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Original article

Interaction between propionibacteria and starter / non-starter lactic acid bacteria in Swiss-type cheeses

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Abstract — Thermophilic lactic acid bacteria, propionibacteria (PAB) and facultatively heterofermentative lactobacilli (FHL) form the main flora of Swiss-type cheeses. The aim of this work was to investigate their interactions and impact on product quality, and in particular on the defect of late fermentation. For this purpose Emmental model cheeses were produced according to a twice-replicated 2^4 full factorial experimental design. The four factors were: (1) The type of cultures of PAB, one with a weak (Prop 96) and the other one with a strong aspartase activity (Prop 90). (2) The addition or not of a culture of FHL composed of 3 *Lactobacillus casei* strains. (3) The addition or not of a culture of 4 *Lactobacillus helveticus* strains. (4) The season: winter (hay feeding) or summer (grass feeding). FHL and PAB counts, organic acids, proteolysis, eye formation were followed during ripening. Prop 90 showed a higher growth and fermentation role resulting in a higher number of eyes and an increased risk of late fermentation compared to Prop 96. PAB growth was also favoured in cheeses manufactured with winter milk which had a slightly higher water content. The addition of *L. helveticus* tended to increase the risk of late fermentation. On the other hand the addition of FHL inhibited lactate fermentation, with a more marked effect on Prop 96 culture. In conclusion the defect of late fermentation can be prevented by using PAB with weak aspartase activity, by adding FHL and by omitting *L. helveticus*.

Emmental cheese / propionibacteria / aspartase activity / *Lactobacillus casei / Lactobacillus helveticus*

Résumé — Les bactéries lactiques thermophiles, les bactéries propioniques (PAB) et les lactobacilles hétérofermentaires facultatifs (FHL) constituent les principales flores de l'écosystème des fromages de type emmental. L'objectif de ce travail était de déterminer les interactions entre ces flores et leurs conséquences sur la qualité du fromage, en particulier sur le défaut de fermentation tardive. Dans ce but, des fromages emmental modèles étaient produits selon un plan factoriel expérimental complet répété 2 fois, avec les 4 facteurs suivants : (1) le type de levain propionique, l'un avec une

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faible (Prop 96), l'autre avec une forte activité aspartase (Prop 90). (2) L'addition ou non d'une culture FHL composée de 3 souches de *Lactobacillus casei*. (3) L'addition ou non d'une culture de 4 souches de *Lactobacillus helveticus*. (4) La saison : hiver (alimentation en foin), ou été (alimentation en herbe). La croissance des FHL et des PAB, ainsi que les teneurs en acides organiques, la protéolyse et la formation de l'ouverture étaient suivis au cours de l'affinage. La culture Prop 90 montrait un taux de croissance et une vitesse de fermentation plus élevés que Prop 96, ce qui conduisait à un nombre supérieur d'ouvertures et un risque accru de fermentation tardive. La croissance des PAB était également favorisée dans les fromages d'hiver, légèrement plus humides. En revanche, l'addition de FHL inhibaient la fermentation propionique, avec un effet plus marqué sur la culture Prop 96. De même la présence de *L. helveticus* augmentait le risque de fermentation tardive. En conclusion, le défaut de fermentation tardive peut être évité en utilisant un levain propionique à faible activité aspartase, en ajoutant des FHL et en omettant *L. helveticus*.

emmental / fromage / *Propionibacterium* / activité aspartase / *Lactobacillus casei* / *Lactobacillus helveticus*

1. INTRODUCTION

Propionibacteria (PAB) are used in the Swiss cheese industry for the manufacture of Emmental to achieve the characteristic eyes and nutty flavour. Strain diversity of the natural propionibacterial flora is great which, fortunately, has not been influenced by the wide use of commercially available cultures [15].

Three different metabolic pathways (Fig. 1) have been described for the utilisation of lactate as energy source and aspartate as electron acceptor, both of which are available in cheese [7, 11–13]. In the presence of aspartate, the fermentation of lactate is coupled with the fermentation of aspartate to succinate, and no propionate is produced (C). During the ripening of Swiss-type cheese, aspartate is rapidly metabolised and L(+)-lactate is preferably used [10, 23]. The role of pathway B (formation of succinate by fixation of CO_2) is certainly of minor importance, but it has not yet been clarified [26].

