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The surface flora of bacterial smear-ripened cheeses from cow's and goat's milk

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Abstract

Debaryomyces hansenii was found to be the predominant yeast in all stages of ripening in several studies on semi-hard Tilsit cheese, soft Chaumes cheese and semi-hard goat's cheese. 75–95% of the bacterial flora was made up of coryneform bacteria. *Brevibacterium linens* was found at 0–15%. Yellow pigmented coryneform isolates (1–30%) belonged to *Arthrobacter nicotianae*. Non pathogenic staphylococci (mainly *Staphylococcus equorum*) were found at 5–15% of the total flora. Model systems were used to study the microbial interactions for growth, colour and aroma development within the surface flora to be able to formulate a defined starter culture.

Commercially available surface starters do not reflect the microbial composition of the cheese surface. Too much emphasis is put on *B. linens* because of the orange pigmentation. New results showed that the red–brown or orange pigments are most likely due to the yellow pigmented *Arthrobacter* sp. in the surface flora. The mechanisms of developing the different shades of red are not yet understood. *B. linens* may be more important for aroma development, due to a highly efficient sulfur metabolism which also affects colour development. The importance of commercial *S. xylosus* is not clear since *S. equorum* was predominant instead, on all cheese varieties analysed. Brines were determined to be a natural reservoir for salt-tolerant *S. equorum*. The successful use of a defined 5-strain starter (*D. hansenii*, *B. linens*, *A. nicotianae*, *Corynebacterium ammoniagenes* and *S. sciuri*) for Tilsit cheese ripening was demonstrated on a 10 kg scale. Further improvement is currently being tested within an EU funded project. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Smear cheeses; Tilsit; Chaumes; *Staphylococcus*; *Brevibacterium*; *Corynebacterium*; *Arthrobacter*

1. Introduction

In a small proportion of the cheese varieties sold worldwide, the surface is covered by a layer of yeast and bacteria. These aerobic micro-organisms have a strong impact on the appearance, flavour and texture development of the cheeses, and usually lead to shorter ripening periods of several weeks rather than months. The well-known examples are Tilsit and Romadour cheeses. Apart from the influence of the physical and chemical parameters of the cheese milk, starter-bacteria 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, is essential during ripening because the cheese surfaces are exposed to an unsterile environment. Undesirable contaminants will grow immediately if the balance of the cheese microflora is disturbed. Understanding the microbial ecology of the cheese surface is prerequisite to the development of surface starter cultures and for control of surface ripening (Bockelmann, 1997, 1999).

Bacterial smear-ripened cheeses have a long tradition. Without the knowledge of the bacterial nature of the surface flora, a large variety of smear cheeses was produced long before 1900 (Fox, 1993). When cheeses produced from raw milk—an important source for surface micro-organisms—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 (Chapman & Sharpe, 1990). More than 100 years ago, Laxa (1899) had already isolated yeasts and yellow-pigmented bacteria from the surface of smear cheeses. The importance of yellow-pigmented *A. nicotianae* for the development of the typical reddish-brown colour of semi-hard smear cheeses has recently been confirmed (Bockelmann, Fuehr, Martin, & Heller,

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1997a). The sources of growing surface micro-organisms are cheese milk, brine baths, the air of ripening rooms, ripening pads and humans. 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, Rajkowski, & Stabel, 1997), the dependance on an intact "house microflora" and starter cultures has increased considerably.

Surface-ripening of smear cheeses begins with the growth of yeasts (e.g. *D. hansenii*) which utilise the lactate and increase the surface pH of the cheese (Busse, 1989; Eliskases-Lechner & Ginzinger, 1995; Keller & Puhan, 1985; Reps, 1993). When the pH increases to >6, *B. linens*, other coryneform bacteria and staphylococci (micrococci) begin to grow and eventually cover the whole surface of the cheese (Eliskases-Lechner & Ginzinger, 1995; Bockelmann, Krusch, Engel, Klijn, Smit, & Heller, 1997c). Proteolysis is considerably stronger at the cheese surface compared with the core of cheeses (Bockelmann, Hoppe-Seyler, Lick, & Heller, 1998).

