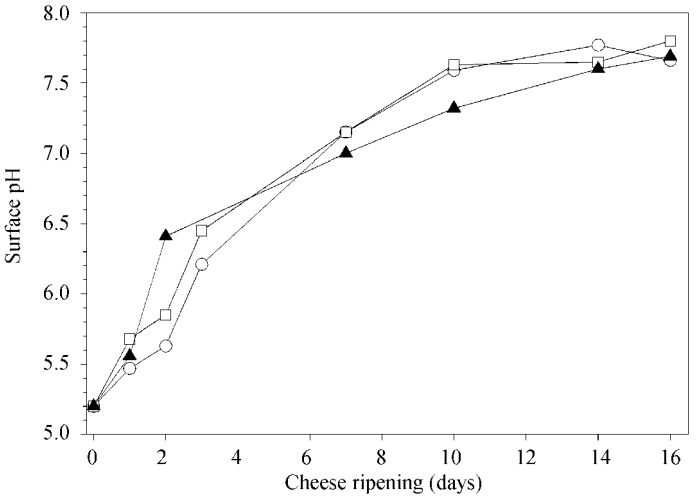

Fig. 21.3 Deacidification of the surface of smear-ripened cheeses.

Debaryomyces hansenii was used in the smear (10^7 cfu/ml). Cheeses of batch 3 (mean of four cheeses, filled triangles) were smeared with an old–young smear obtained from a local Tilsit cheese producer (total bacteria cell counts $>10^{10}$ cfu/ml). Sterile brines were used for salting.

The bacterial composition of the experimental cheeses analysed over the ripening period of 6 weeks was quite similar to that of old–young smeared control cheeses (Table 21.4). In the table, Tilsit-like cheeses were smeared with *D. hansenii*, *B. linens*, *M. gubbeenense*, *C. casei* and *S. sciuri* (10^8 cfu/ml for each species). An old–young smear was obtained from a local Tilsit cheese producer. Similar results were obtained in later experiments, but with *S. sciuri* replaced by the more common *S. equorum* and with *D. hansenii* and *S. equorum* excluded from the smear but inoculated into the cheese brines instead (10^6 cfu/ml). Instead of the yellow-pigmented *Microbacterium gubbeenense*, yellow-pigmented *A. nicotianae* strains were used later. Both species occur on smear cheeses, and in

Table 21.4 Total surface cell counts of experimental cheeses

Surface cell counts (cfu/cm^2)	Defined surface starter			Old–young smeared 6 weeks
	1 week	2 weeks	6 weeks	
D. hansenii	9.1×10^6	6.9×10^5	3.9×10^5	2.9×10^5
Bacterial counts	1.7×10^9	8.1×10^8	2.6×10^9	3.3×10^9
C. ammoniagenes	76%	74%	90%	90%
M. barkeri	7.2%	12%	1%	2%
B. linens	3%	2%	1%	6%
S. sciuri	13.3%	12%	8%	2%

the experimental cheese trials they seemed to possess similar technological properties. By strain identification using pulsed field gel electrophoresis of bacteria isolated from the experimental cheese surfaces after 2 and 8 weeks of ripening (60 isolates), it was confirmed that the surface flora consisted of near 100% of the starter strains (Bockelmann *et al.*, submitted for publication).

After numerous cheese trials it can now be concluded that semi-soft cheese ripening proceeds appropriately if the pH is greater than 7 and the total bacterial counts are above 10^9 cfu/cm^2 after 7 days of ripening; the pH was measured with a flat surface electrode, and for sampling thin slices of 20–30 cm^2 were cut from the surface, 10 g of a slice homogenised in 90 ml of Ringer's solution being taken as 10^{-1} dilution (Hoppe-Seyler *et al.*, 2000).

Cultures for soft cheeses

Less information is available on the requirements for defined surface cultures for smeared soft cheeses. Culture development for these cheeses is currently being studied in a German project conducted at the Federal Dairy Research Centre, Kiel, Germany (AIF-FV 12780N, 2001–2003). The mature flora of smeared soft cheeses showed distinct differences from that of semi-soft cheeses (Table 21.3). A very high proportion of yellow-pigmented coryneform bacteria, *M. gubbeenense* as well as *A. nicotianae* (see above), and the presence of a second yeast species, *Geotrichum candidum*, are typical for the microflora of Limburg- and Romadour-like cheeses (Bockelmann, 2002b).