Facultatively heterofermentative non-starter lactic acid bacteria (FHL) are used in the Swiss artisanal cheese industry to slow down propionic acid fermentation [27]. Jimeno et al. [19] found growth inhibition of PAB in cheese of up to 80% compared to the control growth without FHL (*Lactobacillus casei* and *Lb. rhamnosus*). As a consequence, less propionic acid is produced. The observed inhibition could not be reproduced in co-cultures, suggesting that bacteriocin production is not responsible for this effect. Citrate metabolism most probably plays the key role, since citrate (–) mutants were shown to inhibit PAB much less than the corresponding citrate (+) strains [20]. FHL

(A) Classical propionic acid fermentation:
3 mol lactate $\rightarrow$ 2 mol propionate + 1 mol acetate + 1 mol CO_2 + 1 mol ATP

(B) Formation of succinate during propionic acid fermentation by CO_2-fixation:
3 mol lactate $\rightarrow$ (2 - x) mol propionate + 1 mol acetate + (1 - x) mol CO_2 + x mol succinate
Wood-Werkman-pathway

(C) Fermentation of aspartate to succinate during propionic acid fermentation:
3 mol lactate + 6 mol aspartate $\rightarrow$ 3 mol acetate + 3 mol CO_2 + 6 mol succinate + 6 mol NH_3 + 3 mol ATP

Figure 1. Metabolic pathways for the utilisation of lactate by propionic acid bacteria according to Crow and Turner [12] and Sebastiani und Tschager [26].

metabolise all the citrate initially present in cheese to acetate, formate and CO_2. *Lb. rhamnosus* also produces small but appreciable amounts of diacetyl which has a lethal effect on PAB. Acetate and formate seem to have an inhibitory effect on PAB growth. In addition, the metabolism of citrate leads to a release of the complexed copper. The relative concentration of citrate and copper play an important role in the observed inhibition [22].

During cheese ripening, proteolysis is very important for the development of the texture and flavour characteristics. Intensified proteolysis generally leads to accelerated ripening of the product which is desired as long as no effect on the storage quality is encountered. In Emmental production, strong proteolysis together with intense propionic acid fermentation may, however, be the primary cause of late fermentation [1, 5]. Several investigations have shown that thermophilic lactic acid bacteria (LAB), especially *Lb. delbrückii* and *Lb. helveticus*, were able to stimulate PAB growth [9, 21–23]. Baer [4] found poor growth of PAB on milk alone or with added rennet, but good growth in the presence of LAB alone or with added rennet. It was concluded that propionibacterial growth depends on the presence of free amino acids or small peptides. In a later work, Baer and Ryba [5] found that PAB clearly prefer free amino acids to peptides. They concluded that growth of PAB, and thus the intensity of propionic acid fermentation and the risk of late fermentation, is correlated with the amount of free amino acids. Piveteau et al. [23] described the liberation of a heat resistant stimulatory compound by *Lb. helveticus* which might be the free amino acid aspartate or a peptide containing it. The absence of nutrients is, in contrast, not the reason why PAB fail to grow in milk when inoculated at $<10^5$ cfu·mL^{-1}. The same authors gave evidence for an inhibitory substance in milk, which is heat-stable and of low molecular mass [24]. It is removed by *Lb. helveticus* strains as a result of proteolysis, but not by *Lb. delbrückii* nor *Lb. lactis* strains. The activation of PAB growth may consequently be the result of stimulation by the proteolytic activity of lactobacilli liberating peptides and free amino acids and/or the removal of an inhibitory substance by the action of *Lb. helveticus* [21].

The above mentioned micro-organisms are often found or even employed in Swiss-type cheese manufacture. The aim of this work was to investigate and understand their interactions and their impact on product quality.

2. MATERIALS AND METHODS

2.1. Starter and non-starter cultures

Two different cultures of *Propionibacterium freudenreichii* sp. *shermanii* were used, one with a weak (Prop 96) and one with a strong aspartase activity (Prop 90). The Prop 96 culture is composed of 2 *Propionibacterium* strains, isolated from Appenzeller cheese (Switzerland), which in vitro metabolised not more than 100 nmol aspartate in 1 min·mg^{-1} protein in cell extract. The culture is produced at the FAM (Swiss Dairy Research Station, Liebefeld, Bern, Switzerland) and sold in liquid form to cheese factories manufacturing Swiss type cheese. The Prop 90 culture (not for sale to cheese factories) contains a single *Propionibacterium* strain isolated from Gruyère cheese. This strain was able to metabolise in vitro up to 800 nmol aspartate in 1 min·mg^{-1} protein in cell extract.

The *Lb. helveticus* culture XMK1168 is a mixture of 3 strains (a, b and f) of *Streptococcus thermophilus*, as well as of 4 strains of *Lb. helveticus*. Three of the *Lb. helveticus* strains were isolated from whey of a Swiss cheese factory which produces Tilsit cheese from raw milk, and the fourth strain was isolated from whey of a Swiss cheese factory which produces Appenzell cheese. Since this is still a test culture, it cannot yet be purchased from the FAM.