Correct handling and storage of smear cheeses during ripening is essential. Ripening temperatures ranging from 14°C to 19°C, and the humidity should be at least 95%. Excessive ventilation should be avoided (Kammerlehner, 1995). In addition, repeated turning of cheeses and surface treatment by repeated smearing (brushing) are most important for ripening. The smear is applied to the cheese using a circular, rotating brush, which is wetted when moving through the smear liquid placed at the bottom of the machine. The smear liquid is made up in water or whey containing $\sim 3\%$ salt to mirror the salinity of the cheeses and is generally inoculated by commercially available surface starters. Only few species, e.g. *D. hansenii* and *B. linens*, are commercially available, which does not reflect the microbial complexity found on the surface of smear cheeses.

Generally, cheese producers do not rely on these cultures alone. Traditionally, mature cheeses are smeared before young ones, which is called "old–young" smearing. Thus, all necessary micro-organisms have been transferred to the smear bath when smearing of young cheeses is started. A disadvantage of this approach will also be that undesirable contaminants will be transferred to the smear bath and will be spread around the whole factoryhahn. Smearing machines were found to be an important source of *Listeria* contamination (Hahn & Hammer, 1993). Due to the long tradition in producing surface-ripened cheeses, it is known that with good manufacturing practice these contaminants will not grow to cell counts posing a risk to the consumers. It was demonstrated by Bockelmann, Hoppe-Seyler, Krusch, Hoffmann and Heller (1997b) that it is possible to use defined surface starter cultures to replace the old–young smearing.

This paper reviews results of the Federal dairy research centre, Kiel, on bacterial surface cheese ripening including new, unpublished results on semi-hard cow's and goat's cheeses as well as soft Chaumes cheese. New ideas for a better understanding of the function of the complex surface flora of smear cheeses and for formulating better surface starter cultures are presented.

2. Results and discussion

2.1. Recent hierarchic classification of cheese smear bacteria

New results from molecular bacterial taxonomy revealed that there is no taxon "coryneform bacteria" (Stackebrandt, Rainey, & Ward-Rainey, 1997). Today, the family names Micrococcaceae, Brevibacteriaceae and Corynebacteriaceae should be used with corresponding genera (Table 1). The genus *Staphylococcus* is not grouped into Micrococcaceae but belongs to a different subdivision (Table 1). For practical reasons, the expression coryneform bacteria is still widely used to group *Brevibacterium* sp., *Corynebacterium* sp., and *Arthrobacter* sp. and will be used in this paper, too.

2.2. Composition of the surface flora

For bacteriological analysis, three types of cheese were selected: the well-known semi-hard Tilsit cheese with irregular holes and a light-brown surface, smeared semi-hard goat's cheeses (farmhouse cheeses) with a slightly more orange appearance, and the French soft cheese Chaumes characterised by a bright orange

Table 1
Current hierarchic classification of bacterial families and genera with relevance for smear cheeses. Species from genera printed in bold were used for defined starters in this study

Actinobacteria
Micrococcineae
Micrococcaceae
Arthrobacter, *Micrococcus*, *Kocuria*, *Renibacterium*, etc.
Brevibacteriaceae
Brevibacterium
Microbacteriaceae
Microbacterium/Aureobacterium, *Clavibacter*, *Curtobacterium*, etc.
Dermabacteriaceae
Brachybacterium, Dermabacter
Corynebacterinea
Corynebacteriaceae
Corynebacterium, *Turicella*
Nocardiaceae
Rhodococcus, *Nocardia*
Firmicutes with low GC content of DNA
Staphylococcus, *Enterococcus*, *Listeria*, *Bacillus*

