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Propionic acid bacteria as protective cultures in fermented milks and breads

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Abstract — Strains of lactic acid bacteria (LAB) and propionic acid bacteria (PAB) were screened as biopreservatives against food spoilage yeasts, molds and *Bacillus* spp. singly and in combination. A combination of *Lactobacillus rhamnosus* strain LC705 and *Propionibacterium freudenreichii* ssp. *shermanii* strain JS was found to be the most active against yeasts, molds and *Bacillus* spp. The combination was tested for its activity against yeasts and molds in different food applications and the best results were obtained in fermented milks and in bakery products. An initial level of 10^7 cells·g^{-1} fermented milk product and a level of 10^8 cells·g^{-1} sour dough were found to be effective against yeasts and *Bacillus* spp. Technology for the use of the combination in different applications was developed. © Inra/Elsevier, Paris.

propionic acid bacteria / lactic acid bacteria / protective culture / antimicrobial activity

Résumé — Des bactéries propioniques comme agent de conservation dans les laits fermentés et les pains. Des souches de bactéries lactiques (LAB) et de bactéries propioniques (PAB) ont été testées séparément ou en culture mixte, comme agent de conservation pour leur activité antimicrobienne envers des contaminants comme les levures, moisissures et *Bacillus*. La combinaison des souches *Lactobacillus rhamnosus* LC705 et *Propionibacterium freudenreichii* ssp. *Shermanii* JS s'est révélée la plus active à l'encontre des levures, moisissures et *Bacillus*. L'activité de cette combinaison a été testée envers les levures et les moisissures dans différents produits alimentaires et les meilleurs résultats ont été obtenus sur des laits fermentés et des produits de boulangerie, Un niveau initial de 10^7 cellules·g^{-1}de lait fermenté et de 10^8 cellules·g^{-1} de pâte à pain acidifiée étaient efficace contre les levures et *Bacillus*. La technologie d'emploi de cette combinaison bactérienne a été développée pour différentes applications. © Inra/Elsevier, Paris.

bactérie propionique / bactérie lactique / culture protectrice / activité antimicrobienne / conservation

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1. INTRODUCTION

Traditionally, propionic acid bacteria (PAB) are known for their ability to convert lactate to propionate, acetate and CO_2, which is responsible for the formation of eyes in Swiss-type cheese [10]. A potential new role for PAB in food preservation has been recently introduced. 'Microgard ™' is a well known PAB based biopreservative in which inhibitory activity has been associated with diacetyl, propionic, acetic and lactic acid and to a heat-stable 700–Da peptide [5]. 'Microgard ™' inhibits most Gram-negative bacteria and some fungi [3]. Bacteriocins of PAB have been studied and reviewed recently by Barefood and Grinstead [4]. Other uses of PAB in the control of undesirable micro-organisms have been reported by Odame-Darkwah and Marshall [13] who showed that the bread spoilage bacterium, *Bacillus pumilus,* was inhibited by *P. freudenreichii* ssp. *shermanii*. Propionic acid and its salts are accepted as preservatives for industrial use in bread manufacture because of their inhibitory activity against mold and *Bacillus* ssp. [8]. In addition to food applications, PAB and propionic acid have also been used as preservatives in silage [9].

Most of the research and development work in biopreservation has concentrated on lactic acid bacteria (LAB). These produce various antimicrobial compounds such as organic acids, H_2O_2, diacetyl, bacteriocins and bacteriocin-like substances. Studies on bacteriocin-producing LAB were reviewed by De Vuyst and Vandamme [7]. The activity of LAB cultures and their bacteriocin production has been studied in various food systems and some bacteriocins (nisin and pediocin) are already used in food preservation. Unfortunately, Gram-negative bacteria, yeasts and molds are not inhibited by LAB bacteriocins, although yeasts and molds are frequently the cause of hygienic problems in fermented milk products and bread.

The objective of this work was to develop a protective culture which would improve the shelf life of fermented milks and bread, and which would replace chemical additives such as sorbic and acetic acids in different applications. Further aims were to optimize the amount of antagonistic culture needed for the inhibition of undesirable microbes and to develop a technology for using the culture in fermented milks and in wheat bread.