The appearance of the cheeses with a soft, white and dry layer of yeasts with streaks or spots of orange bacterial growth indicates the presence of *G. candidum* and smear bacteria (Fig. 21.1). Perhaps due to the growth of *G. candidum* covering large areas of the cheese, the bacterial cell counts on the cheese surface are lower than for Tilsit-type cheeses; 10^8 cfu/cm^2 of smear bacteria are usually found on commercial soft smear cheeses after 2 weeks of ripening when the cheeses are packaged (Bockelmann, 2002b). During this time commercial cheeses are old–young sprayed up to six times depending on the visible smear development (personal communication from several German cheese producers).

The deacidification of traditionally old–young sprayed soft cheeses by smear bacteria is quite different from that of semi-soft cheeses, showing a delay in the pH increase of 2–5 days. Then, deacidification proceeds rapidly and a surface pH of 7 is also achieved after one week as for semi-soft smear cheeses (Fig. 21.4). In the figure, for the five experimental cheese batches (filled symbols) the cheese milk was inoculated with *Geotrichum candidum* (10^2 cfu/ml). The cheese brines contained *Debaryomyces hansenii* and *Staphylococcus equorum* (10^6 cfu/ml). Cheeses were smeared with several combinations of *Arthrobacter nicotianae*, *Microbacterium gubbeenense*, *Brevibacterium linens* and *Halomonas variabilis* (10^7 cfu/ml in smear). The deacidification of commercial (old–young smeared) cheeses was measured in three German cheese plants (open symbols).

The use of a typical Tilsit surface culture for soft cheese ripening did not lead to typical deacidification, growth of smear bacteria, colour and aroma

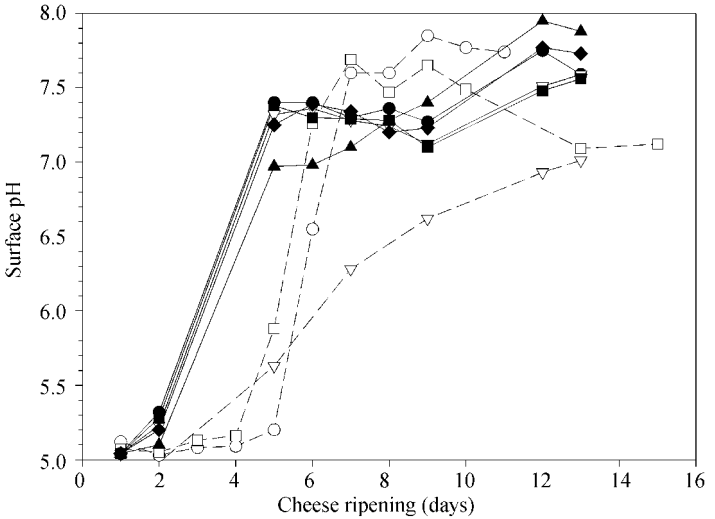

Fig. 21.4 Ripening of soft Limburg cheese.

development (Bockelmann, 2002b). The addition of *G. candidum* to the cheese milk (10^2–10^3 cfu/ml) in combination with a Tilsit starter led to a quite typical flavour. However, the appearance, smell and taste were further improved when the smear consisted of strains and species isolated from smeared soft cheeses (unpublished results, Table 21.3). Deacidification of the cheese surface by defined surface cultures lacked the delay of the commercial cheeses, the important high surface pH value of 7 being generally reached earlier (Fig. 21.4). At the moment pilot-scale cheese trials are being performed in cooperation with an industrial partner to test the suitability of the cultures in an industrial background.

Cultures for acid-curd cheese

Culture development is least developed for acid-curd cheeses, probably because of the limited local importance for some parts of Germany. A project on culture development for acid-curd cheeses was recently started (AIF-FV 13018, 2001–2003). It is quite difficult to maintain the high quality of acid-curd cheeses, since the production of the raw material (low-fat quarg) and the ripening of cheeses are performed independently by separate companies. In this process, microbiological ripening is already occurring when the quarg is transported to the cheese companies over many hours, often under conditions of insufficient cooling. The quarg is usually further stored at 10–15ºC for a couple of days before the cheeses are produced from combined batches of different quarg producers. At this stage the quarg shows yeast cell counts of 10^5–10^7 cfu/g with a very aromatic smell (ester, alcohol, fruity, yeasty; Engel and Roesch, 1995).