The FHL culture MK3008 is composed of 3 strains of *Lactobacillus casei* isolated from a ripe Emmental cheese of good quality. It is part of the culture collection of the FAM and is generally sold to cheese factories in order to prevent late fermentation of Emmental cheeses or to enhance eye formation in semi-hard cheeses with no propionic acid fermentation.

2.2. Manufacture of model cheeses

The Emmental cheeses were produced in the pilot plant of the FAM according to the manufacturing protocol shown in Figure 2.

Eight cheeses were produced per day. The pH of the cheeses was measured after 2, 4 and 24 h and at the end of ripening. Total lactic acid, galactose, water and leucine aminopeptidase activity were determined after 24 h, as well as the titers of propionibacteria, FHL, non-fermenting and salt-tolerant bacteria. Free short-chain acids, carbon dioxide, citrate, propionibacteria and FHL were determined after 40 and 180 d. Water, fat, total nitrogen (TN), water soluble nitrogen (WSN), non-protein nitrogen (NPN) which is the 12% trichloroacetic acid soluble fraction, free amino acids, succinate, lactate and sensory characteristics were determined at the end of maturation (180 d). The cheeses were also X-rayed (65 kV, 20 mAs, 1.6 s on a Philips Practix 21) after 40 and 180 d in order to count the eyes; and the duration of eye formation was documented.

2.3. Microbiological and chemical analyses

The non-fermenting bacterial flora were analysed on sugar-free agar without penicillin (3 d at 30 °C) [25]. Propionibacteria were analysed on lactate agar (10 d at 30 °C) [16]. Salt-tolerant bacteria were analysed on mannite-NaCl agar (2 d at 37 °C) [25]. Facultatively heterofermentative lactobacilli were enumerated on FH agar with mannite, incubated anaerobically for 3 d at 38 °C [18].

TN, WSN and NPN were determined by the Kjeldahl method according to the IDF Standard 20A [17] with a Büchi B-435 digestion unit and a Büchi B-339 distillation unit (Büchi, Flawil, Switzerland). RP-HPLC was used to analyse free amino-acids after a pre-column derivatisation with o-phthalaldehyde [8].

Carbon dioxide produced during fermentation was measured after stabilisation of samples with 50 $mmol \cdot L^{-1}$ NaOH. Measurements were performed by infra-red photometry of gas released from the sample after the addition of 2.5 $mol \cdot L^{-1}$ H_2SO_4 according to Bosset et al. [6].

After isolation by steam distillation, free short-chain fatty acids were determined by gas-chromatograph using a flame ionisation detector. The method has been described in detail by Badertscher et al. [3].

L- and D-lactic acid, citrate, succinate and galactose were analysed enzymatically according to the instructions protocol by the kit manufacturer (Roche diagnostics, Mannheim, Germany).

2.4. Experimental design

The experimental design was based on a twice-replicated 2^4 full factorial treatment structure with four blocks of eight vats each. This allowed an efficient use of resources, to study several treatments simultaneously. The cultures of propionibacteria (Prop 96/Prop 90), of XMK1168 (+/–) and of MK3008 (+/–), as well as the season of milk production (feeding of grass or hay) each represented a treatment factor at two levels. The combinations of these factor

120 L milk (35 g·kg^{-1} fat)
↓
Addition of water (10 L) and 13 mL copper sulphate (39.3 g of copper(II)-sulfat-pentahydrate in 1 L water)
↓
Pre-ripening of vat milk at 31 °C for 30 min
↓
Addition of fresh starter culture (130 mL/~30 °SH)
↓
Addition of old starter culture or XMK1168 (130 mL/~45 °SH)
↓
Coagulation at 32 °C for 35 min
↓
Addition of water (2 L) and rennet extract (18 mL, Bichsel, Switzerland)
↓
Addition of propionibacteria (Prop 96 or Prop 90 grown on peptone whey) to a final concentration of approximately 1.5×10^{3} cfu·mL^{-1} milk
↓
Addition of 0.6 mL (or none) FHL (MK3008) culture
↓
Cutting (grain size: 4–8 mm)
↓
Stirring for 25 min
↓
Scalding to 53 °C during 30 min (+3 L water)
↓
Stirring for 35 min at 53 °C
↓
Pressing room
(50 °C for 2 h, ramping from 50°C to 35 °C over 5 h, 35 °C for 10 h, ramping from 35 °C to 25 °C over 1 h, 25 °C for 4 h)
Pressure: 6 bar for 15 min, 7.5 bar for 5 h, 2 bar for 14 h
↓
Brining for 1 d at 11–13 °C
↓
Ripening in the cold room for 10 d at 11–13 °C (75–85% relative humidity)
↓
Ripening in the warm room for ~60 d at 21–23 °C (75–85% relative humidity)
↓
Maturation at 11–13 °C (65–75% relative humidity)

