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## The bacterial flora of surface-ripened cheeses with special regard to coryneforms

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**Summary** — Randomly-selected Austrian bacterial surface-ripened cheeses were examined for changes in the microbiological composition of the smear. The bacterial counts of the Tilsit cheeses from 14 cheese plants and of 3 types of soft cheeses selected varied from  $10^4$  to  $10^9$  cfu/cm<sup>2</sup> smear 3 d after manufacture and from  $10^8$  to  $10^9$  cfu/cm<sup>2</sup> smear after a ripening period of 3 weeks. The flora tolerated a NaCl content of at least 80 g/kg in the plate count agar. A total of 386 isolates of coryneform bacteria were identified. The bacterial flora proved to be of mixed population. However, *Brevibacterium linens* accounted for a large share of the flora, comprising 30% of the total bacterial count. Besides *Brevibacterium linens*, the other main types found to be present in the heterogeneous flora were cream-coloured and yellow-pigmented coryneforms, which were predominantly identified as *Arthrobacter globiformis* and *Brevibacterium ammoniagenes*. The coryneforms isolated from the cheeses 3 d after manufacture were more proteolytic than those isolated at later stages of ripening.

**Tilsit cheese / smear-ripened soft cheese / smear / coryneform bacteria / cheese ripening**

**Résumé** — Flore bactérienne de surface des fromages à pâte molle, en particulier les corynéformes. L'évolution de la flore microbienne sur la croûte des fromages autrichiens emmorgés a été examinée. Des Tilsit provenant de 2 fromageries ainsi que 3 différents types de fromage à pâte molle fabriqués par la même fromagerie ont été étudiés pendant toute la période de l'affinage, de même que des Tilsit provenant de 12 fromageries à 3 différents stades d'affinage. Le nombre des germes aérobies mésophiles dans la morge était compris entre  $10^4$  et  $10^9$  ufc par cm<sup>2</sup> de morge 3 j après la production et entre  $10^8$  et  $10^9$  ufc par cm<sup>2</sup> après 3 sem d'affinage respectivement. La flore a toléré une teneur en NaCl de 80 g/kg dans le milieu. Au total, 386 souches de bactéries corynéformes ont été identifiées à l'aide de 52 tests biochimiques. La flore de ces morges se trouve être une population mixte. *Brevibacterium linens*, considéré comme dominant dans la morge, représentait 30% de la flore bactérienne totale. Les autres espèces principales de cette flore hétérogène étaient de couleur jaune ou non pigmentées. Elles ont été identifiées en grande partie comme *Arthrobacter globiformis* et *Brevibacterium ammoniagenes*. Les bactéries corynéformes isolées de la morge des fromages de 3 j étaient plus protéolytiques que celles isolées plus tardivement.

**Tilsit / fromage à pâte molle / morge / bactérie corynéforme / affinage**

## INTRODUCTION

On the surfaces of red smear cheeses, bacterial counts of between  $10^9$  and  $10^{11}$  cfu/g smear ( $10^7$ – $10^9$  cfu/cm<sup>2</sup> surface) are reached within the first 2 weeks of ripening and remain constant until the time of consumption (Accolas *et al.*, 1978; Keller and Puhani, 1985; Grand *et al.*, 1992). The variations between different cheese varieties or cheese factories are low. Only during the initial period of ripening do differences occur with regard to the rapidity of the change from yeast dominance to bacterial dominance.

The importance of the coryneforms for the surface ripening of smear cheeses is often stated, but only a few studies have been made of the identity of these bacteria. The following species were found on the surfaces of different cheese varieties: *Arthrobacter aurescens*, *Arthrobacter citreus*, *Arthrobacter globiformis*, *Arthrobacter nicotianae*, *Arthrobacter protophormiae*, *Arthrobacter uratoxydans*, *Arthrobacter variabilis*, *Arthrobacter* spp, *Brevibacterium ammoniagenes*, *Brevibacterium erythrogenes*, *Brevibacterium helvolum*, *Brevibacterium linens*, *Brevibacterium oxydans* and *Corynebacterium* spp (Seiler, 1986; Sauter, 1986; Mayer, 1990; Piton and Fontanier, 1990; Piton-Malleret and Gorrieri, 1992). Among these species, the grey, the white and the cream-coloured ones formed the main part (40%), the yellow strains 36% and the orange-pigmented strains only 22% (Sauter, 1986), in spite of using *Brevibacterium linens* cultures for each smearing. This is why some commercial cultures for use in smear-cheese ripening presently contain different coryneforms besides *Brevibacterium linens* such as *Brevibacterium* spp and *Arthrobacter* spp.