Based on these observations several cheese trials were performed to assess the importance of quarg ripening, i.e. the presence of yeasts, for the whole process (Bockelmann *et al.*, in press). Cheeses produced from fresh quarg (total bacterial and yeast cell counts below 10^2 cfu/g) were quite sensitive towards fungal contamination and could not be ripened appropriately by spraying cheese with smear bacteria. When the cheese milk was inoculated with two yeasts (*K. marxianus* and *C. krusei*, $>10^3$ cfu/ml) and the quarg was ripened (stored in air-tight plastic bags) for about 7 days, no mould growth occurred on the cheeses within a week even if no smear was applied to the surface. The maximum cell counts of *K. marxianus* were observed during quarg ripening, while the cell counts of *C. krusei* increased during cheese ripening. The ripened quarg showed the typical intense aromatic profile described above and cheeses produced from ripened quarg had a quite typical appearance, taste and smell after 2 weeks of ripening and storage (Table 21.4). This confirmed the results of Engel and Roesch (1995) who found that these two species could always be isolated from acid-curd cheeses and should be essential for a typical cheese ripening.

Staphylococcus saprophyticus is a common undesirable contaminant on commercial acid-curd cheeses (Bockelmann *et al.*, 2002). Cheese trials revealed that the acid- and salt-tolerant *S. equorum* added to the ripened quarg together with ripening salts had a beneficial effect on cheese ripening, especially on the texture of experimental cheeses. Similar effects, the improvement of the texture of cheese, are claimed for commercial staphylococci (*S. xylosus*, *S. carnosus*) by starter companies. The ripening of cheeses was typical and reproducible when they were produced under the described conditions and with *B. linens* sprayed on the surface at the first day. The deacidification was faster compared to commercial acid-curd cheeses (Fig. 21.5: see below). The typical shrinking of the white quarg zone starting at the surface was already quite advanced after 5 days (ripening at >30ºC and 15ºC for 2 days, storage at 8ºC for 3 days; Table 21.2, Fig. 21.6: see below). Studies concentrating on selecting appropriate surface smear cultures are currently being performed.

In Fig. 21.5, for the experimental cheese batches the milk was inoculated with *K. marxianus* and *C. krusei* at $>10^3$ cfu/ml. The quarg (dry mass 32%) was incubated in closed plastic bags at 16ºC for 7 days before the quarg was mixed with salts and the cheeses were formed. The first data point of the curves resembles the quarg pH before the addition of ripening salts, the second the surface pH of the cheeses 12 h (24 h) after the addition of salts. *B. linens* was sprayed onto the surface after moulding (10^6 cfu/ml).

In Fig. 21.6, the quarg used for production was ripened with *K. marxianus* and *C. krusei* (added to the cheese milk) at 16ºC for 7 days. Cheese ripening was performed at >30ºC and 97% r.h. for 24 h, then after spraying with smear cultures the cheeses were incubated at 12ºC and 80% r.h. for 24 h before being packed in foil and stored at 8ºC. The photograph was taken after 3 days of storage. The speed of ripening, clearly seen by the shrinking of the quarg core, is comparable to that of commercial acid-curd cheeses which are old–young

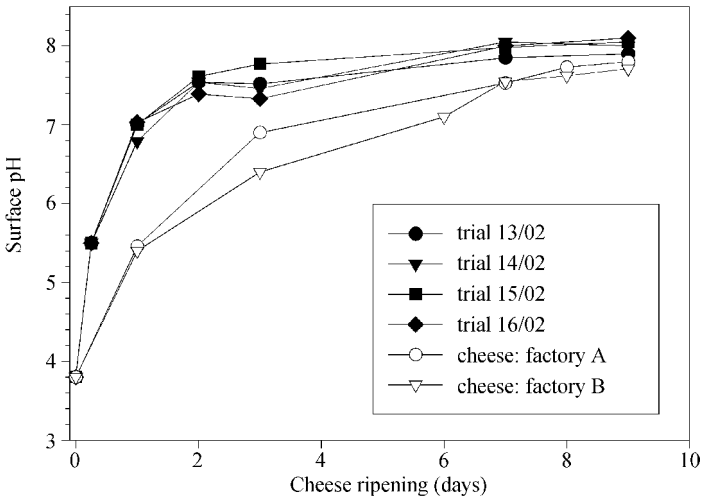

Fig. 21.5 Ripening of acid-curd cheese.