Table 2
The bacterial surface flora of different commercial cheese varieties; sample size for Tilsit cheese ($n = 20$), semi-hard goat's cheese ($n = 5$); Chaumes, analysis of single cheeses[a]

Surface micro-organisms	Tilsit cheese (%)	Soft Chaumes cheese			Semi-hard goat's cheese (%)
		1996 (%)	1998 (%)	1999 (%)	
D. hansenii	Predominant	n.d.	n.d.	n.d.	Predominant
Non-pigmented corynef. Bact.	70–90	54	65	68	71–80
Arthrobacter (yellow)	5–10	27	28	1.5	0.2–7
B. linens	0.1–15	<0.1	—	0.1	0.1–17
Staphylococci	5–15	<0.1	2	5	0.2–5
Micrococci	—	18	5	25	—

[a] n.d. = not determined, (–) = not detected; the fraction "non-pigmented coryneform bacteria" contained *Corynebacterium ammoniagenes*, *Brevibacterium casei* and other unidentified species.

surface. Identification of isolates was performed using classical bacteriological methods according to Bergeys manual of determinative bacteriology. Results were confirmed by 16 s rRNA analysis performed by the NIZO, Ede, The Netherlands (Bockelmann et al., 1997c). Corynebacteria, brevibacteria, *Arthrobacter* and staphylococci grew on all smear cheese varieties studied (Table 2). All yellow-pigmented isolates selected belonged to the *Arthrobacter* genus, mainly *A. nicotianae*. The soft cheeses with a very orange appearance seemed to have a very high proportion of yellow *A. nicotianae* in 2 of 3 studies (1996, 1998). However, in the third study (see Section 2.3.3) the same cheese variety with the same orange appearance had a very low proportion of *A. nicotianae* in the last study. The orange colour is most likely not due to the presence of brevibacteria which were only present at <1% of the flora. In general, *B. linens* was found at 0.1–15% of the total bacterial flora. Only for soft chaumes cheeses were a percentage of diplococci found. Two isolates were identified as *Micrococcus luteus* and *Kocuria cristinae* (both non-pigmented) using biochemical identification kits (ID32 Staph, BioMérieux). It is possible that these genera are typical for soft cheeses. Micrococci were not isolated from any of the other semi-hard cheeses. The flora of more soft cheese varieties is currently being studied. Most important for a complete smear layer for all studied cheese varieties were non-pigmented white or cream-coloured coryneform bacteria. Abundant species identified so far were *C. ammoniagenes* (Table 2) and *B. casei* (unpublished results).

Of all isolated staphylococci, *Staphylococcus equorum* was more or less predominant on all cheeses studied. High cell counts of this species were always found in the brines in several cheese factories (approx. 10^5 cfu* mL^{-1}) indicating that brines are a natural source for cheese surface staphylococci. Detection of *S. xylosus* on goat's cheeses was due to the use of a commercial preparation in the smear. The appearance of *S. saprophyticus* on goat's cheeses was highly undesirable, the species is classified in the risk category 2 being "potentially harmful to humans" which stresses the importance of better surface starters which may help to suppress naturally occurring contaminants (Table 3).

Many yeast species can be isolated from the surface of smear cheeses. Among 19 yeast species identified by Rohm, Eliskases-Lechner, and Brauer (1992) *D. hansenii* occurred most frequently, followed by *Geotrichum candidum*, and *Issatchenkia orientalis*, and *Kluyveromyces marxianus*. Several *Candida* species and *Yarrowia lipolytica* were also isolated. On all cheeses tested *D. hansenii* was predominant, cell counts of other yeast species were negligible (Table 2).

