JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

The Addition of *Propionibacterium freudenreichii* to Raclette Cheese Induces Biochemical Changes and Enhances Flavor Development

ANNE THIERRY,*,† MARIE-BERNADETTE MAILLARD,† PASCAL BONNARME,‡ AND EDMOND ROUSSEL§

UMR 1253 Science et Technologie du Lait et de l'Oeuf, INRA-Agrocampus, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France, UMR 782 Génie et Microbiologie des Procédés Alimentaires, INRA-Ina PG, 78850 Thiverval Grignon, France, and Standa-Industrie, 14050 Caen, France

Two mixtures of *Propionibacterium freudenreichii* commercial strains were tested as adjunct cultures in pasteurized milk Raclette cheese to investigate the ability of propionibacteria (PAB) to enhance flavor development. Cheese flavor was assessed by a trained sensory panel, and levels of free amino acids, free fatty acids, and volatile compounds were determined. The PAB level showed a 1.4 log increase within the ripening period (12 weeks at 11 °C). Eye formation, which was not desired, was not observed in PAB cheeses. PAB fermented lactate to acetate and propionate and produced fatty acids by lipolysis, branched chain volatile compounds derived from isoleucine and leucine catabolism and some esters. One of the experimental cheeses received the highest scores for odor and flavor intensity and was characterized by higher frequencies of detection for some minor notes ("propionic" and "whey" odor, "sweet" taste). PAB can therefore be considered as potential adjunct cultures to enhance or modify cheese flavor development.

KEYWORDS: ***Propionibacterium freudenreichii*; Raclette cheese; adjunct culture; flavor; volatile compound**

INTRODUCTION

Most cheese types develop their characteristic flavor during ripening through the gradual breakdown of carbohydrates, fats, and proteins. Microorganisms and their enzymes play a major role in these biochemical changes. They include starter bacteria, adjunct "starters", and nonstarter microflora. The primary function of starters is to ensure consistent acid formation during cheesemaking. Adjunct starters include lactic and nonlactic microorganisms added to specific cheese varieties to perform various functions such as opening (*Propionibacterium freudenreichii* in Swiss cheese) and rind formation (*Brevibacterium linens* in smear-ripened cheeses). Nonstarter microflora can originate from the milk, especially if raw milk is used, or from the cheesemaking environment and can also contribute to the development of cheese flavor. Cheeses made from raw milk generally have a more intense flavor than pasteurized or microfiltered milk cheeses (*1*), thus stimulating the interest in the development of adjunct cultures for addition to pasteurized milk for cheese flavor enhancement.

Adjunct cultures have been defined as selected strains of cheese-related microorganisms that are added to the cheese milk to improve the development of cheese sensory quality (*2*). Mesophilic species of *Lactobacillus*, which are the predominant species of nonstarter bacteria, are the most popular cheese adjunct cultures. The addition of lactobacilli to cheese milk can have various effects on cheese flavor depending on the species and strains used, as well as on the type of cheese. Hence, lactobacilli adjunct can improve cheese flavor in some cases, although they can also lead to the formation of atypical flavors or indeed have no significant effect (*3*). Besides lactobacilli, other microorganisms have also been proposed as adjunct cultures, such as *Enterococcus*, *Pseudomonas*, smear bacteria, yeasts (*3*), and *P. freudenreichii* (*4*).

P. freudenreichii is commonly used as an adjunct starter in Swiss type cheeses, a variety of cheeses with characteristic round "eyes" (*5*) such as Emmental and Maasdam cheeses, where this species grows during the ripening and constitutes one of the major microflora (*6*). Propionic acid bacteria (PAB) are involved in the formation of the characteristic flavor and opening of this variety of cheeses, via the fermentation of lactate to ethanoate (acetate), propanoate (propionate), and CO_2 (*7*). Because of their ability to produce a high amount of CO_2, PAB can also be involved in undesirable fermentation reactions and defects observed in several varieties of hard and semihard cheeses, such as Comté and Italian cheeses (*8*−*10*). As a result, PAB have rarely been tested for use as adjunct cultures in cheese. However, *P. freudenreichii* NCDO 853 has been successfully used in

* To whom correspondence should be addressed. Tel: 33(0)2 23 48 53 37. Fax: 33(0)2 23 48 53 50. E-mail: anne.thierry@rennes.inra.fr.
† UMR 1253 Science et Technologie du Lait et de l'Oeuf.
‡ UMR 782 Génie et Microbiologie des Procédés Alimentaires.
§ Standa-Industrie.

10.1021/jf0481195 CCC: $30.25
Published on Web 04/23/2005

experimental Cheddar cheese manufacture. Cheeses containing PAB received better flavor and texture scores than the control cheeses (*4*). In ultrafiltered milk cheese, the addition of *P. freudenreichii* cells did not modify the levels of proteolysis but induced an increase in some volatile compounds (*11*). Intracellular crude extracts of PAB were also added to ultrafiltered milk cheese and to Ras cheese. In ultrafiltered milk cheese, the addition of PAB extracts did not significantly modify either the level of proteolysis or the profile of volatile compounds (*11*). In Ras cheese, the addition of PAB extracts increased the degree of proteolysis and the intensity of flavor and bitterness, in comparison to the control cheese (*12*).

The aim of this study was to test the ability of *P. freudenreichii* to enhance the flavor of pasteurized Raclette cheese, a semihard cheese. This cheese was originally exclusively manufactured in the Swiss mountains from raw milk, but it is now largely produced from pasteurized milk in industrial plants (*13*).

MATERIALS AND METHODS

Cheese Manufacture. Raclette cheeses were produced in an industrial plant according to the usual cheesemaking process, using 16000 L vats of pasteurized milk. Direct vat set cultures of homofermentative mesophilic lactic acid bacteria (*Lactococcus lactis* subsp. *lactis* and *cremoris*) were added (50−75 g per 1000 L) during milk storage. Milk was heated to 32 °C and inoculated by *Lactobacillus helveticus* and two mixtures of lyophilized *P. freudenreichii* strains, consisting of commercial strains (PAL/ITG, from Standa Industrie, Caen, France) and TL strain (from the collection of the laboratory Science et Technologie du Lait et de l'Oeuf, INRA, Rennes, France), were used as adjunct cultures in experimental cheeses (coded A and B) at an overall level of 1.6×10^6 cfu (colony-forming unit) mL^{-1} of cheese milk. In cheese A, the adjunct was a mixture of four strains, PAL/ITG P10, PAL/ITG P18, PAL/ITG P20, and TL162, inoculated at 4.1×10^5 cfu mL^{-1} each, while in cheese B, the adjunct was composed of three other strains, PAL/ITG P9, PAL/ITG P19, and TL160, 5.5×10^5 cfu mL^{-1}. All of these strains were chosen because they have been shown to be salt resistant (*14*) (E. Roussel, Standa Industrie, personal communication). As no data were available on the ability of these PAB strains to grow at low temperature under conditions that are far from their optimal growth conditions (11 vs 30 °C), mixtures of strains rather than pure cultures were used to maximize the likelihood of observing the growth of at least one strain of PAB in Raclette cheese. Traditional rennet (520 mg L^{-1} chymozyne) was added (4 L per 16000 L) at 32 °C and at pH 6.55−6.60. A control batch of cheeses was manufactured under the same conditions using the same milk, without added PAB (cheese C). Cheeses were salted by immersion in a NaCl-saturated brine at 12 °C for an average of 24 h, with variation of 3−4 h due to the brining device used (the cheeses entering the salt bath first were the last to be removed). The resultant cheeses were ripened at 11 °C for 12 weeks. One cheese was used for sampling at each ripening time point. Some previously obtained data showed that there was only low variability in cheese gross composition within the cheeses from the same batch (e.g., <0.5% for dry matter and fat), except for NaCl (up to 25% of difference, due to the variations in brining time). The inner part of the cheese was aseptically sampled for microbiological analyses after brining (3 days) and at 6, 8, 10, and 12 weeks of ripening, to follow PAB growth throughout the ripening. Samples were also taken from middle-aged (6 weeks) and ripened (12 weeks) cheeses for biochemical analyses and were frozen at −80 °C until use.