Figure 2. Flow sheet of the manufacturing procedure for Emmental cheese at the Swiss Dairy Research Station.

levels defined the treatment which was applied to an experimental unit, which is the vat (Tab. I). Since the pilot plant equipment consisted of only eight vats, the repetition of the experiment in each season had to be carried out on two different days. On each day, the eight treatment combinations were allocated to the vats at random

The variance of the response variables were analysed with SYSTAT (Systat for Windows, Version 9.0, SPSS, Chicago 1999) using a GLM (general linear model).

Table I. Full factorial experimental design of the model cheese production.

Treatment	Propionibacteria	*Lb. helveticus* (XMK1168)	*Lb. casei* (MK3008)	Season	Day
1, 12	Prop 90	+	+	grass	1+2
2, 15	Prop 90	–	+	grass	1+2
3, 10	Prop 90	–	–	grass	1+2
4, 14	Prop 90	+	–	grass	1+2
5, 9	Prop 96	+	+	grass	1+2
6, 11	Prop 96	–	+	grass	1+2
7, 16	Prop 96	+	–	grass	1+2
8, 13	Prop 96	–	–	grass	1+2
25, 21	Prop 96	+	+	hay	3+4
26, 23	Prop 96	–	+	hay	3+4
27, 20	Prop 96	+	–	hay	3+4
28, 17	Prop 96	–	–	hay	3+4
29, 24	Prop 90	+	+	hay	3+4
30, 19	Prop 90	–	+	hay	3+4
31, 22	Prop 90	–	–	hay	3+4
32, 18	Prop 90	+	–	hay	3+4

3. RESULTS

3.1. Factor "feeding"

Milk produced during the hay feeding season (winter) is by experience less "ripe" and more prone to the defect of late fermentation during cheese ripening than milk produced during the grass feeding season (summer). Cheese producers generally observe a slightly lower rate of acidification of the winter milk with a resulting lower pH and, thus, higher content of water and lactate in the cheese after 24 h. This fact was also observed in our experiment: in winter, the acidification was slower (Tab. II, higher pH after 2 h) with resulting higher lactate and water contents, and a lower pH after 24 h and at the end of maturation (Tab. II). A low pH leads to a slower propionic acid fermentation, since the optimal pH range lies between 6 and 7. Only with proteolysis can a change in pH be anticipated. Thus, a higher PAB concentration is needed in order to start propionic acid fermentation under this disadvantageous pH condition. This may be the cause of higher PAB counts which lead to more lactate consumption and therefore more propionic acid and CO_2 production (Tabs. IV–VI).

The higher fat and lower protein contents of the cheeses in winter are rather incidental, but not the higher proteolytic parameters (Tab. III). They have to be looked at in the context of acidification. The slower rate of acidification leads to a higher water content which is advantageous for enzymatic reactions such as proteolysis.

The slightly elevated concentration in capronate and butyrate might be the result

Table II. Water, lactate and pH in Emmental cheese grouped by the four factors tested in the model cheese production.

Factor	N	Water ($g \cdot kg^{-1}$)		Lactate ($mmol \cdot kg^{-1}$)		D-Lactate ($mmol \cdot kg^{-1}$)		pH		
		1 d	180 d	1 d	180 d	1 d	180 d	2 h	24 h	180 d
Feeding										
grass	16	372.5	326.2	125.6	26.9	53.7	12.7	5.93	5.30	5.83
hay	16	373.9	331.4	131.3	25.5	52.3	12.9	5.99	5.28	5.72
PAB										
Prop 96	16	372.9	328.2	128.3	34.5	52.4	15.8	5.96	5.29	5.77
Prop 90	16	373.5	329.4	128.6	17.9	53.5	9.8	5.96	5.29	5.78
Lb. casei (MK3008)										
yes	16	372.9	328.9	128.9	51.2	52.9	24.9	5.95	5.30	5.76
no	16	373.5	328.7	127.9	1.2	53.0	0.7	5.97	5.29	5.79
Lb. helveticus (XMK1168)										
yes	16	372.8	328.5	127.9	25.4	55.5	11.3	5.97	5.30	5.78
no	16	373.6	329.1	129.0	26.9	50.4	14.3	5.95	5.29	5.77
ANOVA										
Feeding		*	***	***	-	-	-	***	**	***
PAB		-	-	-	***	-	***	-	-	-
Lb. casei		-	-	-	***	-	***	*	-	***
Lb. helveticus		-	-	-	-	***	*	**	-	**

- not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

of an increased share of milk from cows at the end of lactation which is typical of hay feeding (Tab. V). This milk is usually more prone to lipolysis.