In Austria it is common practice to use *Brevibacterium linens* as the sole culture organism. All other microorganisms found on the cheese surfaces, both the bacteria

and the yeasts, are caused by contamination during the manufacturing and ripening processes. In a previous paper (Eliskases-Lechner and Ginzinger, 1995), we reported on the yeast flora of surface-ripened cheeses. The aim of the present study was the quantification and characterization of the bacterial flora in the smear of these cheeses.

## MATERIALS AND METHODS

### *Origin of cheese samples*

The smears of Tilsit cheeses from 2 plants (A, B) and 3 varieties of soft cheeses from 1 plant (C) were examined during the ripening period. In addition, Tilsit cheeses from 13 Austrian dairy plants were examined at 3 stages of ripening, on the 3rd, 7th and 21st d after manufacture.

### *Enumeration and isolation*

The smear film of 100 cm<sup>2</sup> of the cheese surface was scraped off with a sterile spatula, diluted serially in Ringer solution and plated on plate count agar + 30 g/kg NaCl, 80 g/kg NaCl and 150 g/kg NaCl (PCA; Merck 5463); it was then incubated at 30°C for 12 d (Seiler, 1986) in daylight to make pigment production possible for those coryneforms which are colourless after growth in darkness (Mulder *et al.*, 1966; El-Erian, 1969). The yeast development on the plates was suppressed by Pimaricin; before use 0.1 ml of a suspension (60 mg Pimaricin/3 ml H<sub>2</sub>O) was plated on the surface of the PCA (Sauter, 1986; Engel, 1993). Three pigmented groups (orange, yellow, cream) were counted separately. All counts are given per cm<sup>2</sup> smear surface; the use of g smear surface as a unit would result in values approximately 100 x higher. Five colonies of each morphological type were selected randomly from the PCA plates, purified and stored on plate count agar slants.

Yeast populations were detected on yeast extract-glucose-chloramphenicol-agar (YGCA; Merck 1600) with 10 µg/kg bromophenol blue

(Rapp, 1974) and incubated at 25°C for 5 d (IDF, 1990).

### Identification

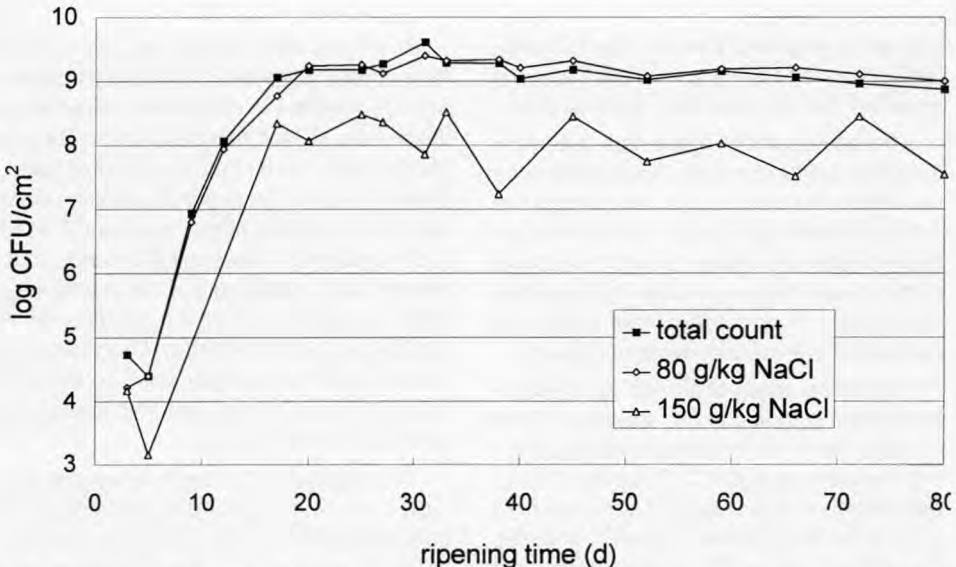
Three hundred eighty-six coryneforms were identified using 52 biochemical characteristics described by Seiler *et al* (1980), Seiler (1983, 1986) and Valdés-Stauber and Seiler (1990, personal information), including assimilation of sugars, amino acids and acids, production of acid from carbon sources, nitrate reduction, NaCl tolerance, KOH test (Grecz and Dack, 1961), hydrolysis of xanthine, starch, gelatine, casein and catabolism of tyrosin. A microtitre tray technique and the computer program by Seiler and Braatz (1988) were used for the identification routine.

A screening of proteolytic activities on calcium-caseinate agar, with a 3-mm layer (Frazier and Rupp; Merck 5409) was made. The diameter of the clear zone around the colony was measured as the parameter of proteolytic activity.