Microbiological Analyses. The outer part of the cheese samples (4 cm under ring) was discarded, and 20 g of cheese was dispersed in 180 g of a 2% sodium citrate solution, homogenized, diluted with peptone saline solution, and plated on specific media. The presence of contaminating nonstarter lactobacilli in experimental cheeses was checked on facultative heterofermentative lactobacilli agar (*15*) incubated at 37 °C anaerobically for 3 days. PAB were enumerated on lithium−glycerol agar (LGA) (*16*) incubated at 30 °C anaerobically for 6 days. The strains that grew in cheese were identified by their DNA fingerprints using pulsed field gel electrophoresis (PFGE). To this end, 40 isolates were picked from the LGA plates obtained from samples of cheeses A and B at 10 weeks of ripening. DNA samples were prepared according to Gautier (*17*), from the isolates grown in yeast extract−lactate medium. DNA samples were digested for 4 h at 37 °C using the restriction enzyme *XbaI* (Eurogentec, Liège, Belgium). Restriction fragments were separated by electrophoresis for 20 h at 200 V and 14 °C in a 1% agarose gel, with a pulse time of 2−20 s, using a Chef DRII system (BioRad, Richmond, United Kingdom). PFGE patterns were visually compared. A size marker was loaded in three lanes per gel to facilitate comparison.

Compositional Analysis. Samples were taken at 6 and 12 weeks of ripening for triplicate determination of (i) moisture, by oven drying at 102 °C for 7 h (*18*); (ii) fat, by butyrometry (*19*); (iii) sodium chloride, by potentiometry (*20*); and (iv) pH, measured on grated cheese with a InLab423 pH combination microelectrode (Mettler Toledo SA, Viroflay, France).

Organic acid analysis and amino acids were determined at 6 and 12 weeks of ripening, as follows: a 20 g sample was homogenized with 40 g of distilled water by mixing for 2 min at 20500 rpm using an Ultraturrax blender (Janke & Kunkel, Staufen, Germany). Homogenates were incubated for 1 h at 40 °C, prior to centrifugation at 3600*g* for 30 min at 4 °C. The supernatant was then filtered on Whatman 40 filters and diluted with the same volume of 0.005 M H_2SO_4, before filtering on 0.45 μm pore diameter membranes (Whatman). Lactic acid, acetic acid, and propionic acid were determined by high-performance liquid chromatography on an Aminex A-6 ion exchange column (Bio-Rad, Hercules, Ca) at 55 °C with 0.005 M H_2SO_4 as the eluent at a flow rate of 1.0 mL min^{-1}. Both UV (210 nm) and refractometric detectors were used. For amino acid analysis, 803 mg of sulfosalicilic acid was added to 10 mL of cheese homogenate supernatant. The mixture was incubated for 1 h at 40 °C and centrifuged at 3600*g* at 4 °C for 15 min. A 400 μL amount of supernatant was diluted with 1600 μL of 0.2 M lithium citrate buffer, pH 2.2, before filtering on 0.45 μm pore diameter membranes. Amino acids were analyzed using an amino acid analyzer (AlphaPlus serie 2, Pharmacia, Uppsala, Sweden). All samples were analyzed in duplicate.

Free fatty acids and neutral volatile compounds were analyzed at 12 weeks of ripening. Free fatty acids (from $C_{4:0}$ to $C_{18:3}$) were extracted using ether/heptane (50:50, v/v) at acidic pH, separated from fat on an aminopropyl column, and analyzed by gas chromatography (GC) according to the method of De Jong et al. (*21*). 2-Methylbutanoic acid and 3-methylbutanoic (isovaleric) acid were coeluted by this method and are referred to as "methylbutanoic acids" in the present study. Neutral volatile compounds were detected by dynamic headspace GC-MS analysis. Before analysis, cheese samples were thawed at 4 °C and cut into cubes (2.5 mm × 2.5 mm × 2.5 mm), which were mixed. A 20 g sample was homogenized with 80 g of refrigerated solution of 0.5 M sodium citrate, by mixing for 2 min at 20500 rpm using an Ultraturrax blender. A 7 g sample of this cheese homogenate (±0.05 g) was used for each headspace GC-MS analysis. All samples were analyzed in duplicate. Volatile compounds were trapped on a Vocarb 3000 trap (Supelco, Bella Fonte, PA), thermally desorbed at 250 °C, and cryofocused at −100 °C before being injected into a HP5890 (Hewlett-Packard) gas chromatograph-HP5972A quadrupole mass spectrometer (GC-MS). Volatiles were separated on a HP5 capillary column (60 m × 0.32 mm × 1.0 μm film thickness) and ionized by electronic impact, as previously described (*22*). Twenty-seven volatiles were identified by comparison of mass spectra and retention times with those of authentic standards purchased from Sigma-Aldrich (St. Quentin Fallavier, France) and from Extrasynthèse (Genay, France). The other volatile compounds, for which standards were not available, were tentatively identified on the basis of mass spectral data from the Hewlett-Packard Chemstation NIST 75K mass spectral Database. Peaks were quantified by the areas of the total ion current (TIC) or of selected fragments (*m*/*z*) in the case of two coeluted compounds. Nineteen neutral volatile compounds were quantified using an external calibration method based on the addition of standards to cheese homogenate, to avoid the approximations related to the commonly used internal standard calibration (*23*). We used the regression curves that we previously obtained using spiked Emmental cheese homogenate (*24*), as Raclette

and Emmental homogenates have the same fat content (~5.8 g kg^{-1}) and a close dry matter content (~12.5 and 11.8 g kg^{-1}, respectively, in Emmental and Raclette cheese homogenates).

Six high purity chemicals (3-methylbutanal, 3-methylbutanol, ethyl propionate, ethyl butanoate, 2-heptanone, and dimethyl disulfide), purchased from Sigma-Aldrich, were used as external standards. Stock solutions of standard compounds were prepared in high purity (99.8%) methanol at concentrations of 4−7 mg g^{-1} and stored at −20 °C. Amounts of each stock solution were used to prepare a standard mix containing 50−1100 μg g^{-1} of each standard compound. An aliquot (~15 mg) of standard mix was accurately weighted and used to spike a 35 g sample of control cheese homogenate, resulting in final concentrations of 100−2200 μg g^{-1} cheese. Seven additional calibration standard solutions were prepared by further dilution (weight to weight) of the spiked cheese homogenate in blank cheese homogenate, to obtain eight different concentrations covering the following ranges: 0.8−100 (dimethyl disulfide), 1.4−180 (3-methylbutanal), 8−1000 (2-heptanone), and 17−2200 (ethyl propionate, ethyl butanoate, and 3-methylbutanol) ng g^{-1} cheese. Six compounds were quantified from the regression curve of the corresponding standard. Thirteen other compounds were quantified from the regression curves of a standard having the same chemical function and a close molecular mass as follows: 2-propanol, 1-propanol, 2-butanol, 2-methylpropanol, 1-butanol, and 2-methylbutanol from 3-methylbutanol; 2-methylbutanal from 3-methylbutanal; *n*-propyl acetate from ethyl propionate; propyl propionate and propyl butanoate from ethyl butanoate; 2-pentanone and cyclopentanone from 2-heptanone; and dimethyl trisulfide from dimethyl disulfide.