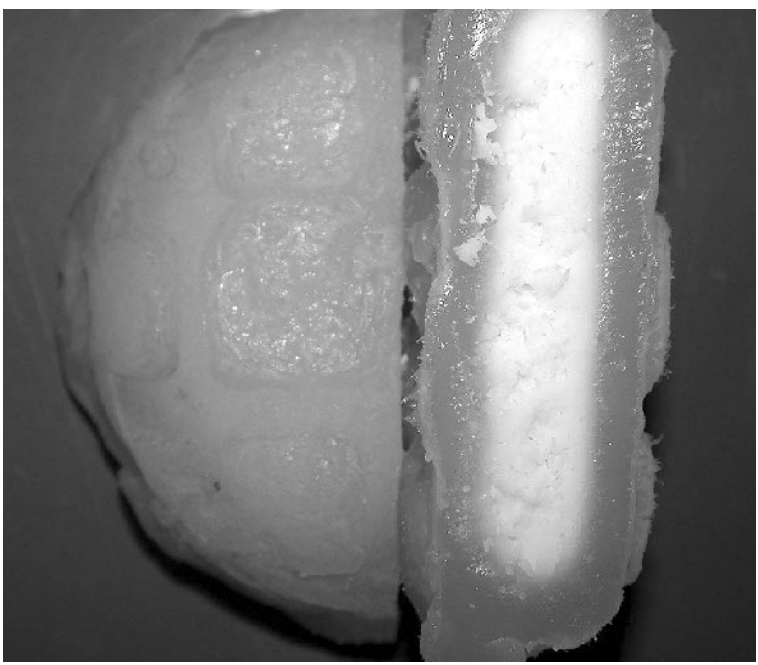

Fig. 21.6 Ripening of acid-curd cheese with defined cultures.

ripened. Contaminants (moulds, enterococci, enterobacteria) were below the detection limit (10^2 cfu/cm^2).

Brine microflora

Cheese brines which are not pasteurised regularly (semi-soft cheeses, e.g. Tilsit) develop a typical salt-tolerant microflora with a composition depending on the house microflora of the factory (Bockelmann, 2002a). Apart from many fungal and bacterial species which can be found at low cell counts ($<10^2$ cfu/ml), typical smear microorganisms can be detected at 10^4–10^6 cfu/ml in aged brines. Salt-tolerant yeasts (*D. hansenii*) and staphylococci (*S. equorum*) are usually predominant. Sometimes cheese brines can be contaminated with *Penicillium*, *Mucor* and other moulds. Since the surface of smear cheeses is not covered by any artificial means (wax, foil) after brining, it can be imagined that the brine microflora has a significant impact on the development of the surface microflora and product quality of smear cheeses. Obviously, no effect is present when dry salting is used, or for acid-curd cheese when quarg is mixed with solid ripening salts.

The control of the brine microflora is widely neglected. However, results of Jaeger *et al.* (2002) showed a clear mould-inhibiting effect of high concentrations of *D. hansenii* and *S. equorum* ($>10^6$ cfu/ml) during cheese ripening and a beneficial effect on deacidification in the first 2 days (Fig. 21.7). In the figure, four experimental cheeses were brined in the presence of *D. hansenii* and *S. equorum* (cell counts > 10^6 cfu/ml for each species ▲), and four control cheeses were salted in sterile brines (●). For the control cheeses a complete smear culture consisting of five species (see the start of Section 21.3.3) was used for smearing. Smearing of the other cheeses was performed with the three species *B. linens*, *C. casei* and *M. gubbeenense*. Deacidification of all cheeses proceeded as

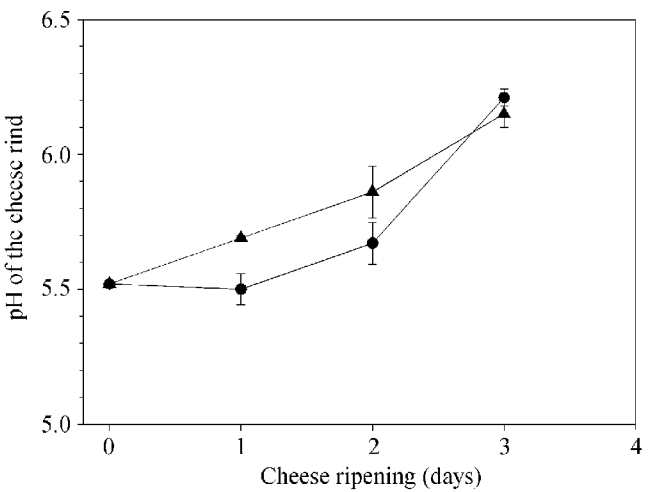

Fig. 21.7 Deacidification of the surface of Tilsit-type cheese.

shown in Fig. 21.3 after 3 days. Laboratory-scale cheese trials showed that both *D. hansenii* and *S. equorum* could be excluded from the smear culture if they were present in the brines without a negative effect.