## RESULTS

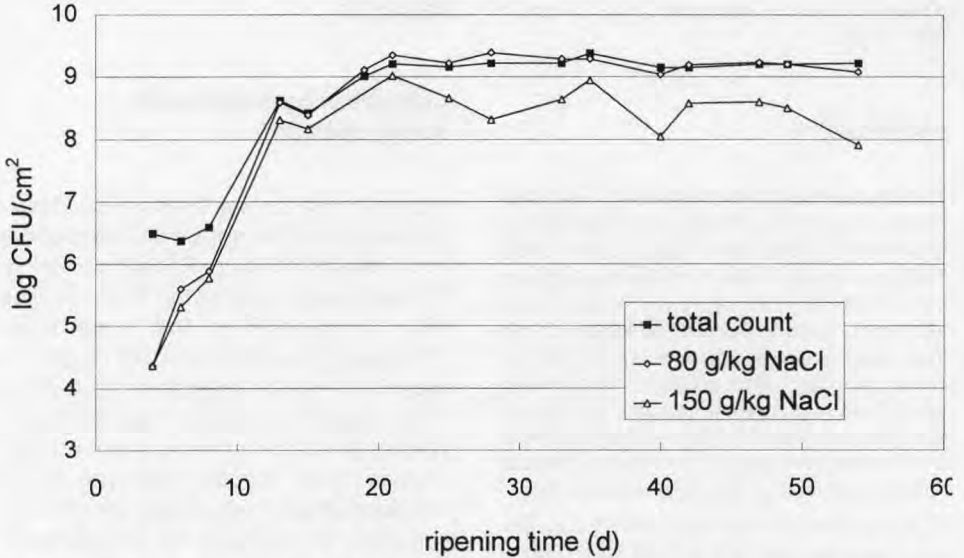
### Changes in bacterial counts during ripening

Figures 1 and 2 show the increase in bacterial counts on the surface of Tilsit cheeses from 2 plants (codes A, B) during ripening. The bacterial counts of the Tilsit cheeses after removal from the brine were  $10^4$  cfu/cm<sup>2</sup> in plant A and  $10^6$  cfu/cm<sup>2</sup> in plant B. It must be considered that at this stage of ripening no "smear" had developed. Within 20 d, the maximum count,  $10^9$  cfu/cm<sup>2</sup>, was reached and the count remained at this level until the time of consumption. The counts of the 80 g/kg-NaCl-resistant bacteria were equivalent to the total number of cheese surface bacteria; accordingly, the bacterial flora can be character-



**Fig 1.** Development of the 80 g/kg-NaCl- and 150 g/kg-NaCl-tolerant bacteria compared with the total bacterial count of Tilsit cheese during the whole ripening period (plant A).

*Évolution de la flore bactérienne tolérante des teneurs de 80 g/kg et 150 g/kg NaCl comparée à la flore totale au cours de la maturation de Tilsit (fromagerie A).*



**Fig 2.** Development of the 80 g/kg-NaCl- and 150 g/kg-NaCl-tolerant bacteria compared with the total bacterial count of Tilsit cheese during the whole ripening period (plant B).

*Évolution de la flore bactérienne tolérante des teneurs de 80 g/kg et 150 g/kg NaCl comparée à la flore totale au cours de la maturation de Tilsit (fromagerie B).*

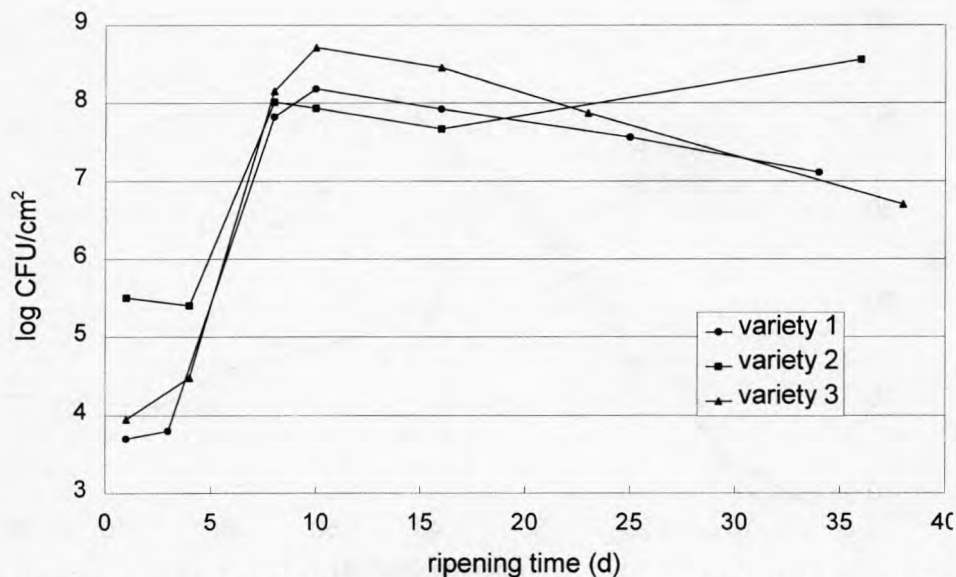
ized as 80 g/kg-NaCl tolerant. The 150 g/kg-NaCl-tolerant bacteria counts were approximately 1 log unit lower than the total count.