Flavor Assessment. Sensory evaluations of ripened cheeses were performed by Les Maisons du Goût (Bourg-en-Bresse, France). A panel of 21 highly experienced trained assessors participated in the sensory analysis. All panelists were selected for their ability to memorize various odor and flavor descriptors, trained over 10 sessions to recognize the presence of these descriptors for many types of cheese, and retrained and controlled at least every 2 weeks. The cheeses were evaluated in duplicate by each assessor, at 1 week intervals (after 12 and 13 weeks of ripening, respectively). Cheeses were coded with randomly selected three digit numbers for presentation, and the order of tasting was balanced. On the day of assessment, the outer layer of cheese (5 mm) was discarded. Samples (~20 mm × 20 mm × 50 mm) were presented in disposable flasks and were allowed to warm to 15 °C for 1.5 h before evaluation (referred to as "cold evaluation"). As Raclette cheese is traditionally consumed in a melted form, samples were also served hot after being heated at 200 °C for 5 min in glass ramequins without a cover and then for 1 additional min with an aluminum foil cover to concentrate odors (referred to as "hot evaluation"). All assessments were conducted in individual booths under conditions complying with French standard for the design of test rooms (AFNOR NF V09.105). Assessors were also provided with water and bread to cleanse their palates between tasting. Cheese odor (nasal perception) and flavor (retronasal perception) intensities were rated using a 10 cm undifferentiated scale anchored at both ends with extremes of the intensities (0 = none and 10 = intense). Four descriptors were used to describe the taste of cheese. Fifty-three descriptors (**Table 1**) were used to describe the odor and the aroma of Raclette cheese in cold evaluation. These descriptors were chosen from lists of descriptors most frequently reported in the published literature about cheese. Pure chemical compounds chosen among those present in cheese (45 varieties) and reminiscent of each descriptor were used in training. Two additional descriptors were selected for use during hot evaluation of cheese: toasted and oily. For each of these descriptors, the assessors indicated whether the corresponding odor, aroma, and taste were perceived or not, allowing a frequency of perception (number of "presence" responses out of the total number of responses, i.e., 21 assessors × 2 replicates = 42), expressed as a percentage, to be calculated. Data were collected and analyzed using FIZZ software (Biosystèmes, Couternon, France).

This method of sensory evaluation was devised by Les Maisons du Goût to show a maximum of odor and aroma notes, whereas classical quantitative sensory evaluation considers only a limited number of odor/aroma descriptors. This method proved useful to compare the impact of different starters or to show the sensory particularities of PDO cheeses (JF Clément, personal communication).

Table 1. List of the Odor and Flavor Descriptors Used by the Assessors to Describe the Odor and the Aroma of Raclette Cheese

family	descriptors[a]		
lactic	acetaldehyde	butter	fresh milk
	blue cheese	fresh cream	whey
	boiled milk		
floral	floral	honey	
fruity	apricot/peach	lemon	pineapple
	hazelnut	pear	walnut
vegetable	cabbage (cooked)	mushroom	straw
	garlic	onion (cooked)	wet box
	grass	potato	wood
	hay	resin	
spicy	clove	nutmeg	pepper
	cumin		
toasted	bread crust	coffee	oily[b]
	burnt	malty	toasted[b]
	caramel		
animal	animal	shed	sweat socks
	meat broth		
others	acetic	moldy	rancid
	alcohol	pharmaceutical	soap
	ammoniac	plastic	sulfurous
	butyric	propionic	yeast
	ground	putrid	

[a] French terms were translated according to Bérodier (*37*). Some descriptors were not described and were tentatively translated as follows: moldy (cave), wet box (buis mouillé), yeast (levure de boulanger), sweat socks (pied), ground (terre), and grass (herbacé). [b] Oily and toasted were used only for "hot evaluation" (see Materials and Methods).

Statistical Analyses. Concentration data of duplicate analyses of each compound were used for statistical analysis. One way analyses of variance (ANOVA) was performed by using the General Linear Model procedure of Statgraphics Plus (Statistical Graphic Corp., Englewood Cliffs, NJ) to determine the effect of the addition of *P. freudenreichii* (mixture A or B) on the concentration data of each compound. The Fisher's least significant difference (LSD) test was used to determine which means were significantly different at a 95% confidence level. Sensory data were analyzed by using FIZZ software. The ability of odor and flavor intensity scores to discriminate between cheeses was investigated using one way ANOVA for both types of evaluation (cold and hot evaluations). The Newman−Keuls test was used to determine which means were significantly different at a 95% confidence level. For each odor/aroma descriptor having a perception frequency over 10% for at least one cheese, the ability of the frequency of this descriptor to discriminate between cheeses was investigated using a χ^2 test.

RESULTS

Cheese Gross Composition. The gross composition of the cheeses did not significantly differ between the three cheeses and was consistent with the expected values. At the end of ripening (12 weeks), cheeses contained 59.4 ± 1.5% total solids, 48.7 ± 1.2% fat in dry matter, and 57.1 ± 1.8% moisture in nonfat cheese. The pH increased from 5.31 ± 0.04 (at the end of brining) to 5.53 ± 0.04 at the end of ripening. The NaCl concentrations slightly differed between cheese samples, with values of 27.0 ± 0.1, 21.5 ± 0.2, and 26.3 ± 0.1 g kg^{-1}, respectively, for cheeses A, B, and C, which corresponded to values of salt-in-moisture of 6.7, 5.3, and 6.5% of salt-in-moisture, respectively. These differences can result from differences of brining times, which ranged from 18 to 27 h between cheese batches.

Growth of Propionibacteria and Nonstarter Bacteria. In both experimental cheeses, PAB were 8.5 × 10^6 cfu per g cheese

Table 2. Growth of Propionibacteria in Raclette Cheeses Inoculated (A and B) or Not (C) with *P. freudenreichii* as Adjunct Culture

ripening time	cheese A	cheese B	control cheese (C)
3 days	8.5×10^6	8.5×10^6	ND[a]
6 weeks	4.9×10^7	5.2×10^7	3.0×10^3
8 weeks	3.0×10^7	5.4×10^7	3.6×10^2
10 weeks	1.2×10^8	1.1×10^8	3.0×10^3
12 weeks	2.0×10^7	1.5×10^8	ND

[a] Not determined.

Table 3. Identification of *P. freudenreichii* Strains Present in Two Experimental Cheeses Inoculated with *P. freudenreichii* as Adjunct Culture at 10 Weeks of Ripening[a]

cheese	strains	no. of isolates (percentage)	counts (cfu g^{-1})
cheese A	TL162	18 (53)	6.4×10^7
	P18	9 (26)	3.2×10^7
	P20	6 (18)	2.1×10^7
	P10	1 (3)	3×10^6
	total	34 (100)	1.2×10^8
cheese B	P19	19 (50)	5.7×10^7
	TL160	15 (39)	4.5×10^7
	P9	4 (11)	1.2×10^7
	total	38 (100)	1.1×10^8

[a] The initial counts of each strain (after brining) were 2×10^6 and 3×10^6, respectively, in cheeses A and B.

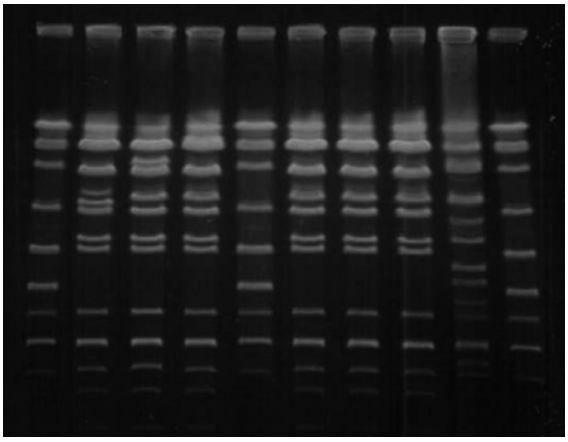

Figure 1. PFGE separation of *Xba*I restriction fragments of genomic DNA from five of the 34 propionibacteria clones isolated at 10 weeks of ripening from Raclette cheese A. This cheese contained a mixture of four strains of *P. freudenreichii*. Lanes 1, 5, and 10, TL size standard; lanes 2–4, 6, and 7, isolates from cheese A, identified as TL162; lane 8, TL162; and lane 9, ITGP10.

at day 3 and grew to 1.1×10^8 cfu g^{-1} at 10 weeks of ripening (**Table 2**). The microbial development somehow differed in cheeses A and B after 10 weeks of ripening. In cheese A, the PAB population showed a 0.8 log decrease from week 10 to week 12, whereas it slightly increased in cheese B, reaching 1.5×10^8 cfu g^{-1} at 12 weeks of ripening (**Table 2**). In the control cheeses, the contaminating PAB remained below 3×10^3 cfu g^{-1} throughout ripening. In all cheeses, nonstarter lactic acid bacteria were not detectable at 6 weeks and ranged from 2×10^6 to 7×10^6 cfu g^{-1} by the end of ripening. The results of the identification of PAB strains are summarized in **Table 3**. An example of PFGE patterns obtained for five clones isolated from cheese A at 10 weeks of ripening is given in **Figure 1**. Three out of the four strains inoculated grew in cheese A, with