As shown in Table 2 the microflora of all tested cheeses was similar. It was assumed that it should be possible to define a surface starter for smear cheeses on the basis of these results. Four bacterial and one yeast species were selected for screening of the technological

Table 3
Bacterial surface flora on two smeared semi-hard goat's cheeses[a]

	Batch 1 (bad quality) (%)	Batch 2 (better quality) (%)
Coryneform bacteria (non-pigmented)	99	64
Arthrobacter sp. (yellow)	0.2	7
B. linens	0.3	29
Staphylococci	0.5	2
S. saprophyticus		40
S. xylosus		30
S. equorum		20
S. kloosii		10

[a] Deacidification of batch 1 was poor (<pH 7) the smear layer was thin and incomplete. Batch 2 had an orange appearance. Commercial *Brevibacterium linens* and *Staphylococcus xylosus* preparations had been used for smearing (pers. communication of cheese manufacturer). Classification of 20 *Staphylococcus* isolates of batch 2 revealed the presence of the non food-grade *S. saprophyticus* (risk category 2). "Coryneform bacteria" were not further classified.

parameters growth, aroma- and colour development: *A. nicotianae*, *B. linens*, *C. ammoniagenes*, *S. sciuri*, and *D. hansenii*.

2.3. Screening of surface starter strains in model systems

Three model system were developed for screening of suitable strains regarding growth, sensory and pigmentation properties (shake liquid cultures, agar plate cultures and sterile 5 g "mini" cheeses in centrifuge bottles; Bockelmann et al. (1997b). The most simple system, shake liquid cultures based on milk, proved to be suitable to study interspecies interactions, which are essential for cheese ripening.

2.3.1. Growth

Initially *D. hansenii* was included in the studies but it was found that yeasts were only necessary for deacidification of growth media (initial pH 5.5). This effect was simulated in further experiments by reducing the addition of lactic acid to an initial pH 6.0. The non-proteolytic *C. ammoniagenes* did not grow in the milk model in pure culture, but in combination with proteolytic species growth to high cell densities was observed. An effect of *C. ammoniagenes* on the growth of other smear bacteria was not observed.

The proteolytic *S. sciuri* stimulated growth of the yellow proteolytic *A. nicotianae* (Table 4). Both strains grew to similar cell counts ($>10^{10}$ cfu* mL^{-1}) in mixed liquid culture within four days. *B. linens* had a similar effect on *A. nicotianae*. However, after four days of growth, cell counts of *B. linens* were only 5% of the values found for *A. nicotianae*. Addition of amino acids (casein hydrolysate) to pure cultures of *A. nicotianae* had a small growth promoting effect only (Table 4). At 30°C all strains had similar growth rates. Under cheese ripening conditions, at 21°C and below, growth rates of *B. linens* strains were lower compared with the other surface bacteria (data not shown). This may explain the low proportion (1–30%) of *B. linens* found on smear cheeses (Bockelmann et al., 1997c; Eliskases-Lechner & Ginzinger, 1995; Busse, 1989). This is probably desirable, since *B. linens* produces a very intense ammoniacal smell which is not acceptable for most consumers at high concentrations.

Table 4
Growth of smear bacteria in shake liquid milk culture (initial pH 6.0, 21°C, Bockelmann et al., 1997b)[a]

Culture	Species	Cell counts (cfu mL^{-1})
Pure culture	*Arthrobacter sp.* (prt+, yellow)	6.0×10^{8}
+casein-hydrolysate	*Arthrobacter sp.* (prt+, yellow)	6.8×10^{8}
Mixed culture	*Arthrobacter sp.* (prt+, yellow)	5.2×10^{10}
	B. linens (prt+ + +, cream-coloured)	2.7×10^{9}
Mixed culture	*Arthrobacter sp.* (prt+, yellow)	4.1×10^{10}
	Staphylococcus sp. (prt+ + +, cream coloured)	2.3×10^{10}

[a] prt+ = weakly proteolytic, prt + + + = strongly proteolytic according to fluorimetric assays using FITC-labelled total casein (data not shown).