The development of the total bacterial count of 3 different soft cheese varieties is characterized by a maximum of  $> 10^8$  cfu/cm<sup>2</sup> (fig 3) after 10 d. In the following ripening periods, a slight decrease in the counts of the smears of 2 varieties occurred, but considerable differences between the 3 varieties were not found.

The percentages of orange- and yellow-pigmented colonies in the smears of Tilsit cheeses from 2 cheese plants during ripening are shown in figure 4. The proportion of pigmented colonies reached a maximum of 43% of the total bacterial count in cheeses from plant A, and of 35% in those from plant B. Furthermore, a difference was found between the smears from the 2 plants in the relation of orange- to yellow-pigmented colonies.

By means of the results just given, 3 different times of ripening (3, 7 and 21 d after manufacture) were chosen to characterize the smears of Tilsit cheeses from 14 cheese plants. From each plant, the cultures used, the kind and the frequency of smearing were recorded. All plants other than E and F used a *Brevibacterium linens* culture; plant L additionally used a yeast culture. Moreover, in all plants except E and F, the repeated smearing began with the older cheeses and moved onto the younger ones so that the younger cheeses were contaminated by the smear of the older ones.

The variation in the bacterial counts was high in the initial period of ripening, with counts between  $10^4$  and  $10^9$  cfu/cm<sup>2</sup> smear being found (table I). After 3 weeks, the counts in the smears of all plants were  $> 10^8$  cfu/cm<sup>2</sup>. In the smears of the 3- and 7-d-old cheeses, pigmented colonies occurred only sporadically, and were found in levels



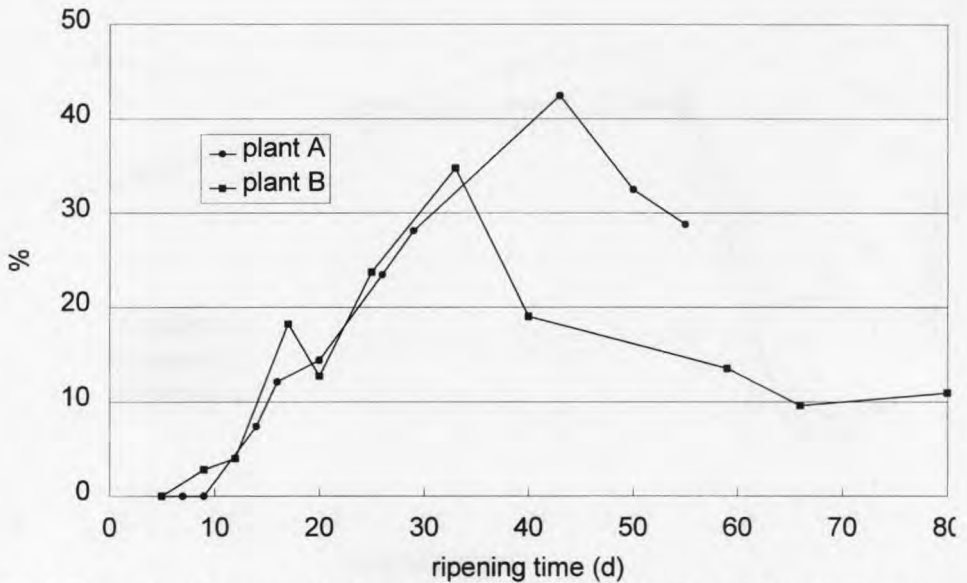
**Fig 3.** Development of the total bacterial count in the smears of 3 different soft cheese varieties during the whole ripening period (plant C).

*Évolution de la flore totale dans la morge de 3 différents types de fromages à pâte molle pendant toute la période de l'affinage (fromagerie C).*

**Table I.** Yeast counts and total bacterial counts in the smears of Tilsit cheeses from 14 cheese plants, 3, 7 and 21 d after manufacture, and the percentages of pigmented bacterial colonies after 21 d. *Nombre de levures et flore totale bactérienne dans la morge de Tilsit de 14 fromageries, 3, 7 et 21 j après la fabrication, et pourcentages des colonies pigmentées après 21 j.*