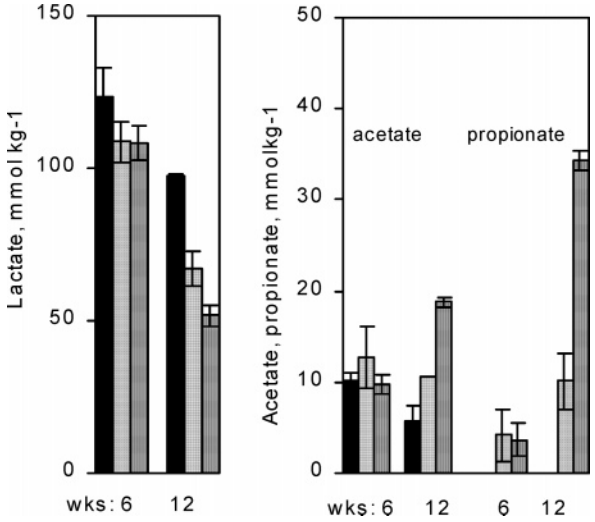

Figure 2. Concentration of lactate, acetate, and propionate at two stages of ripening in Raclette cheeses inoculated (A, thin hatched bars; B, horizontal hatched bars) or not (C, solid bars) with *P. freudenreichii*. Bars and error bars show the means and the standard deviation of duplicate analyses.

the strain TL162 reaching the highest populations. The three strains inoculated grew in cheese B, but strain PAL/ITG P9 achieved lower populations than the two other strains. The differences of behavior of PAB strains could not be explained by the known data about the strains used. For example, PAL/ITG P9 was one of the most salt resistant in mini Swiss type cheese but showed the slowest growth in Raclette cheese B (*14*). Other factors, such as temperature of ripening, could also have induced variations in growth between strains.

Propionic Fermentation. The fermentation of lactate to acetate and propionate occurred in cheeses A and B and was concomitant with PAB growth. **Figure 2** shows the concentrations of these acids in the three cheeses at 6 and 12 weeks of ripening. The concentrations of lactate decreased in all of the cheeses during the ripening but was more marked in both experimental cheeses than in the control cheese. About 4 mmol kg^{-1} propionate was detected after 6 weeks of ripening in both experimental cheeses. At week 12, the concentration of propionate was 10 and 34 mmol kg^{-1} in cheeses A and B, respectively, whereas the propionate level remained undetectable in the control cheese C (**Figure 2**). The production of propionate and acetate in experimental cheeses from week 6 to week 12 was compared to those that could be predicted from Fitz's equation describing PAB metabolism (2 mol of propionate and 1 mol of acetate for 3 mol of lactate). In cheese B, 31 mol of propionate and 9 mol of acetate were produced for 57 mol of lactate consumed (**Figure 2**), whereas the production of acetate and propionate in cheese A was markedly lower than those expected from lactate consumption. It is likely that the observed concentrations result from metabolism by various microflora, including surface microflora. It should also be noted that the values observed for duplicate analyses varied by up to 30% due to coelution phenomena.

Free Amino Acids. The presence of PAB did not significantly affect the total amount of free amino acids in cheeses, which showed average concentrations of 5.2 ± 0.88 g kg^{-1} at 6 weeks and 9.7 ± 0.63 g kg^{-1} at 12 weeks of ripening. As the values for duplicate analyses differed by 2−15%, only differences above 30% between cheeses were considered as significant. Hence, cheese B contained less Asp, Asn, and more γ-amino-butanoic acid (gaba) than the control cheese C (86 and

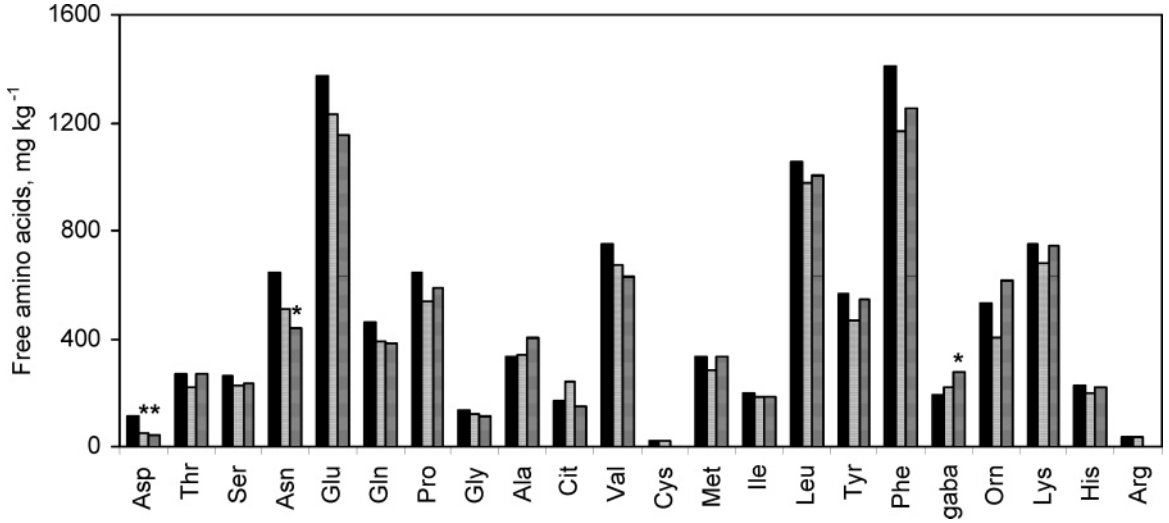

Figure 3. Free amino acids at 12 weeks of ripening in Raclette cheeses inoculated (A, thin hatched bars; B, horizontal hatched bars) or not (C, solid bars) with *P. freudenreichii*. The values for duplicate analyses did not differ by more than 15%. Differences above 30% between experimental and control cheeses are marked with * and are considered as significant.

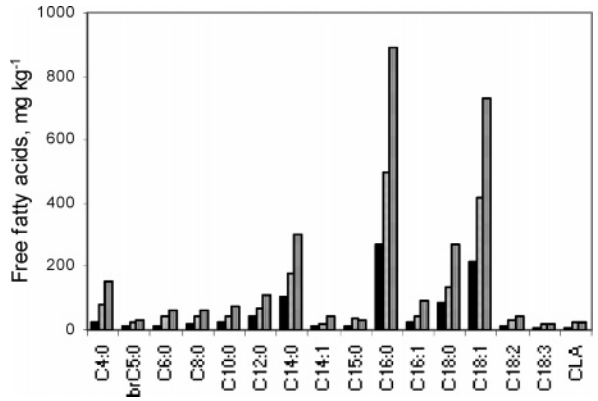

Figure 4. Free fatty acids in Raclette cheeses inoculated (A, thin hatched bars; B, horizontal hatched bars) or not (C, solid bars) with *P. freudenreichii* at 12 weeks of ripening; $brC_{5:0}$, 2-methyl- plus 3-methylbutanoic acids; CLA, conjugated linolenic acid. The values for duplicate analyses did not differ by more than 10%.

39% less and 36% more, respectively), whereas cheese A contained less Asp than cheese C (79% less) (**Figure 3**). Regardless of which cheese, five amino acids (Phe, Leu, Glu, Lys, and Val) accounted for about 50% of the total free amino acids detected. In addition to the amino acids originating from casein, all cheeses contained gaba, deriving from Glu decarboxylation, and citrulline (Cit) and ornithine (Orn), two products of Arg catabolism.