3.2. Factor "aspartase activity of propionibacteria"

Figure 3 shows clearly the differences in aspartate metabolism of PAB cultures Prop 96 and Prop 90. Culture Prop 90 with a strong aspartase activity metabolises 12 mmol more aspartate and asparagine releasing, as a consequence, 12 mmol more succinate (Tabs. III and IV and Fig. 1). It is often observed that the ability to metabolise aspartate is coupled with a stronger growth rate of PAB leading to higher PAB counts and higher concentrations of propionate, acetate and CO_2. The same observation has already been described in a prior work [2, 28].

The number of eyes at the end of maturation and the height of loaves seem to be correlated: the shorter the stay in the warm room, the faster eyes are formed, and the greater the number of eyes and loaf height (Tab. VI).

PAB culture Prop 90 and the absence of FHL lead to not only a higher number of

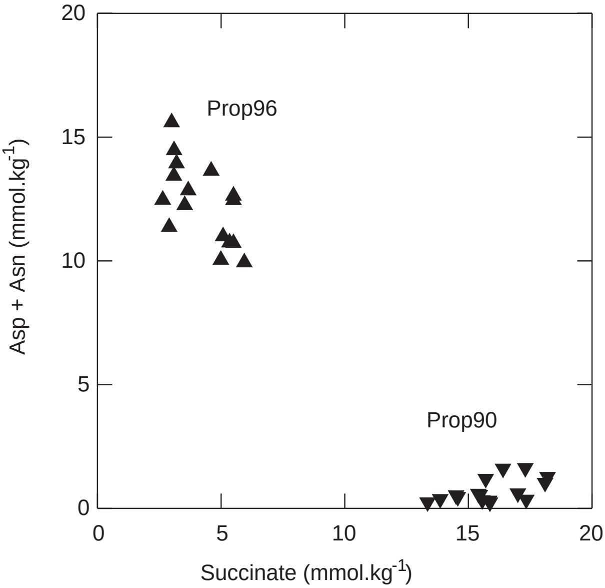

Figure 3. Influence of the aspartate metabolism on succinate liberation in the Emmental cheeses of 6 months (Prop 96: weak aspartase activity; Prop 90: strong aspartase activity).

eyes, but also to a larger size of the same as a consequence of excessive CO_2 production. These are signs of the beginning of late fermentation which is not desired by the producers [1]. In Table VII it can be seen that conservation is judged to be slightly lower for the Emmental cheeses made with culture Prop 90.

The PAB-culture displays another major quality: culture Prop 90 with high aspartase activity enhances flavour intensity, evidence for which was observed previously by Wyder et al. [28]. The reason for this is not to be found in proteolysis itself, but in amino acid catabolism and consequently the production of more volatile components.

3.3. Factor "facultatively heterofermentative Lactobacilli"

Already after 40 d of ripening, the FHL had also increased in numbers in the cheeses where they had not been added. Thus, they must have originated from raw milk and consist of less than 10% of the final FHL concentration in the cheeses with the addition of MK3008 (Tab. IV). The addition of FHL to the cheese milk is usually aimed at controlling the growth of raw milk flora during cheese ripening.

Citrate is mostly metabolised by FHL. As Table IV shows, citrate is consumed within the first 40 days of ripening. Starting from 9 $mmol \cdot kg^{-1}$ in the cheese, non-starter FHL utilise approximately 3 mmol and the starter FHL metabolise all available citrate to formate and acetate. The main reason for the use of FHL in the production of Emmental cheese is the inhibition of PAB and, therefore, to control propionic acid fermentation. Ever since FHL cultures were introduced to Switzerland in 1989, the defect of late fermentation has decreased considerably. The mechanism of inhibition is not yet conclusively clarified. According to Jimeno et al. [19] excess formate and acetate probably have an inhibitory effect on PAB.

Table III. Fat content and proteolytic parameters in Emmental of 180 d grouped by the four factors tested in the model cheese production.