Plant	Log yeast count/cm <sup>2</sup>			Log bacterial count/cm <sup>2</sup>			% Pigmented colonies (21 d)
	3 d	7 d	21 d	3 d	7 d	21 d	
A	3.5	6.5	7.7	6.3	6.8	9.3	24
B	< 2.0	< 2.0	4.8	4.5	7.0	9.1	13
D	5.9	4.1	5.9	6.2	6.3	8.4	30
E	6.3	5.6	5.4	8.0	8.9	9.0	0
F	> 6.5	6.8	6.6	9.5	9.3	9.5	10
G	2.8	6.4	4.4	5.3	9.4	9.2	0
H	< 2.0	6.7	5.9	5.2	8.8	9.0	0
I	5.2	7.1	6.8	5.4	8.4	8.6	5
J	4.1	7.1	5.8	7.0	8.8	9.7	0
K	2.2	7.1	6.9	5.4	8.4	8.8	0
L	5.5	6.7	6.3	5.9	8.1	9.2	20
M	3.1	6.0	6.5	5.0	8.5	9.6	20
N	5.3	6.4	2.6	7.1	8.6	9.0	0
O	5.3	7.0	5.8	5.9	8.1	9.0	5





**Fig 4.** Percentages of orange- and yellow-pigmented colonies of the total bacterial count in the smears of Tilsit cheeses from 2 cheese plants during ripening.  
*Pourcentages des colonies de couleur jaune et orange présentes dans la flore totale de la morge de Tilsit provenant de 2 fromageries pendant l'affinage.*

of between 0 and 30% of the total bacterial count after 3 weeks of ripening.

In the smears of the 3 different soft cheese varieties, a change from yeast dominance to bacterial dominance occurred within the first week. This change was not typical for the Tilsit cheeses: all smear samples 3, 7 and 21 d after manufacture showed lower yeast counts than bacterial counts. The percentages of yeasts were lower in the smear samples of the older cheeses than in the younger ones.

#### **Identification results**

Three hundred eighty-six isolates of coryneform bacteria were isolated from the smears of the previously mentioned cheese samples and were identified. Table II outlines

the occurrence of the different species in the different cheese plants. Thirteen different species were found. Besides *Brevibacterium linens*, the other main types found present in the heterogeneous flora were cream-coloured and yellow-pigmented coryneforms, which were identified as *Arthrobacter citreus*, *Arthrobacter globiformis*, *Arthrobacter nicotianae*, *Arthrobacter variabilis*, *Brevibacterium ammoniagenes*, *Brevibacterium fuscum*, *Brevibacterium helvolum*, *Brevibacterium imperiale*, *Brevibacterium oxydans*, *Corynebacterium betae*, *Corynebacterium insidiosum* and *Corynebacterium poinsettiae*. In the bacterial flora in plants G and N, *Brevibacterium ammoniagenes* was dominant, while in plants A, B and L *Brevibacterium ammoniagenes* and *Brevibacterium linens* dominated. In the smears from plants C, D and I, a predominance of *Arthrobacter globiformis*

**Table II.** Subdivision according to species of coryneforms isolated from the surfaces of cheeses from 15 plants.

*Fréquence des espèces corynéformes isolées de la morge des fromages fabriqués dans les 15 fromageries.*

Species	Cheese plants														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
<i>A citreus</i>	1		12							1			3	2	
<i>A globiformis</i>		1	72	19			1		8			1			
<i>A nicotianae</i>	1		1					1	2			1	4		
<i>A spp</i>	4	1	5		4	8	1	1		1	6	3	9		
<i>A variabilis</i>	1						4			3	2			2	2
<i>B ammoniagenes</i>	8	6		1	1		11	4		1	2	7		12	
<i>B fuscum</i>	1											1			
<i>B helvolum</i>	1														
<i>B imperiale</i>	1	3			1							2		1	
<i>B linens</i>	20	5		1	8	13	1	5		1		5	13		5
<i>B oxydans</i>											3				
<i>B spp</i>	1											2		1	1
<i>C betae</i>															4
<i>C insidiosum</i>		1			3		1			1	1				1
<i>C poinsettiae</i>	1	1			1		1	2			1				5
<i>C spp</i>	2	1			4	4	2	6		1		2		3	
Total no of isolates	42	19	90	21	22	25	22	19	10	9	15	24	29	21	18

A: *Arthrobacter*; B: *Brevibacterium*; C: *Corynebacterium*

was characteristic. *Brevibacterium linens* in combination with *Arthrobacter* spp was typical for the smears from plants E, F and M. In the remaining 4 plants, no single species was found to dominate the flora.

### Biochemical properties of the bacterial flora

In tables III and IV the biochemical properties of the species are outlined as the percentage of positive results. The isolates tested showed a wide range of combinations of characteristics.

### Proteolytic activity

Changes in the frequency of proteolytic bacteria during the ripening were found (table V). The percentage of strains showing medium and high levels of proteolytic activity was higher at the beginning of ripening than after 21 d.