Free Fatty Acids. The concentrations of all free fatty acids derived from lipolysis were 2−5-fold higher in the experimental cheeses than in the control cheese (C) at 12 weeks of ripening. The highest concentration of total free fatty acids was observed in the experimental cheese B (2.93 g kg^{-1} as compared to 1.69 and 0.88 g kg^{-1}, respectively, in cheeses A and C) (**Figure 4**). The levels of the short chain fatty acids showed the highest increase in the presence of PAB, with butanoic acid concentrations of 27, 77, and 155 mg kg^{-1}, respectively, and hexanoic acid concentrations of 10, 40, and 62 mg kg^{-1}, respectively, in cheeses C, A, and B. In addition, the concentrations of conjugated linolenic acid (CLA) were 4.4-fold higher in the experimental cheeses than in the control cheese (22, 22, and 5 mg kg^{-1}, respectively, in cheeses A, B, and C). The CLA peak corresponded, under the chromatographic conditions used, to a mixture of positional and geometric isomers of CLA, including the main isomer (rumenic acid, $C_{18:2}$, 9-cis, 11-trans), which generally makes up to ∼80% of the total CLA in dairy products (*25*). Apart from the fatty acids resulting from fat hydrolysis, methylbutanoic acids (3-methylbutannoic and 2-methylbutannoic acid), which originate from the catabolism of branched chain amino acids leucine and isoleucine, respectively, were also detected. The concentrations of methylbutanoic acids were significantly higher in cheeses A and B than in the control cheese (**Figure 4**).

Volatile Compounds. The main volatile compounds in the three Raclette cheeses and the results of one way ANOVA are reported in **Table 4**. Nine alcohols, three aldehydes, seven esters, 10 ketones, and two sulfur-containing compounds were identified. The concentrations of 16 of these volatiles were significantly affected in the presence of PAB (**Table 4**). Except for ethanol and pentanol, which showed similar concentrations in the three cheeses, alcohol concentrations were significantly increased in cheese B and, to a lesser extent, in cheese A, as compared to the control. The level of 1-propanol was increased 20-fold in cheese A and 93-fold in cheese B, which also contained the highest levels of the corresponding propyl esters. Two branched chain alcohols, 3-methyl-1-butanol and 2-methyl-1-butanol, were also detected at significantly higher concentrations in experimental cheeses than in the control cheese (**Table 4**). Five of the seven esters detected were present at significantly higher concentrations in cheese B: three propyl esters, ethyl butanoate, and ethyl propionate. The increase in propyl ester concentration in cheese B can be related to the increase in concentration of the corresponding alcohol (1-propanol), whereas the increase in ethyl ester can be related to the increase in concentration of the corresponding acids (propionate, butanoate) in this cheese, in comparison to the control cheese. Regarding the other volatile compounds, no obvious clear-cut effect of PAB adjuncts could be identified on the concentration of ketones, although PAB B seemed to slightly increase the formation of methyl-ketones (2-pentanone and 2-heptanone). In contrast, PAB B adjuncts decreased the concentration of the two sulfur-bearing aroma compounds detected in Raclette cheeses, dimethyl disulfide and dimethyl trisulfide (**Table 4**).

Sensory Assessment. The results of intensity of odor and flavor, averaged across assessors and replicates, as well as the results of one way ANOVA are shown in **Table 5**. For both

Table 4. Volatile Compounds of Three Raclette Cheeses Inoculated (A and B) or Not (C) with *P. freudenreichii* as Adjunct Culture Extracted by Head Space GC-MS at 12 Weeks of Ripening

RI[a]	compound[b]	concentration in cheese[c] A[d]	B[d]	C	stat[e]	odor threshold value (ng g^{-1})[f]
482	ethanol$	545.3	429.7	415.8	0.269	
499	2-propanol	11.7 a	28.7 b	12.9 a	**<0.001**	1500000[1]
551	1-propanol	177.2 b	817.5 c	8.8 a	**<0.001**	800000[1]
581	2,3-butanedione$	10.0	6.4	9.9	0.062	
590	2-butanone$	25.7	25.3	21.0	0.313	
597	2-butanol	2.9 a	11.7 b	5.3 a	**0.005**	16000[1]
609	ethyl acetate$	4.7	6.5	4.6	0.256	
622	2-methyl-1-propanol	7.0 b	7.0 b	4.7 a	0.058	200000[1]
647	3-methylbutanal†	2.3	1.1	3.5	0.142	
653	3-methyl-2-butanone$*	0.7	0.6	0.6	0.412	
657	2-methylbutanal	2.0 b	6.2 c	0.3 a	**<0.001**	1250,[1] 40–130,[2] 130[3]
657	1-butanol	5.8 a	25.1 b	10.5 a	**0.039**	200000,[1] 500[2]
680	2-pentanone	27.0 ab	32.9 b	19.3 a	**0.048**	30000,[1] 500[3]
688	2,3-pentanedione$*	1.5	0.9	1.0	0.085	
705	ethyl propionate†	3.2 b	11.3 c	<0.4 a	**<0.001**	4.9–9.9[2]
708	*n*-propyl acetate	1.4 b	2.8 c	<0.4 a	**0.028**	unk
727	3-methyl-1-butanol†	217.3 b	148.0 ab	41.3 a	**0.046**	300–4750,[2] 3200[3]
732	2-methyl-1-butanol	19.3 b	102.9 c	2.9 a	**<0.001**	65000,[1] 5500,[2] 6250[3]
733	4-methyl-2-pentanone$	1.7	1.2	1.3	0.217	
741	dimethyl disulfide†	56.4 ab	17.5 a	101.5 b	**0.032**	120[2]
747	3-methyl-2-pentanone$	1.3	0.9	1.3	0.660	
761	1-pentanol$	0.5	0.2	0.5	0.312	
782	2-hexanone$	0.5 ab	0.6 b	0.3 a	0.092	
796	cyclopentanone*	1.0 b	0.6 a	0.6 a	**0.040**	unk
792	ethyl butanoate†	9.0 a	16.5 b	11.4 a	**0.008**	400,[1] 0.13–450,[2] 16[3]
801	propyl propionate	<0.2	1.5	<0.2	**<0.001**	unk
883	2-heptanone†	11.8 ab	18.0 b	9.3 a	0.071	2000,[1] 140–3000,[2] 700[3]
887	propyl butanoate	<0.2	1.7	<0.2	**0.002**	unk
894	heptanal$	0.3	<0.1	0.3	0.103	
958	3-methylbutyl propionate$	0.9	0.4	0.1	0.123	
973	dimethyl trisulfide*	7.6 c	2.0 a	3.8 b	**0.002**	0.1[3]

[a] RI, retention indices. [b] Volatiles were identified by comparison of mass spectra and retention times with those of authentic standards. Four compounds marked with * were tentatively identified by comparison of mass spectral data with those of the NIST 75K database. [c] Volatiles were quantified by TIC excepted for 2-methyl-butanal and 1-butanol, for which the selected fragments 56 and 57, respectively, were used. Values are means of duplicate analyses and are expressed in ng g^{-1}, except for 12 compounds marked with $, expressed in arbitrary units. Six compounds were quantified by a regression curve obtained using authentic standards (marked with †), and 13 other compounds were quantified using the regression curve of a closely related standard, as described in the Materials and Methods. Values in the same row with the same letter do not significantly differ by LSD test ($\alpha < 0.05$). [d] See **Table 2** for a description of PAB cultures added in cheeses A and B. [e] Results of the analysis of variance: probability of *F*-test. Values below 0.05 are in bold. [f] Flavor threshold, given for compounds showing a significant difference in concentration in the presence of propionibacteria, evaluated in: 1, beer; 2, water; 3, milk; from refs *35* and *38–40*; unk, unknown.

Table 5. Results of ANOVA on Intensity of Odor and Aroma of Three Raclette Cheeses Inoculated (A and B) or Not (C) with *P. freudenreichii*, Evaluated at Room Temperature (at 20 °C) or after Heating (at 200 °C)

samples		means (standard deviations) of odor and flavor intensity scores[a] cheese A	cheese B	cheese C	*p*
not heated	odor	5.55 (1.44) b	5.63 (1.79) b	4.84 (1.74) a	0.016
	flavor	4.93 (1.97) a	5.56 (1.74) b	5.64 (1.37) b	0.025
heated	odor	4.82 (1.84) ab	5.37 (1.84) b	4.43 (1.78) a	0.011
	flavor	5.05 (1.77) b	5.33 (1.84) b	4.57 (1.42) a	0.007

[a] Scores on a 0 (none) to 10 (intense) scale. Values are means of 42 assessments (21 assessors, duplicate evaluations). Values in the same row with the same letter were not significantly different according to the by Newman–Keuls multiple comparison test ($\alpha < 0.05$).

cold and hot evaluations, cheese B received the highest scores for odor and flavor intensity. The scores received by cheese A were more variable and difficult to interpret (**Table 5**).