Factor	N	Fat ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	WSN (% of TN)	NPN (% of WSN)	FAA	Asn ($mmol \cdot kg^{-1}$)	Asp
Feeding								
grass	16	331.0	46.5	23.2	61.9	175.5	4.68	1.30
hay	16	334.8	44.7	25.0	64.6	199.1	4.93	2.16
PAB								
Prop 96	16	333.0	45.5	24.6	63.1	196.8	9.51	2.83
Prop 90	16	332.7	45.6	23.6	63.2	177.8	0.09	0.62
Lb. casei (MK3008)								
yes	16	333.0	45.6	24.5	63.3	191.9	5.02	1.88
no	16	332.8	45.6	23.7	63.0	182.7	4.59	1.57
Lb. helveticus (XMK1168)								
yes	16	333.3	45.6	24.0	64.5	196.0	4.80	1.88
no	16	332.5	45.6	24.2	61.8	178.5	4.80	1.57
ANOVA								
Feeding		***	***	***	***	**	-	**
PAB		-	-	**	-	**	***	***
Lb. casei		-	-	*	-	-	-	-
Lb. helveticus		-	-	-	***	*	-	-

- not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; PAB propionibacteria; Asn asparagine; Asp aspartate; FAA free amino acids.

A measurable consequence of the addition of MK3008 is that approximately 23 mmol (~70%) less propionic acid are produced after 40 d which corresponds to the classical pathway to 11 mmol CO_2. This is approximately the difference found after 40 d in Emmental cheese with and without the addition of MK3008 (Tab. VI). The FHL therefore prolong the stay of the cheese in the warm room. The number of eyes after 40 and 180 d do not always correlate: citrate fermentation by FHL takes place before propionic fermentation. Nearly all the citrate is used after 40 d by FHL (Tab. IV). Since citrate metabolism also leads to the production of CO_2, this might be the reason why the number of eyes after 40 d is higher. Later during ripening, however, much more CO_2 is produced by PAB.

The interaction between FHL and PAB found in this experiment confirms what is already known by cheesemakers: culture Prop 96 together with MK3008 leads to the longest stay in the warm room and culture Prop 90 without addition of MK3008 to the shortest stay. In other words: culture Prop 96 is inhibited by FHL much more than culture Prop 90. The question arises as to whether Prop 96 is more sensitive to formate and acetate than Prop 90. The interaction shows that both cultures produce

Table IV. Facultative heterofermentative (FHL) and propionic acid bacteria (PAB), as well as citrate an succinate in Emmental cheese grouped by the four factors tested in the model cheese production.

Factor	N	FHL (log cfu·g^{-1})			PAB (log cfu·g^{-1})			Citrate (mmol·kg^{-1})		Succinate (mmol·kg^{-1})
		1 d	40 d	180 d	1 d	40 d	180 d	40 d	180 d	180 d
Feeding										
grass	16	1.97	7.83	7.24	3.44	7.86	8.12	3.6	4.2	9.8
hay	16	2.32	7.83	7.23	3.62	8.91	8.03	3.1	3.3	10.4
PAB										
Prop 96	16	2.18	7.84	7.30	3.63	8.17	7.56	3.1	3.8	4.2
Prop 90	16	2.11	7.83	7.17	3.42	8.59	8.59	3.6	3.7	15.9
Lb. casei (MK3008)										
yes	16	3.82	8.29	7.53	3.50	8.34	7.95	0.3	0.2	9.3
no	16	0.48	7.37	6.94	3.56	8.42	8.20	6.4	7.4	10.8
Lb. helveticus (XMK1168)										
yes	16	2.19	7.79	7.30	3.49	8.49	7.97	3.2	3.6	10.2
no	16	2.11	7.87	7.17	3.57	8.27	8.18	3.5	3.9	9.9
ANOVA										
Feeding		-	-	-	-	***	-	*	***	-
PAB		-	-	-	-	**	***	-	-	***
Lb. casei		***	***	***	-	-	-	***	***	***
Lb. helveticus		-	-	-	-	-	-	-	*	-

- not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; PAB propionibacteria.

approximately the same amounts of propionic acid (180 d), but with the addition of MK3008, culture Prop 96 produces ~40% less propionic acid and culture Prop 90 ~20% less propionic acid. This is why culture Prop 90 is generally more prone to provoking late fermentation (Fig. 4).

Concerning the sensory analysis, the MK3008 culture is striking. It is generally judged by the sensorial panel to be responsible for a slightly poorer quality of the cheeses, e.g. in flavour. A possible reason is the elevated amounts of acetate.

3.4. Factor "*Lb. helveticus*"

Lb. helveticus theoretically releases a racemic mixture of DL-lactic acid in a ratio of 1:2. This is why more D-lactate is found in the cheeses with the addition of XMK1168 (Tab. II). At a rough estimate the contributions of the different bacteria to the lactate pool are as follows: *Lb. helveticus* 15 mmol·kg^{-1}, *Str. thermophilus* 50 mmol·kg^{-1} and *Lb. lactis* 60 mmol·kg^{-1}. *Lb. helveticus* obviously did not dominate in the Emmental cheeses.