### DISCUSSION

At the beginning of ripening, the bacterial counts in the smears of the cheese surfaces varied from  $10^4$ – $10^9$  cfu/cm<sup>2</sup>. In the follow-



ing period, the counts increased to  $10^8$ – $10^9$  cfu/cm<sup>2</sup> and the differences between the smears from the different plants were equalized. In analysing Limburger (El-Erian, 1969), Tilsit (Keller and Puhon, 1985), Weinkäse (Sauter, 1986), Gruyère and Beaufort (Accolas *et al.*, 1978; Piton-Malleret and Gorrieri, 1992), counts of this level were regularly found on the surfaces of smear-ripened cheeses. Pigmented coryneforms showed a similar development as the total bacterial count but in lower numbers, occurring at levels of between 0 and 43% of the total counts. Grand *et al.* (1992) found counts

of pigmented strains more than 1 log unit lower.

The bacterial flora can be characterized by means of a marked resistance to NaCl. Thus, no difference between the total number of bacteria and the 80 g/kg resistant bacteria was found. Only with 150 g/kg NaCl in the medium were the counts 1 log unit lower than the total number. The cheese surface can therefore be described as an ecosystem in which NaCl-tolerant bacteria predominate. The salt tolerance of the surface flora of smear-ripened cheeses was also noted by El-Erian (1969), Accolas *et*

**Table III.** Percentages of positive tests of the *Arthrobacter* spp most commonly isolated from the cheese surfaces.

*Pourcentage d'essais positifs concernant Arthrobacter spp les plus fréquemment isolés sur la croûte des fromages.*

Character	Arthrobacter citreus (n = 19)	Arthrobacter globiformis (n = 102)	Arthrobacter variabilis (n = 14)
<i>Assimilation of:</i>			
Acetate	100	99	100
Propionate	100	99	100
Valerate	94	91	83
Capronate	0	3	0
Heptanoate	81	89	83
Caprylate	56	55	83
4-Amiobutyrate	88	100	75
4-Aminovalerate	100	86	75
Malonate	56	90	17
Succinate	100	100	92
DL-malate	100	99	83
Adipinate	31	66	0
Citrate	75	93	83
DL-lactate	100	100	100
Fumarate	94	100	100
Levulinat	75	66	58
4-Hydroxybenzoate	31	46	0
Glyoxylate	25	79	0
D-gluconate	100	98	83
2-Keto-gluconate	44	95	0
Glycine	88	92	17
L-leucine	81	99	50
L-proline	94	99	92

*al* (1978) and Grand *et al* (1992), who also equated the halotolerant flora with the total bacterial count. It is possible to select the bacterial flora by varying the salt concentration on the cheese surface. In practice, the salt content on the cheese surface can be varied using different concentrations in the smear water. At the beginning of ripening this concentration should not be too high because of the yeasts. A NaCl concentration of 50 g/kg in the smear water is ideal (Sauter, 1986). The NaCl concentration in the smear water can be increased parallel to

the increase in pH to 120 g/kg (Busse, 1989).

No significant difference was found between the bacterial status on Tilsit cheese and that on the 3 soft cheese varieties, with the exception of the relation between yeasts and coryneforms. Whereas the characteristic change from yeast dominance to bacterial dominance was found in the smears of the 3 different soft cheese varieties, in the smears of Tilsit cheeses from all 14 plants, the bacteria were dominant at all stages of ripening (Eliskases-Lechner and Ginzing, 1989).

**Table III.** (continued)

L-threonine	94	98	25
L-asparagine	100	100	58
L-aspartate	88	71	17
L-arginine	94	99	8
L-histidine	100	100	17
D-xylose	50	100	50
D-ribose	81	97	33
L-arabinose	13	94	8
D-galactose	100	98	67
L-rhamnose	67	96	8
D-xylitol	56	91	8
M-inositol	5	86	0
2,3-Butyleneglycol	88	95	25
Glycerol	81	100	25
Acetamide	13	87	0
<i>Acid from:</i>			
D-xylose	38	16	0
D-glucose	75	92	75
Sucrose	63	61	8
Lactose	50	59	0
Starch	0	1	0
Dextrin	0	16	0
<i>Hydrolysis of:</i>			
Xanthine	19	39	0
Starch	19	46	9
Gelatin	69	88	9
Casein	19	72	0
Catabolism of tyrosine	60	84	0
Resistance to 70 g/kg NaCl	100	99	100
Nitrate reduction	94	82	25
Pigment with KOH	0	0	0

**Table IV.** Percentages of positive tests of the *Corynebacterium* spp and *Brevibacterium* spp most commonly isolated from the cheese surfaces.  
*Pourcentage d'essais positifs concernant Corynebacterium spp et Brevibacterium spp les plus fréquemment isolés sur la croûte des fromages.*