2.3.2. Aroma development

Pure cultures of all strains did not produce Tilsit-like smell (a complex aromatic profile dominated by sulfur compounds, Table 5) in the shake liquid milk model. None of the yeast strains tested contributed noticeably to the development of cheese flavour, indicating that the main role of yeasts is deacidification of cheese surfaces. Staphylococci did not contribute much to Tilsit-like flavour; however, proteolytic and non-proteolytic strains in combination with yeasts produced a smell characteristic for young commercial cheeses after brining. Identification of micro-organisms in several brine baths showed that *D. hansenii* and *Staphylococcus* spp. were predominant (10^5 cfu mL^{-1}). Growth of these micro-organisms in cheese brines is unlikely because of the high salt concentrations, but when young cheeses are immersed in brines, the cheese surface still has lower concentrations of salt, and might provide growth substrates for the microflora present in the brine. One part of the salt-tolerant microflora may be washed off the surface and survive in the brines until the next batch of young cheeses is immersed. This could explain the build-up of the endogenous microflora of cheese brines.

Volatile aromatic sulphur compounds originating from cysteine and methionine are probably key components of smear cheese flavour, and contribute to the garlic note. The thioesters (*S*-methylthioacetate, thiopropanoate, thiobutyrate, etc.) are also important for the overall aroma Cuer, Dauphin, Kergomard, Dumont, & Adda, 1979). *B. linens* was shown to produce H_2S, methanethiol, dimethyldisulphide, *S*-methylthioacetate, 4-trithiapentane, and ethional. Dias and Weimer (1998) purified and characterised a *L*-methionine-γ-lyase responsible for the conversion of methionine to methanethiol, α-ketobutyrate, and ammonia. The authors found that the enzyme was active under cheese conditions.

Table 5
Volatile flavour development in the liquid milk model system

Strains	Volatile flavour
B. linens	Fishy, ammonia
A. nicotianae	Urine-like
B. linens + *A. nicotianae*	Fruity, Tilsit-like
B. linens + Met/Cys	Tilsit-like (methanethiol, methylthioacetate, methylthiopropionate, H_2S, etc.)

Various combinations of strains did not produce a true Tilsit-like smell in the selected model system. When brevibacteria dominated the flora, an intensive ammoniacal, fishy flavour was observed. Smear cheese-like smell was produced in mixed cultures consisting of one strain each of the yeast *D. hansenii*, *A. nicotianae*, *S. sciuri*, *C. ammoniagenes* and *B. linens* (data not shown). During experiments, *A. nicotianae* and *B. linens* were found to be most important for the development of a typical smell. A typical smell was developed in liquid model systems without the use of the other three species (Table 5). Recent experiments showed that *A. nicotianae* could be replaced by addition of sulfur-containing amino acids. The presence of methionine and cysteine in the milk model system led also to a typical smear cheese smell, probably due the liberation of methanethiol and other compounds. Together with growth stimulation of non-proteolytic yeasts and *Corynebacterium* sp. as well as *A. nicotianae* (Table 4), this demonstrates the importance of *B. linens* for the ripening of smear cheeses, even at the low proportions found in the surface microflora (Bockelmann et al., 1997a, b, c). Sometimes no *B. linens* were isolated from mature cheeses of normal quality. It may be that other species such as the closely related non-pigmented *B. casei* could replace *B. linens* for typical aroma development. However, identification is only possible with molecular methods and no data exist on the proportion of *B. casei* to the non-pigmented coryneform bacteria.

2.3.3. Colour development

Coryneform bacteria form the major part of the surface flora. Yellow and orange strains have always been found in the surface flora, but is has been reported that a large proportion of the coryneform strains isolated produce white or cream coloured colonies (Bockelmann et al. 1997c; Eliskases-Lechner & Ginzinger, 1995; Seiler, 1986). *B. linens* is generally found to be essential for colour development on smear-ripened cheeses, since many strains possess bright cell-bound orange pigments (Busse, 1989; Reps, 1993). Considering the low percentage of *B. linens* found in the surface microflora in this study, it is doubtful that these pigments are actually responsible for the red–brown colour of Tilsit cheeses. The absorbance spectra of the red–brown Tilsit pigments and orange *B. linens* pigments were also shown to be different (Bockelmann et al., 1997a).