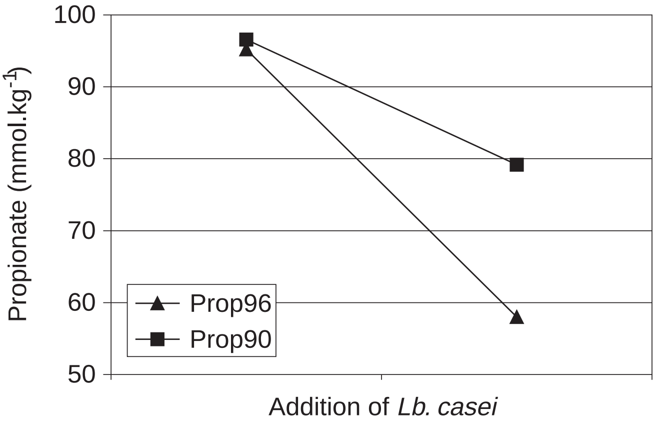

Figure 4. Two way interaction between propionibacteria of different aspartase activity and *Lb. casei* for propionate in Emmental cheese of 180 d (Prop 96: weak aspartase activity; Prop 90: strong aspartase activity).

Table V. Free short chain acids (FSCA in mmol·kg^{-1}) in Emmental cheese grouped by the four factors tested in the model cheese production.

Factor	N	Formiate		Acetate		Propionate		Butyrate		Capronate		FSCA	
		40 d	180 d	40 d	180 d	40 d	180 d	40 d	180 d	40 d	180 d	40 d	180 d
Feeding													
grass	16	2.1	1.7	17.7	41.5	16.7	75.8	0.29	0.87	0.06	0.32	36.9	120.4
hay	16	2.5	2.4	25.7	51.6	30.8	88.7	0.45	1.17	0.09	0.36	59.6	144.4
PAB													
Prop 96	16	2.4	2.3	20.0	43.3	20.9	76.6	0.37	1.03	0.07	0.32	43.7	123.8
Prop 90	16	2.2	1.8	23.4	49.8	26.7	87.9	0.36	1.02	0.07	0.36	52.8	141.0
Lb. casei (MK3008)													
yes	16	4.1	3.5	23.9	47.3	12.1	68.6	0.36	1.05	0.07	0.33	40.6	121.1
no	16	0.5	0.6	19.5	45.8	35.4	95.9	0.37	0.99	0.08	0.35	55.9	143.7
Lb. helveticus (XMK1168)													
yes	16	2.3	2.2	22.0	47.3	25.0	82.0	0.35	1.02	0.08	0.34	49.7	133.0
no	16	2.3	1.9	21.4	45.9	22.6	82.5	0.38	1.02	0.07	0.34	46.8	131.8
ANOVA													
Feeding		*	*	***	***	***	***	***	***	***	**	***	***
PAB		-	*	**	***	*	***	-	-	-	**	*	***
Lb. casei		***	***	***	-	***	***	-	-	-	-	***	***
Lb. helveticus		-	-	-	-	-	-	-	-	-	-	-	-

- not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; PAB propionibacteria.

Table VI. Eye formation in Emmental cheese grouped by the four factors tested in the model cheese production.

Factor	N	Days in the warm room	Number of eyes		Size of eyes (mm)		Height (cm)	Carbon dioxide ($mmol \cdot kg^{-1}$)
			40 d	180 d	40 d	180 d	180 d	40 d
Feeding								
grass	16	59.50	56.4	153.8	11.0	7.3	21.0	18.2
hay	16	63.69	55.9	88.3	10.8	9.4	26.0	24.8
PAB								
Prop 96	16	65.75	59.8	101.7	10.6	8.3	22.2	19.1
Prop 90	16	57.44	52.5	140.4	11.3	8.4	24.8	24.0
Lb. casei (MK3008)								
yes	16	64.69	82.1	101.1	10.7	9.8	22.6	17.8
no	16	58.50	30.1	141.1	11.2	6.9	24.4	25.2
Lb. helveticus (XMK1168)								
yes	16	60.44	67.4	128.6	11.0	8.5	23.3	21.8
no	16	62.75	44.9	113.6	10.9	8.2	23.7	21.3
ANOVA								
Feeding		***	-	***	*	***	***	*
PAB		***	-	*	***	-	-	-
Lb. casei		***	**	*	***	***	-	*
Lb. helveticus		**	-	-	-	-	-	-

- not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; PAB propionibacteria.