Character	<i>Corynebacterium</i> <i>poinsettiae</i> (n = 12)	<i>Brevibacterium</i> <i>ammoniagenes</i> (n = 53)	<i>Brevibacterium</i> <i>linens</i> (n = 77)
<i>Assimilation of:</i>			
Acetate	0	35	99
Propionate	15	89	99
Valerate	0	9	99
Capronate	38	0	97
Heptanoate	0	5	94
Caprylate	0	4	76
4-Amiobutyrate	8	94	97
4-Aminovalerate	0	7	94
Malonate	0	17	92
Succinate	8	98	99
DL-malate	0	83	95
Adipinate	30	0	83
Citrate	0	13	80
DL-lactate	73	98	99
Fumarate	0	98	99
Levulinate	0	4	76
4-Hydroxybenzoate	0	0	24
Glyoxylate	0	2	74
D-gluconate	92	86	91
2-Keto-gluconate	31	86	38
Glycine	0	0	53
L-leucine	85	20	80
L-proline	23	62	73

1995). It can therefore be concluded that the knowledge concerning smear-ripened soft cheeses is not in every case applicable to semi-hard cheeses. Keller and Puhan (1985) also found that the yeast counts on Tilsit cheese were never greater than the bacterial counts. For soft cheese varieties, this change is of great importance, and should occur at a certain stage of ripening to avoid the growth of undesired moulds (Sauter, 1986; Mayer, 1990).

In 12 of the 14 cheese plants, *Brevibacterium linens* cultures were used. Nevertheless, *Brevibacterium linens* was not a dominant species within the bacterial flora of

the cheese smear. It reached a maximum level of approximately 30% of the total flora on the 21st d of ripening. The species *Arthrobacter globiformis* and *Brevibacterium ammoniagenes* took a dominant position. All others of the 13 different species found occurred only sporadically, or were typical for the smear from a single plant. Although the morphology of the colonies was the main criterion for selection, the exact percentages in the smear are difficult to specify. Additionally, the change in composition of the bacterial flora during the ripening period is also difficult to specify, although tendencies are distinguishable.

L-threonine	0	48	86
L-asparagine	0	96	100
L-aspartate	0	59	73
L-arginine	7	13	99
L-histidine	7	89	96
D-xylose	92	46	49
D-ribose	54	83	78
L-arabinose	54	28	8
D-galactose	54	34	96
L-rhamnose	0	9	61
D-xylitol	31	5	10
M-inositol	0	11	35
2,3-Butyleneglycol	0	5	77
Glycerol	85	41	99
Acetamide	38	13	43
<i>Acid from:</i>			
D-xylose	38	11	5
D-glucose	85	89	68
Sucrose	62	20	19
Lactose	77	26	18
Starch	0	0	0
Dextrin	0	4	4
<i>Hydrolysis of:</i>			
Xanthine	15	6	66
Starch	0	24	3
Gelatin	64	22	97
Casein	83	33	71
Catabolism of tyrosine	15	41	64
Resistance to 70 g/kg NaCl	100	91	100
Nitrate reduction	38	93	55
Pigment with KOH	8	0	98

**Table V.** Proteolytic activity of coryneforms isolated from Tilsit cheese at 3 stages of ripening.  
*Activité protéolytique des bactéries corynéformes isolées de fromage Tilsit à 3 stades d'affinage.*

Time of isolation (days)	No of isolates	Activity %		
		Low	Medium	High
3	87	33.3	41.4	25.3
7	87	42.5	34.5	23.0
21	89	55.0	27.0	18.0

The comparison with literature data is difficult, because often no identification was made, a differentiation by means of morphological characteristics being carried out instead. For example, Mulder *et al* (1966) and El-Erian (1969) found 65-80% grey-white strains on the surface of Limburger cheese, whereas isolates of the type *Brevibacterium linens* made up between 9 and 24%. Sauter (1986) found, on the surface of Weinkäse, 42% white to cream-coloured coryneforms, 36% yellow-pigmented coryneforms and only 22% showing the typical pigmentation of *Brevibacterium linens*. Seiler (1986) and Mayer (1990) defined the isolated strains as *Arthrobacter* spp. On the surfaces of different cheese varieties, eg Romadur, Limburger cheese, Weinkäse, Harzer Handkäse and Camembert, *Arthrobacter nicotianae*, *Arthrobacter variabilis* and *Brevibacterium ammoniagenes* were found. The species *Arthrobacter protophormiae* and *Arthrobacter uratoxydans* occurred in the smear of Vacherin Mont-d'Or (Anonymous, 1988; Hug-Michel *et al*, 1989). Sauter (1986) identified the coryneforms of Weinkäse as *Arthrobacter aurescens*, *Arthrobacter citreus*, *Arthrobacter globiformis*, *Brevibacterium linens*, *Brevibacterium helvolum* and *Brevibacterium oxydans*.