It has now been demonstrated in model systems that the typical red–brown colour of Tilsit cheese can be produced without the orange pigments of *B. linens* (Table 6). Only the presence of yellow-pigmented *Arthrobacter* strains, which developed red–brown pigments in mixed culture with the proteolytic *B. linens* was essential for typical colour production (Table 5). This was independent of the pigmentation of the selected *Brevibacterium* strain (data not shown). The same effect could be produced in mixed cultures of *A. nicotianae* and the proteolytic non-pigmented *S. sciuri* when the pH was kept at neutral or alkaline values (Table 6). Differences between the yellow and red–brown pigments deriving from *A. nicotianae* and the orange pigments of *B. linens* were determined by Bockelmann et al. (1997a) who found that the two types of pigments had different absorbance spectra. The red–brown colour was also produced by *A. nicotianae* in pure culture when casein hydrolysate or sulfur-containing amino acids (methionine) were added to the medium, demonstrating the importance of free amino acids not only for aroma but also for colour production. Thus, the proteolytic properties of smear bacteria might be more important for typical colour development than the orange pigments of *B. linens*.

Table 6
Colour development by smear bacteria in the liquid milk model system[a]

Strains	Colour of growth medium
B. linens (orange) prt+ + + (pH 7)	Red–brown
A nicotianae (prt + yellow) + *B. linens* (orange) prt + + + (pH 7)	Red–brown
Arthrobacter (prt + yellow) + *S. sciuri* prt + + + (pH 6)	Yellow
Arthrobacter (prt + yellow) + *S. sciuri* prt + + + (pH 7)	Red–brown
Arthrobacter (prt + yellow) + caseinhydrolysate (Merck) (pH 7)	Red–brown
Arthrobacter (prt + yellow) + cysteine/methionine	Red–brown

[a] prt+ = weakly proteolytic, prt+ + + = strongly proteolytic according to fluorimetric assays using FITC-labelled total casein (data not shown).

2.4. Cheese making with defined starters

Six batches of four Tilsit cheeses (2.5 kg each) were produced in an environment without a typical "Tilsit house microflora" (glass containers of 1 m^3). The first and second batch were smeared with pure commercial suspensions of *D. hansenii* and *B. linens*. For the first batch, final concentrations of cell counts in the smear liquid were chosen according to recommendations given by the starter culture supplier. In the first few days, serious problems with fungal growth occurred which persisted until the end of ripening. In the second batch when higher concentrations of *D. hansenii* and *B. linens* were used in the smear ($>10^8$ cfu mL^{-1}) growth of moulds was delayed, however, it could not be suppressed. In spite of smearing with a 2-strain culture, the bacterial surface flora of both batches consisted of the same bacterial groups typical for commercial smear

Table 7
Surface flora of 4 batches of Tilsiter cheese produced in laboratory scale (10 kg) and ripened in a 1 m^3 container which was chemically disinfected at the beginning of ripening but could not be kept under sterile conditions during ripening[a]