2. MATERIALS AND METHODS

2.1. Bacterial strains and preparation of the protective culture

Lactobacillus rhamnosus LC705 (DSM 7061) and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7067) were from the Valio culture collection. The protective culture, called Bioprofit, was produced by growing both strains together in a medium consisting of 5 % (w/v) whey permeate (Valio Ltd, Helsinki, Finland), 2 % (w/v) casein hydrolysate (Valio Ltd), and 1 % (w/v) yeast extract (LAB M, Bury, UK) in a BIOSTAT (B. Braun, Melsungen, Germany) fermentor connected to a microfiltration system (Millipore, Bedford, USA) for 48 h at 30 °C at pH 5.5. The protective culture was used in applications as a fresh concentrated culture.

Commercial starters for quark (Probat 505) and for yogurt (V2) were obtained from Wiesby GmbH, Niebull, Germany. The DL-culture for quark was composed of undefined multiple species of *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis*, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* ssp. *cremoris* (Probat 505). The yogurt starter consisted of different strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

Rhodotorula rubra RHO and *Pichia quilermondii* PQ were both isolated from spoiled fermented milk products and were from the Valio culture collection. They were grown in Bacto YM-broth (Difco Laboratories, Detroit, USA) at 25 °C for 2 d before use. *Bacillus subtilis* P.2.94 and *Bacillus licheniformis* P.1.94 were isolated from a ropy wheat bread. *Bacillus* strains were sub-cultured twice in BHI-broth (LAB M) for 24 h at 37 °C before use. Spores were obtained by growing these *Bacillus* strains on nutrient agar

plates fortified with 0.003 % (w/v) $MnSO_4 \cdot 4H_2O$ and 0.025 % (w/v) KH_2PO_4, aerobically at 37 °C for 48 h. The spores were collected by centrifugation at 4 500 *g* for 10 min at 4 °C, washed twice with sterile water and then resuspended in sterile water (50 mL) to form a stock solution. The stock solution was heated at 80 °C for 10 min before storage at 4 °C. The stock solution contained 9×10^9 spores·mL^{-1}.

2.2. Manufacture of quark

Quark was manufactured according to the thermo-quarg process [15] using Probat 505 as starter. The protective culture concentrate was mixed with the fresh fermented quark mass, after manufacture at levels of either 0.01 % (v/v) or 0.1 % (v/v). Controls were prepared without and with calcium sorbate which was suspended in water and added to the fresh fermented quark mass at a final concentration of 0.06 % (w/v). The quark masses were then artificially contaminated with *R. rubra* RHO before packing. Yeasts, molds, LAB and PAB were analyzed once per week during storage at 6 °C for 5 weeks and organic acids were analyzed at the beginning and after 3 weeks of storage at 6 °C.

2.3. Manufacture of yogurt

Control yogurt was fermented with a commercial yogurt culture (V2). Test yogurt was prepared as for control yogurt with the addition of the protective culture to give an initial level of > 10^7 cells of protective culture·g^{-1} yogurt. The protective culture was added at the same time as the starter. Yogurt was fermented at 42 °C for about 3 h until the pH reached pH 4.5. Strawberry jam was contaminated either with *R. rubra* RHO or *P. quilermondii* PQ at concentrations of 100–500 cells·g^{-1}. Contaminated jam was added to yogurt at 14 % (v/v). Basic starters, LAB, PAB and yeasts were enumerated once per week during storage at 6 °C for 4 weeks and organic acids were analyzed at the beginning and after 3 weeks of storage at 6 °C.

2.4. Manufacture of wheat bread with sour dough

Bread was made by a natural sour dough process without intentional addition of starter. Test bread was manufactured by mixing equal amounts of wheat flour and water with the protective culture to a homogenous mass, which was then fermented for either 4, 10 or 20 h at 30 °C. This sour dough was added at different levels to the final dough. Control bread was manufactured for each fermentation time in the same way but without the protective culture. The final dough was made of 1 700–2 100 *g* wheat flour, 170 *g* liquid salt (26 % w/v), 170 *g* liquid yeast (67 % w/v), 96 g butter, 500–800 g water, 172 g liquid sugar (77 % w/v) and 400–1 200 g sour dough. An inoculum of 3×10^2 *B. subtilis* and *B. licheniformis* spores g^{-1} dough was added to each dough. The final doughs were baked at 220 °C for 30 min. Breads were stored at 20–22 °C at an RH of < 20 % at 28–30 °C and at an RH of 70 % for 6 d. The bread was analyzed for *Bacillus* spp., organic acids, pH and total titratable acidity (TTA) during storage for 6 d.