The results concerning the proteolytic activity of the coryneforms correspond to the descriptions given by Mulder *et al* (1966) and El-Erian (1969), who classified the majority of the grey-white coryneforms of the cheese surface as nonproteolytic, and found the flora at earlier stages of ripening more proteolytic than at later stages.

In conclusion, it is remarkable that *Brevibacterium linens* is never the dominant bacterium despite its regular use as a culture. It is to be assumed that the majority of those coryneforms, which were not added as culture organisms, are just as important as *Brevibacterium linens* for the ripening process, eg by their proteolytic activity, for

the production of aroma and the appearance of the cheese surface.

## REFERENCES

- Accolas JP, Melcion D, Vassal L (1978) Étude de la flore superficielle des fromages de Gruyère et de Beaufort. 20<sup>e</sup> Congr Int Lait, Paris, France, 773-774
- Anonymous (1988) Untersuchung über die Dynamik der Oberflächenflora beim Vacherin Mont-d'Or. Tätigkeitsbericht FAM 205-207
- Busse M (1989) Die Oberflächenflora von geschmierem Käse. *Milchwirtsch Ber* 99, 137-141
- El-Erian AFM (1969) Bacteriological studies on Limburger cheese. Thesis, Agricultural University, Wageningen, Netherlands
- Eliskases-Lechner F, Ginzinger W (1995) The yeast flora of surface-ripened cheeses. *Milchwissenschaft* 50,458-462
- Engel G (1993) Hemmung von Hefe- und Schimmelpilzwachstum beim quantitativen Nachweis von Bakterien. *Milchwissenschaft* 48, 325-327
- Grand M, Weber A, Perret J, Zehntner U, Glättli H (1992) Evolution de la flore d'affinage sur des fromages de Gruyère. *Schweiz Milchwirtsch Forschung* 21, 3-5
- Grecz N, Dack GM (1961) Taxonomically significant color reactions of *Brevibacterium linens*. *J Bacteriol* 82, 241-246
- Hug-Michel C, Barben S, Kaufmann U (1989) Hemmung von *Listeria* spp durch Mikroorganismen aus der Rinde von Rotschmiereweichkäse. *Schweiz Milchwirtsch Forschung* 18, 46-49
- IDF (1990) Milk and milk products enumeration of yeasts and moulds colony count technique at 25°C. Standard 94 B
- Keller A, Puhani Z (1985) Entsäuerung der Käse mit Schmiere am Beispiel des Tilsiters. *Schweiz Milchwirtsch Forschung* 14, 3-11
- Mayer W (1990) Einflussfaktoren auf die Oberflächenflora von Rotschmierekäsen - Versuchsergebnisse. IX. Käseertechnologischer Sonderlehrgang, Staatl Lehr- und Versuchsanstalt Kempen/Allgäu
- Mulder EG, Adamse AD, Antheunisse J, Deinema MH, Woldendorp JW, Zevenhuizen LPTM (1966) The relationship between *Brevibacterium linens* and bacteria of the genus *Arthrobacter*. *J Appl Bacteriol* 29, 44-71
- Piton C, Fontanier C (1990) Caractérisation d'une collection de souches de bactéries corynéformes de la morge du gruyère de Comté. *Lait* 70, 383-398
- Piton-Malleret C, Gorrieri M (1992) Nature et variabilité de la flore microbienne dans la morge des fromages de Comté et de Beaufort. *Lait* 72, 143-164

- Rapp M (1974) Indikatorzusätze zur Keimdifferenzierung auf Würze- und Malzextrakt-Agar. *Milchwissenschaft* 29, 341-344
- Sauter H (1986) Die Oberflächenflora von Weinkäse. Reifungsfehler und ihre Ursachen. Thesis, Technical University, Munich, Germany
- Seiler H (1983) Identification key for coryneform bacteria derived by numerical taxonomic studies. *J Gen Microbiol* 129, 1433-1471
- Seiler H (1986) Identification of cheese-smear coryneform bacteria. *J Dairy Res* 53, 439-449
- Seiler H, Braatz R (1988) Computerprogramme MCOMPARE und CLUSUM zur Coryneformenidentifizierung. Bakteriologisches Institut, südd Versuchs- und Forschungsanstalt für Milchwirtschaft Weihenstephan
- Seiler H, Braatz R, Ohmayer G (1980) Numerical cluster analysis of the coryneform bacteria from activated sludge. *Zbl Bakteriol Mikrobiol Hyg Abt I Org C* 1, 357-375
- Valdés-Stauber N, Seiler H (1990) Identifizierung von Coryneformen mit Mikrotiterplatten. Bakteriologisches Institut, südd Versuchs- und Forschungsanstalt für Milchwirtschaft Weihenstephan