Batch	Tilsit smear (in brackets: not added to smear)	Cell count in smear liquid (cfu mL^{-1})	Cell count on mature cheese (cfu cm^{-2})
1	*D. hansenii*	4.0×10^5	2.6×10^6
	Total bacterial count		5.2×10^7
	B. linens	8.0×10^7	0.01%
	(Coryneform bacteria)	Not added	81%
	(Staphylococci)	Not added	19%
Comments: fungal growth on cheese surface; bad appearance and smell; flat taste			
3	D. hansenii	3.3×10^9	8.0×10^5
	Total bacterial count		3.7×10^8
	Coryneform bacteria	1.0×10^{10}	84%
	B. linens	2.8×10^8	11%
	Staphylococci	1.2×10^{10}	5%
Comments: complete commercial smear from cheese plant; typical Tilsit cheese			
5	*D. hansenii*	7.0×10^8	3.4×10^8
	Total bacterial count		8.2×10^8
	Coryneform bacteria	1.0×10^8 (prt−)	49%
	B. linens	1.0×10^7 (prt+)	1%
	Staphylococci	4.0×10^8	49%
Comments: slow aroma development, typical Tilsit cheese			
6	*D. hansenii*	3.0×10^8	1.2×10^7
	Total bacterial count		1.8×10^{10}
	Coryneform bacteria	2.6×10^8 (prt+/prt–1 : 1)	75%
	B. linens	3.1×10^8 (prt+)	4%
	Staphylococci	3.0×10^8 (prt+)	21%
Comments: typical Tilsit cheese, comparable with batch 3			

[a] The total bacterial counts are related to colonies growing on modified Plate-count agar containing 3% salt (Bockelmann et al., 1997c). Cell counts for contaminating bacteria like pseudomonads, coliform bacteria, etc. determined on selective media were several magnitudes less and are not presented in the table. The fraction "coryneform bacteria" contains non-pigmented bacteria like *Corynebacterium* sp., *Brevibacterium* sp., *Arthrobacter* sp. and perhaps other species.

cheeses after 6 weeks of ripening indicating that the unsterile air of the ripening facilities is a source for cheese surface bacteria (Table 7). These bacteria will grow fast on cheese when the starter growth is too slow.

The third batch of cheese was smeared with a suspension supplied weekly by a local cheese producer. Compared with the defined smear cocktails, micro-organism counts were several magnitudes higher (Table 7). This was achieved by the traditional method to smear old cheeses first before the smear was used on young cheeses. With this batch, no problems with growth of moulds occurred. After 6 weeks, cheeses had developed a typical flavour and appearance. The composition of the surface flora was comparable with commercial Tilsit cheeses.

The fourth batch suffered from a *Enterococcus* contamination in the yeast suspension used for smearing. Cell counts in the smear liquid were 3×10^8 cfu mL^{-1} for *D. hansenii* and *B. linens* and 6×10^8 cfu mL^{-1} for enterococci. After 4 weeks of ripening, the cheese was discarded and not further analysed. However, compared to total surface cell counts the proportion of enterococci on the cheese surface had dropped to 0.1% of total cell counts after 4 weeks showing that smear bacteria can inhibit the growth of contaminants.

In the fifth batch,three bacterial strains were used, a non-pigmented proteinase-negative *C. ammoniagenes* strain, a proteinase-negative *S. equorum* and an orange pigmented proteolytic *B. linens* strain. During the first 2 weeks, a minor problem with fungal growth was observed which did not persist further when the smear layer was complete. Aroma development was slow, after 6 weeks of ripening, cheeses tasted like young but typical Tilsit cheeses. The flavour was improved by additional storage of the cheeses at 4°C for 6 weeks.

Based on results from model systems, different strains were selected for the sixth batch. *A. nicotianae* was included, the proteinase-negative *S. equorum* was replaced by the proteolytic *S. sciuri*. *B. linens* strain BR18 (commercial strain) was replaced after 2 weeks of ripening by a strain showing higher proteinase activity and less intensive orange pigments (strain BR 5, isolate from Tilsit cheese). No problems with fungal growth

were observed. Ripening appeared to be slightly slower compared with batch 3 for which a commercial smear had been used. After 6 weeks of ripening, good cheese quality was determined. Additional storage at 4°C for 6 weeks improved the flavour of the cheeses.