21.4 Conclusions and future trends

There is no universal surface culture for all smear cheeses. The surface microflora of the three cheese types mentioned showed distinct differences. Results obtained so far showed that defined customised surface cultures can be used successfully for ripening instead of the described old–young processes. However, future studies will show whether the positive results obtained mainly on a laboratory scale can be transferred to industrial practice. A demonstration project funded by the EU starting at the end of 2002 will concentrate on establishing methods for the ripening of semi-soft Tilsit and related cheeses.

There is no doubt that the use of defined surface cultures will improve food safety by minimising microbial contamination. Microbial analysis of experimental cheeses produced on a laboratory scale showed that the cell counts for enterobacteria, enterococci, pathogenic or saprophytic staphylococci and pseudomonads were generally below the detection limit (unpublished data). With 'old–young' contamination cycles no longer present in factories, which stabilise any contamination occurring, it should be possible to reduce common microbial contaminants significantly. The question remains as to whether the aroma and appearance of these cheeses can be kept unchanged, a topic also studied in the projects mentioned above.

Several measures for improvement could be introduced immediately. Whenever brines are used for salting, the brine microflora should be actively controlled. Fresh brines should be inoculated with the naturally occurring species *D. hansenii* and *S. equorum*. Recently, *S. equorum* became commercially available but it is not clear whether this species may currently be used in cheese production in all European countries. However, replacement of this species by commercial *S. xylosus* does not seem to be practical. Bockelmann and Hoppe-Seyler (2001) showed that the cell counts of *S. xylosus* added to cheese brines dropped, whereas a natural flora of *S. equorum* from the environment developed over weeks. Thus the use of *S. xylosus* in cheese brines may be not only expensive but also inefficient.

Instead of using *B. linens* as the sole smear bacterium for smearing, *Corynebacterium* species which comprise the large part of the surface microflora of semi-soft smear cheeses should be used in addition for these cheeses. The species names of the typical smear bacteria *C. ammoniagenes* and *C. variabile* have recently been changed to *C. casei* and *C. mooreparkense*, respectively (Brennan *et al.*, 2002). However, these species are not yet commercially available.

The yellow-pigmented bacteria *A. nicotianae* and *M. gubbeenense* are of special importance for the ripening of soft cheeses, and should be used more

widely for these products. Studies are currently under way to determine the typical percentage of the two species on the surface of the different smear cheeses. First results indicate a predominance of *M. gubbeenense*.

Even though of minor importance for the European cheese market, acid-curd cheeses can be very interesting foods for consumers who prefer calorie-reduced milk products. Acid-curd cheeses are traditionally produced from low-fat quarg and have very interesting sensory properties. At the moment, their wider distribution is probably prevented by their very traditional production, leading to an almost random surface microflora with unavoidable levels of fungal or bacterial contamination. First results of Bockelmann *et al.* (2002) indicate that culture development (yeasts and bacteria) may yield positive results in the near future to develop mild products which are microbiologically stable over the shelf-life of 7–8 weeks.

21.5 Sources of further information and advice

Apart from data presented in the scientific literature and textbooks on dairy microbiology and dairy processing, further information on culture development for smear cheeses is available on the web pages of European national research institutions in the agricultural sector. The use of defined surface smear cultures features prominently in the work of the Federal Dairy Research Centre, Kiel (Institute of Microbiology, http://www.bafm.de), the Netherlands Dairy Research Institute (http://www.nizo.nl) and the Institute of Food Science and Nutrition of the University of Zürich (Laboratory of Food Microbiology, http://www.mb.ilw.agrl.ethz.ch).

Last but not least, a view of the latest developments can be obtained from brochures or the corresponding web pages of the major starter culture companies (in alphabetical order: Chr. Hansen, Denmark; Danisco-Cultor (formerly Wisby), Germany; Degussa-Bioactives (formerly Sanofi or SKW), Germany; Rhodia-food (formerly Texel), France; Sacco srl, Italy). All companies are aware of the research going on in this area and some are partners in the R&D projects mentioned in this chapter. New cultures may soon be offered for smear cheese varieties.

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