Most *Lb. helveticus* strains usually possess a peptidase- and proteinase-activity that is to a great extent higher than that of other Lactobacilli [14]. This is confirmed by high leucin-aminopeptidase activity (results not shown) measured in the cheeses with addition of XMK1168 and in the higher content of smaller peptides (NPN in Tab. III) and amino acids.

Finally the XMK1168 culture is responsible for a slight shortening of the duration of the cheese in the warm room (Tab. VI). One possible explanation might be that enhanced proteolysis is responsible for a higher pH and consequently better growth conditions for PAB. Also, through proteolysis more asparagine and aspartate are released and these are a source for the aspartate metabolism by PAB. Since there is no difference in the concentrations in the two types of cheese (Tab. III), the additional amounts are probably metabolised by PAB. The texture which becomes shorter and crumbly during proteolysis loses its elasticity and can develop cracks because of the excessive CO_2. This is how *Lb. helveticus* may favour late fermentation and represents, therefore, a risk.

Table VII. Sensory analysis after 180 d in Emmental cheese grouped by the four factors tested in the model cheese production.

Factor	N	Texture (1–6)	Flavour (1–6)	Intensity of Aroma (0–7)	Firmness (2–8)	Conser-vation (1–3)	Sourness (0–7)
Feeding							
grass	16	4.95	4.30	4.59	4.48	2.99	0.43
hay	16	5.23	4.82	4.70	4.26	2.68	0.73
PAB							
Prop 96	16	5.08	4.49	4.36	4.36	2.95	0.60
Prop 90	16	5.11	4.63	4.93	4.37	2.72	0.56
Lb. casei (MK3008)							
yes	16	4.88	4.40	4.75	4.48	2.85	0.65
no	16	5.31	4.73	4.55	4.25	2.82	0.51
Lb. helveticus (XMK1168)							
yes	16	4.94	4.60	4.65	4.65	2.89	0.62
no	16	5.24	4.53	4.64	4.09	2.78	0.54
ANOVA							
Feeding		*	***	-	-	**	*
PAB		-	-	***	-	*	-
Lb. casei		**	***	-	-	-	-
Lb. helveticus		*	-	-	***	-	-

- not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; PAB propionibacteria.

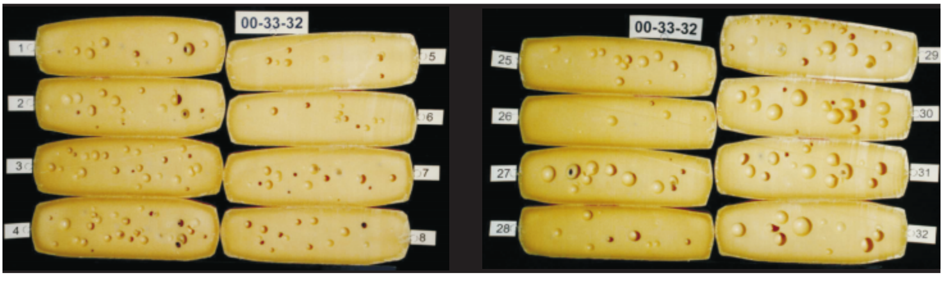

Figure 5 . Emmental cheeses of 6 months of age made with two different propionibacteria (Prop 90 and Prop 96), facultatively heterofermentative lactobacilli (MK3008) and *Lb. helveticus* (XMK1168) during hay as well as grass feeding season (codes for treatment: see Tab. I).

4. DISCUSSION

Much is known on the interactions between propionibacteria, facultatively heterofermentative lactobacilli and *Lb. helveticus*. However, many open questions remain. In this work it was possible to show that aspartate metabolism is coupled with a stronger growth rate of PAB and stronger propionic acid fermentation. Yet it was not possible to answer the question whether the aspartase activity is the cause or just an indicator. FHL obviously inhibited PAB, the culture with low aspartase activity more than the culture with high activity. But an explanation for this difference was not found. Even if *Lb. helveticus* did not dominate in cheese, it was possible to show that it increases the risk of late fermentation due to its proteolytic activity.

Nowadays it is easily possible to control propionic acid fermentation during the ripening of Emmental cheese. Since the introduction of starter lactic acid bacteria in the seventies, of FHL (MK3008) in 1989 and of a PAB culture with weak aspartase activity (Prop 96) in 1996, the defect of late fermentation is practically eliminated in Switzerland. Nevertheless, it is still possible to produce Emmental cheese with eyes made to measure (Fig. 5): large eyes are achievable with the use of *Lb. helveticus* together with a strongly aspartase positive PAB culture. Small eyes are obtained with the use of FHL together with a weakly aspartase positive PAB culture.

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