Forty out of the 55 odor/aroma descriptors used to describe the flavor of Raclette cheeses were cited at a frequency >10%, at least for one type of evaluation (odor/cold, odor/hot, aroma/cold, and aroma/hot). The odor and aroma were perceived as more complex when cheese samples were evaluated cold. Hence, 30 odor and 29 aroma descriptors were found with a frequency of over 10% during cold evaluation vs only 20 and 23, respectively, during hot evaluation (data not shown). Regardless of the evaluation conditions (cold/hot), the descriptors most frequently used to describe the odor and the aroma of cheeses were boiled milk, butter, and, to a lesser extent, bread crust. The aroma and the odors of unheated cheeses were also described with descriptors related to milk (fresh milk, whey), vegetable (hay, straw, grass), floral (apricot/peach), and animal (shed, animal) descriptors. Terms more frequently used to describe heated cheeses were related to toasted (bread crust, oily, toasted) and vegetable (straw, cooked onion, hazelnut). The four taste attributes used (salty, sweet, acidic, and bitter) reached high scores for all cheeses. The results presented in **Tables 6** and **7** contain data for 18 of the 55 odor/aroma descriptors used in this study that achieved frequencies >20% for at least one cheese. Cheeses significantly differed in the frequency of only a few descriptors. Most differences were observed for cold evaluation (**Table 6**). Cheese B was characterized by having a propionic ($p = 0.03$) and whey ($p = 0.05$) odor and a sweet taste ($p = 0.01$) as compared to cheeses A and C. At lower levels of significance, cheese B also differed from the other cheeses by a grass ($p = 0.07$) and hay ($p = 0.15$) odor. Cheese A was characterized by having a more floral odor ($p = 0.005$) and animal odor ($p = 0.20$) and aroma ($p =$

Table 6. Results of Descriptive Analysis of Flavor of Three Raclette Cheeses Inoculated (A and B) or Not (C) with *P. freudenreichii*, Evaluated at Room Temperature (20 °C)[a]

		odor				flavor			
family	descriptors	A	B	C	p^b	A	B	C	p^b
lactic	fresh milk	19	33	26	0.33	17	10	17	0.56
	boiled milk	50	41	55	0.41	38	47	47	0.60
	fresh cream	14	5	17	0.20	14	21	5	0.08
	butter	52	52	48	0.88	60	55	43	0.29
	whey	21	45	29	**0.05**	29	26	19	0.58
	blue cheese	10	0	2	0.07	7	5	5	ND
vegetable	grass	10	29	17	0.07	7	10	19	0.20
	hay	7	21	12	0.15	7	5	14	0.27
	onion (cooked)	14	7	12	0.58	14	17	5	0.20
	straw	7	17	17	0.34	14	19	21	0.70
fruity/floral	apricot/peach	24	14	12	0.30	12	10	0	0.08
	hazelnut	17	14	12	0.82	14	14	19	0.79
	lemon	10	19	10	0.32	19	12	12	0.56
	floral	24	2	7	**0.005**	5	2	2	ND
toasted	bread crust	26	17	12	0.22	21	12	12	0.43
animal	shed	26	21	10	0.13	14	17	14	0.51
	animal	19	7	10	0.20	14	2	7	0.13
others	propionic	0	12	2	**0.03**	10	5	7	0.70
	rancid	7	10	2	0.40	7	12	2	0.24
tastes	salty					45	33	52	0.20
	sweet					36	57	17	**0.01**
	acidic					36	33	48	0.36
	bitter					57	60	71	0.35

[a] Values are perception frequencies, expressed as percentage [number of presence responses divided by the maximal number of responses, i.e., 42 (21 assessors, duplicate evaluations)]. [b] Probability of χ^2 test. Values below 0.05 are in bold.

Table 7. Results of Descriptive Analysis of Flavor of Three Raclette Cheeses Inoculated (A and B) or Not (C) with *P. freudenreichii*, Evaluated after Heating at 200 °C ("Hot Evaluation")[a]

		odor				flavor			
family	descriptors	A	B	C	p^b	A	B	C	p^b
lactic	fresh milk	12	12	7	0.71	5	2	7	ND
	boiled milk	79	55	74	**0.04**	69	69	71	0.96
	fresh cream	21	24	14	0.53	12	14	14	0.93
	butter	76	69	69	0.71	74	69	62	0.50
	whey	7	7	5	ND	19	14	7	0.27
	blue cheese	0	2	2	ND	7	7	7	ND
vegetable	grass	5	5	2	ND	14	14	12	0.93
	hay	14	7	10	0.55	7	12	7	0.68
	onion (cooked)	12	10	14	0.80	26	21	29	0.75
	straw	21	29	21	0.68	14	14	21	0.60
fruity/floral	apricot/peach	0	5	0	ND	7	7	5	ND
	hazelnut	14	21	14	0.60	19	19	24	0.83
	lemon	2	12	2	0.09	24	10	19	0.21
	floral	10	2	0	0.07	2	2	5	ND
toasted	bread crust	38	21	33	0.23	33	43	24	0.18
	oily	71	71	62	0.56	50	52	43	0.67
	toasted	14	17	21	0.69	14	7	29	**0.03**
animal	shed	12	14	10	0.80	21	12	12	0.37
	animal	14	14	10	0.80	21	12	12	0.37
others	propionic	2	5	0	ND	7	2	0	ND
	rancid	5	10	7	0.70	10	7	0	0.14
tastes	salty					64	43	57	0.13
	sweet					33	45	36	0.50
	acidic					24	24	31	0.59
	bitter					41	41	36	0.88

[a] Values are perception frequencies, expressed as percentage [number of presence responses divided by the maximal number of responses, i.e., 42 (21 assessors, duplicate evaluations)]. [b] Probability of χ^2 test. Values below 0.05 are in bold.

0.13) together with a sweeter taste ($p = 0.01$) than the control cheese. At lower levels of significance, both experimental cheeses (A and B) had a more shed odor ($p = 0.13$) and a more apricot/peach aroma ($p = 0.08$) than the control cheese. When cheeses were tested after heating, few significant differences in descriptor frequencies were revealed. Cheese B was characterized by a less marked odor of boiled milk ($p = 0.04$) and a less toasted aroma ($p = 0.03$) than control cheese (**Table 7**). Moreover, both experimental cheeses showed a tendency to have a more rancid aroma ($p = 0.14$).

DISCUSSION

This study shows that *P. freudenreichii* is capable of growth in a semihard cheese under conditions that are much less favorable than those encountered by this species in Swiss cheese. Six of the seven strains used as adjunct, which were chosen for their salt tolerance, effectively grew in Raclette cheese. Their growth rate, however, was rather low. Hence, populations of PAB increased by only ~1 log over 10 weeks of ripening, as compared to a 2−3 log increase within 3−4 weeks of ripening in Emmental cheese. This difference in growth rate could be explained by at least two factors, salt and temperature. The salt-in-moisture content is around 6% in Raclette (as compared to around 1% in Emmental), and the ripening temperature was 11 °C in the present study (as compared to 24 °C in Emmental cheese). The two types of cheeses, however, have comparable pH values. In one of the experimental cheeses (cheese A), a decrease in PAB level was observed at the end of ripening, concomitantly with a reduction in the rate of increase in acetate and propionate concentrations. This observation, which remains to be explained, could be related to the high salt level in cheese A, which contained 25% more than the other experimental cheese (B). The limits of salt concentration tolerated by propionibacteria vary as a function of other growth conditions such as pH. In rich laboratory media (yeast extract lactate medium, for example), 0.79 M NaCl (4.6% salt-in-moisture) was needed to double the generation time of *P. freudenreichii* subsp. *shermanii* CIP103027 (*26*). At cheese pH (5.2−5.5), the critical salt-in-moisture at pH 5.3 for PAB has been evaluated around 3.0% (*14*). The strains used in the present study were chosen from previous results which compared PAB strains for their ability to grow and ferment lactate in mini Swiss cheeses containing different levels of salt-in-moisture (1.0, 1.8, and 3.2%) (*14*). These concentrations are, however, much lower than the levels of salt determined in Raclette cheese (about 6% salt-in-moisture).