2.5. Application of the surface starter in industry

The strain mix described for batch 6 was used on a larger scale for the same type of cheese (Tilsit) by a cheese producer (batch 7). The commercial green cheeses were smeared with the defined surface starter containing cell concentrations used in batch 6 (Table 7). Smearing was performed early in the morning, in smearing machines freshly cleaned each time (young–old smearing). Since the ripening rooms showed a high degree of fungal contamination the defined smear developed too slowly to prevent fungal growth completely. However, the traditionally old–young smeared control cheeses produced on the same day were also affected by mould growth.

Sensory analysis was performed on these cheeses according to the German DLG rules. Appearance, smell, and taste are graded on a scale ranging from 0 (bad) to 5 (perfect). Cheeses of batch 7 were independently graded by taste panels of the cheese producer and the Federal dairy research center. Sensory analyses resulted in comparable grades. The appearance of the old–young smeared cheeses was between 4 and 5, the appearance of the cheeses smeared with a defined smear was given the grading 4 (white fungal spots for both types of cheese). Taste and smell of experimental and control cheeses was considered to be of grade 5. However, all participants agreed that experimental cheeses had a somewhat more distinct flavour with a slightly higher acceptability. This indicated that not all components of the complex house microflora applied to the cheeses through old–young smearing were contributing to desirable aroma components. Of course, an influence of the factory's house microflora on smear development of these cheeses cannot be excluded. Since there were clear differences observed in sensory qualities, it was concluded that the main impact on ripening was indeed due to the different composition of the smears.

3. Conclusions

Results show that it is possible to produce typical Tilsit cheeses using a surface starter flora with only few microbial strains mimicking the essential microbial groups found on cheese surfaces. However, growth of the starter at the beginning of ripening was still too slow to prevent fungal contamination completely. Further improvement of the composition and application of defined surface starters has been started in a new EU project (CT98-PL4220). First results in the project indicate that the performance of defined starters can indeed compete with the traditional "old–young" smear starter.

A preliminary picture about the microbial growth on the smear cheese surface is as follows: during the first few days, yeasts (*D. hansenii*) dominate the flora, detectable often by the yeasty smell. Lactate is metabolised and the pH is increased to pH 7 within 4–7 days. Proteolytic food-grade staphylococci (*S. equorum*) can also grow at acidic pH values. Many strains are proteolytic and may promote growth of the non-proteolytic yeasts, later on, non-proteolytic bacteria (e.g. *Corynebacterium* sp.). A natural source for yeasts and staphylococci are the cheese brines where they can be found at concentrations of about 10^5 cfu mL^{-1}. At pH values > pH 6.0 *Brevibacterium* sp., *Arthrobacter* sp. and *Corynebacterium* sp. start to grow. Very soon, the bacterial flora is dominated by non-pigmented (cream or white colonies) coryneform bacteria, many of the isolates (e.g. *C. ammoniagenes*) being non-proteolytic. They depend on proteolytic bacteria such as some staphylococci and brevibacteria. The main importance of the non-pigmented fraction of the surface microflora seems to be the fast growth—to cover the surface within few days protecting it from growth of contaminants e.g. moulds and pathogenic bacteria. A contribution to flavour development of these coryneform bacteria was not found in the model systems used. Flavour development seems to be mediated by *B. linens* and *A. nicotianae*) even at the low percentage of the microflora observed. In co-culture (liquid model), they develop a typical smear cheese smell. Liberation of methionine seems to play a key role. Both species seem to be responsible for a typical colour development also. The pigments of *B. linens* usually do not contribute to the development of the reddish-brown pigments because the cell counts are often insignificant compared to total surface bacterial counts. Results showed clearly that the conversion of the yellow *Arthrobacter* pigments to red–brown pigments was more important for cheese appearance. As for flavour development, sulfur-containing amino acids play an important role for a typical cheese colour. A difference of soft smear cheeses compared with semi-hard smear cheeses may be a certain percentage of micrococci (*Micrococcus* sp., *Kocuria* sp.) in the surface flora which have never been isolated from semi-hard cheese in these studies. Further investigations are under way.

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