Various biochemical changes were observed in experimental cheeses as compared to the control cheese: the fermentation of lactate to propionate and acetate, a marked enhancement of lipolysis, some modifications of the amino acid profile, the conversion of branched chain amino acid to a variety of aroma compounds, and the increase in other volatile compounds such as esters and ketones. As only three cheese vats were used, all made differently, all differences cannot be explained by the addition of PAB, and it would be necessary to reproduce these experiments to be able to generalize these results. However, the changes that were observed in experimental cheeses are in accordance with the results previously observed in Swiss cheeses where PAB are known to ferment lactate and aspartate and to produce propionate and acetate (*7*). PAB were shown more recently to play a key role in Swiss cheese lipolysis (*24*, *27*), in the production of branched chain compounds, in particular those coming from isoleucine catabolism, and in the formation of esters of propionate (*24*, *28*, *29*). Regarding lipolysis in Raclette cheese, PAB seemed to favor the release of short chain fatty acids and CLA, contrary to that observed in Swiss cheese,

where saturated long chain fatty acids were preferentially released by PAB (*24*, *27*). CLA is formed in the mammary gland and occurs naturally in milk fat. However, CLA can also be formed by conversion of linoleic acid ($C_{18:2}$) by various cheese-related bacteria, including some strains of *P. freudenreichii* (*30*−*32*). This ability to convert $C_{18:2}$ to CLA has only, however, been demonstrated in liquid media, and not in cheese, where CLA concentration is comparable to its concentration in milk fat (*25*, *30*). This study showed that the proportions of free CLA were 0.6, 0.8, and 1.3% of free fatty acids, in the control (C), A, and B Raclette cheeses, respectively, which indicates that free CLA was either more easily released from cheese fat or partly derived from $C_{18:2}$ conversion. Branched chain alcohols and adehydes can originate from the conversion of amino acids, namely, leucine and isoleucine, by PAB, through a transamination step (*33*). The resulting α-ketoacid can then be decarboxylated, giving rise to the corresponding acids, methylbutanoic acids, or to the corresponding aldehydes, 3-methylbutanal and 2-methylbutanal (*34*), which were also detected at low concentrations in the cheeses. Because of the reducing conditions within the cheeses and because of their elevated reactivity, aldehydes are readily reduced to the corresponding alcohols (*35*). Numerous straight chain alcohols (in particular 1-propanol) and methylketones were observed to increase in Raclette cheese in the presence of PAB. The production of these compounds by PAB has not previously been reported in Swiss cheese, but it has been observed for PAB grown in the aqueous phase of Swiss cheese (*29*).

The flavor of cheese was modified in both experimental cheeses by inclusion of PAB as adjuncts. Cheese B, in particular, which contained the highest levels of PAB at the end of ripening, received the highest scores for odor and flavor intensity. The difference in PAB counts between cheeses A and B could be responsible for the difference in intensity of the biochemical and sensory changes observed in the two experimental cheeses. However, an additional effect of the PAB strains cannot be definitely excluded as PAB strains; many abilities of PAB are strain-dependent in Swiss type cheeses (i.e., rate of propionate production, degree of lipolysis, formation of branched chain compounds, and influence on cheese flavor). The increase in flavor observed originates from changes in the volatile and nonvolatile compounds induced by the presence of PAB. However, cheese flavor results from a balance of numerous flavor compounds, and it is very difficult to conclude which compound identified in any of the cheeses produces its distinctive characteristic flavor. From the data available in the literature, it may be hypothesized that propionate, which is thought to contribute to the sweet flavor of Swiss cheese, could be at the origin of the sweet taste of both experimental cheeses and of the propionic odor of cheese B (**Table 6**), since the propionate concentration was 64-fold higher than its odor threshold in water (*35*). Short chain fatty acids derived from lipolysis, e.g., butanoic acid (rancid, cheesy), hexanoic acid (blue cheese, pungent), and octanoic ($C_{8:0}$) acid (soap, wax, rancid, fruity, goat), could also directly contribute to cheese flavor, since they were present at concentrations 5- ($C_{6:0}$ and $C_{8:0}$) to 100-fold ($C_{4:0}$) higher than their odor threshold in water (*35*). These short chain fatty acids could explain the more rancid aroma perceived in experimental cheeses (**Table 7**). The fruity notes perceived in the experimental cheeses may derive from several compounds, including esters, ketones, and methylbutanoic acids, all found at higher levels in the presence of PAB. Out of the above compounds, only esters were present in cheese at concentrations higher than their odor threshold (**Table 4**). Esters are common volatile compounds in cheese, where they are responsible for fruity flavors that can be considered as a flavor defect or contribute positively to cheese flavor (*36*). Although high levels of alcohols were found in Raclette cheese in the presence of PAB, the direct contribution of alcohols to cheese flavor is unlikely due to the high odor threshold values of these compounds.

Eye formation was not observed in Raclette cheese containing PAB, which would have been considered as a defect. The formation of gas holes during Swiss cheese ripening opening results from the fermentation of lactate to acetate, propionate, and carbon dioxide by PAB. However, eye formation is a complex phenomenon, determined by the rates of gas production and diffusion and by the mechanism of hole nucleation and growth in a protein matrix with appropriate structural properties (*6*). It is likely that, in the present study, the low ripening temperature used (11 °C) played a key role in limiting the rate of propionic fermentation and the resulting rate of CO_2 production.

In conclusion, this study shows that propionibacteria can be used as adjunct microflora to enhance cheese flavor, even in some cheese varieties where no opening is desired. These results were obtained in real scale cheeses, manufactured under conditions strictly similar to those usually used by industry (milk treatment, starters, and technological parameters of manufacture and ripening). However, technological parameters, such as salt content, temperature, and time of ripening, could be slightly modified to enhance the growth and the metabolic activity of PAB. The strains of PAB used in the present study were selected for their salt resistance, but adjunct PAB should also be selected on their ability to grow at low temperature. The original sensory evaluation performed in the present study had showed the flavor notes that showed the greatest variability for the treatments and that could be used in quantitative flavor evaluation in further studies. The first results obtained in this study open new possibilities for cheese flavor diversification.

ACKNOWLEDGMENT

We thank J. Hannon for critical review and fruitful discussions.

LITERATURE CITED

(1) Grappin, R.; Beuvier, E. Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese: A review. *Bull. IDF* **1997**, *327*, 16−19.

(2) El Soda, M.; Madkor, S. A.; Tong, P. S. Adjunct cultures: Recent developments and potential significance to the cheese industry. *J. Dairy Sci.* **2000**, *83*, 609−619.

(3) Crow, V.; Curry, B.; Christison, M.; Hellier, K.; Holland, R.; Liu, S. Q. Raw milk flora and NSLAB as adjuncts. *Aust. J. Dairy Technol.* **2002**, *57*, 99−105.

(4) Fernandez-Espla, M. D.; Fox, P. F. Effect of adding *Propionibacterium shermanii* NCDO 853 or *Lactobacillus casei* ssp. *casei* IFPL 731 on proteolysis and flavor development of Cheddar cheese. *J. Agric. Food Chem.* **1998**, *46*, 1228−1234.

(5) Steffen, C.; Eberhard, P.; Bosset, J. O.; Rüegg, M. Swiss-type varieties. In *Cheese: Chemistry, Physics and Microbiology*, 2nd ed.; Fox, P. F., Ed.; Elsevier Applied Science Publishers: London, United Kingdom, 1993; pp 83−110.

(6) Noël, Y.; Boyaval, P.; Thierry, A.; Gagnaire, V.; Grappin, R. Eye formation and Swiss-type cheeses. In *Technology of Cheesemaking*; Law, B. A., Ed.; Sheffield Academic Press Ltd.: Sheffield, United Kingdom, 1999; pp 222−250.

(7) Langsrud, T.; Reinbold, G. W. Flavor development and microbiology of Swiss cheese- A review. III. Ripening and flavor production. *J. Milk Food Technol.* **1973**, *36*, 593−609.

(8) Berdagué, J. L.; Grappin, R. Affinage et qualité du gruyère de Comté VII Caractéristiques de présentation: Ouverture et défaut de lainure des fromages. *Lait* **1989**, *69*, 173−181.
(9) Carcano, M.; Lodi, R.; Todesco, R.; Vezzoli, F. Propionic acid bacteria in milk for Grana cheese. *Latte* **1993**, *18*, 914−919.
(10) Thierry, A.; Madec, M. N. Enumeration of propionibacteria in raw milk using a new selective medium. *Lait* **1995**, *75*, 315−323.
(11) Saboya, L. V.; Goudedranche, H.; Maubois, J. L.; Lerayer, A. L. S.; Lortal, S. Impact of broken cells of lactococci or propionibacteria on the ripening of Saint-Paulin UF-cheeses: Extent of proteolysis and GC-MS profiles. *Lait* **2001**, *81*, 699−713.
(12) Ezzat, N. Accelerated ripening of ras cheese with a commercial proteinase and intracellular enzymes from *Lactobacillus delbrueckii* subsp *bulgaricus*, *Propionibacterium freudenreichii* and *Brevibacterium linens*. *Lait* **1990**, *70*, 459−466.
(13) Klantschitsch, T.; Bachmann, H. P.; Puhan, Z. Influence of milk treatment and ripening conditions on quality of Raclette cheese. *Lait* **2000**, *80*, 51−67.
(14) Richoux, R.; Faivre, E.; Kerjean, J. R. Effet de la teneur en NaCl sur la fermentation du lactate par *Propionibacterium freudenreichii* dans des minifromages à pâte cuite. *Lait* **1998**, *78*, 319−331.
(15) Isolini, D.; Grand, M.; Glättli, H. Selektivmedien zum Nachweis von obligat und fakultativ heterofermentativen Laktobazillen. *Schweiz. Milchw. Forschung* **1990**, *19*, 57−59.
(16) Thierry, A.; Madec, M. N.; Richoux, R. Croissance des bactéries propioniques dans le fromage: Comparaison de deux milieux de dénombrement. *Lait* **1994**, *74*, 161−171.
(17) Gautier, M.; de Carvalho, A. F.; Rouault, A. DNA fingerprinting of dairy propionibacteria strains by pulsed-field gel electrophoresis. *Curr. Microbiol.* **1996**, *32*, 17−24.
(18) IDF. *Standard No. 4A, Determination of the Total Solids Content*; International Dairy Federation: Brussels, Belgium, 1982.
(19) IDF. *Standard No. 152A, Determination of Fat Content (General Guidance on the Use of Butyrometric Methods)*; International Dairy Federation: Brussels, Belgium, 1997.
(20) IDF. *Standard No. 88A, Determination of Chloride Content (Potentiometric Titration Method)*; International Dairy Federation: Brussels, Belgium, 1988.
(21) De Jong, C.; Badings, H. T. Determination of free fatty acids in milk and cheese. Procedures for extraction clean up and capillary gas chromatographic analysis. *J. High Resolut. Chromatogr.* **1990**, *13*, 94−98.
(22) Thierry, A.; Maillard, M. B.; Le Quéré, J. L. Dynamic headspace analysis of Emmental aqueous phase as a method to quantify changes in volatile flavour compounds during ripening. *Int. Dairy J.* **1999**, *9*, 453−463.
(23) Drozd, J.; Vodáková, Z.; Koupil, P. Systematic errors with the use of internal standard calibration in gas chromatographic headspace analysis. *J. Chromatogr. A* **1990**, *518*, 1−8.
(24) Thierry, A.; Maillard, M.-B.; Richoux, R.; Kerjean, J. R.; Lortal, S. *Propionibacterium freudenreichii* strains quantitatively affect production of volatile compounds in Swiss cheese. *Lait* **2005**, *85*, 57−74.
(25) Gnädig, S.; Chamba, J. F.; Perreard, E.; Chappaz, S.; Chardigny, J.-M.; Rickert, R.; Steinhart, H.; Sébédio, J.-L. Influence of manufacturing conditions on the conjugated linoleic acid and the isomer composition in ripened French Emmental cheese. *J. Dairy Res.* **2004**, *71*, 367−371.
(26) Boyaval, P.; Deborde, C.; Corre, C.; Blanco, C.; Begué, E. Stress and osmoprotection in propionibacteria. *Lait* **1999**, *79*, 59−69.
(27) Chamba, J. F.; Perreard, E. Contribution of propionibacteria to lipolysis of Emmental cheese. *Lait* **2002**, *82*, 33−44.
(28) Thierry, A.; Maillard, M. B. Production of cheese flavour compounds derived from amino acid catabolism by *Propionibacterium freudenreichii*. *Lait* **2002**, *82*, 17−32.
(29) Thierry, A.; Maillard, M. B.; Hervé, C.; Richoux, R.; Lortal, S. Varied volatile compounds are produced by *Propionibacterium freudenreichii* in Emmental cheese. *Food Chem.* **2004**, *87*, 439−446.
(30) Sieber, R.; Collomb, M.; Aeschlimann, A.; Jelen, P.; Eyer, H. Impact of microbial cultures on conjugated linoleic acid in dairy products. A review. *Int. Dairy J.* **2004**, *14*, 1−15.
(31) Verhulst, A.; Janssen, G.; Parmentier, G.; Eyssen, H. Isomerization of polyunsaturated long chain fatty acids by Propionibacterium. *Syst. Appl. Microbiol.* **1987**, *9*, 12−15.
(32) Jiang, J.; Björck, L.; Fonden, R. Production of conjugated linoleic acid by dairy starter cultures. *J. Appl. Microbiol.* **1998**, *85*, 95−102.
(33) Thierry, A.; Maillard, M. B.; Yvon, M. Conversion of L-leucine to isovaleric acid by *Propionibacterium freudenreichii* TL 34 and ITGP23. *Appl. Environ. Microbiol.* **2002**, *68*, 608−615.
(34) Yvon, M.; Rijnen, L. Cheese flavour formation by amino acid catabolism. *Int. Dairy J.* **2001**, *11*, 185−201.
(35) Molimard, P.; Spinnler, H. E. Review: Compounds involved in the flavor of surface mold-ripened cheeses: Origins and properties. *J. Dairy Sci.* **1996**, *79*, 169−184.
(36) Liu, S. Q.; Holland, R.; Crow, V. L. Esters and their biosynthesis in fermented dairy products: A review. *Int. Dairy J.* **2004**, *14*, 923−945.
(37) Bérodier, F.; Lavanchy, P.; Zannoni, M.; Casals, J.; Herrero, L.; Adamo, C. Guide to the smell, aroma and taste of hard and semi-hard cheeses−AIR 2039. G.E.CO.TE.F.T.: Poligny, France, 1997; p 76.
(38) Tan, Y.; Siebert, K. J. Quantitative structure−activity relationship modeling of alcohol, ester, aldehyde and ketone flavor thresholds in beer from molecular features. *J. Agric. Food Chem.* **2004**, *52*, 3057−3064.
(39) Sheldon, R. M.; Lindsay, R. C.; Libbey, L. M.; Morgan, M. E. Chemical nature of malty flavor and aroma produced by *Streptococcus lactis* var. *maltigenes*. *Appl. Microbiol.* **1971**, *22*, 263−266.
(40) Sablé, S.; Cottenceau, G. Current knowledge of soft cheeses flavor and related compounds. *J. Agric. Food Chem.* **1999**, *47*, 4825−4836.

Received for review November 10, 2004. Revised manuscript received March 21, 2005. Accepted March 27, 2005. This work was supported by a grant from Ministère de l'Education Nationale, de la Recherche et de la Technologie, Paris, France. We are grateful to Patrick Boyaval who helped in finding the funding for this project.

JF0481195