2.5. Microbiological analyses

Samples were analysed for lactobacilli on MRS-agar (LAB M, Bury, UK) and for *L. rhamnosus* LC705 on MRS supplemented with 0.005 % (w/v) of vancomycin (MRSV) (Sigma Chemical Co., St Louis, USA), for propionic acid bacteria on sodium lactate agar (YEL) with the addition of 1 % (w/v) of β–glycerophosphate (Merck, Darmstadt, Germany), for yeasts and molds on YCG (LAB M) and for *Bacillus* spp. on Phenol Red Egg Yolk Polymyxin agar (LAB M) by spread-plating. MRS and MRSV plates were incubated anaerobically at 37 °C for 3 d, YEL-plates were incubated anaerobically at 30 °C for 7 d, YCG plates were incubated aerobically at 25 °C for 3 d and *Bacillus cereus* plates aerobically at 37 °C for 20–24 h.

2.6. Physico-chemical analyses

pH and total titratable acidity (TTA) were analysed using a modified standard procedure [1] by suspending 10 *g* bread in 2 mL of acetoin to break the dough structure and 98 mL of water and titrating the sample with 0.1 N NaOH to pH 8.5 with a titrator (Mettler DL 20, GWB, Zurich, Switzerland). Lactic acid concentrations were determined enzymatically (Boehringe Mannheim, Mannheim, Germany). Propionic and acetic acids were determined by gas chromatography (Perkin-Elmer Sigma 3B GC coupled to a AS 300 auto-

sampler) using Chromosorb WAW 80/100 as the stationary phase (3 m × 2 mm, id.) [16]. Ethanol and diacetyl were determined by capillary gas chromatography (Carlo Erba GC 6 000 Vega coupled to a HS 250 autosampler) using a 30 m × 0.32 mm (id.) SPB–1 silica column (4.0 mm film, Supelco) as the stationary phase [17]. Benzoic acid was determined by isocratic reversed phase liquid chromatography using a C-18 column (3.2 × 250 mm, packed with 5 mm Spherisorb ODS) equipped with an UV-detector (Waters, Milford, USA) according to [2].

3. RESULTS

3.1. Inhibition of yeasts in fermented milk products

Cell numbers of *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS in the protective culture concentrate were 2×10^{10} cfu·mL^{-1} and 2×10^{10} cfu·mL^{-1}, respectively. A level of 0.1 % (v/v) of the protective culture equivalent to 2×10^{7} cells·g^{-1} of both strains, inhibited the growth of *R. rubra* RHO whereas 0.01 % (v/v), equivalent to levels of 2×10^{6} cells of both strains g^{-1}, had no effect on the growth of the contaminant (*figure 1*). The initial level of yeast was 2 to 3×10^{2} cells·g^{-1} quark which reached 8×10^{6} cells·g^{-1} in the control and in test quark containing 0.01 % (v/v) of the protective culture after storage at 6 °C for 5 weeks. In test quark containing 0.1 % (v/v) of the protective culture, the yeast counts remained at a level of ~1×10^{2} cells·g^{-1} during storage at 6 °C for 5 weeks. The organisms in the protective culture did not grow during the storage (data not shown). The cell numbers of *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS in the protective culture concentrate used for yogurt studies were 4×10^{10} cfu·mL^{-1} and 3×10^{10} cfu·mL^{-1}, respectively. The growth of *R. rubra* RHO was totally inhibited and the growth of *P. quilermondii* PQ was retarded (*figure 2*) by 0.1 % (v/v) of the protective culture during storage for 4 weeks at 6 °C. This level resulted in initial numbers of 4×10^{7} cells·g^{-1} and 3×10^{7} cells·g^{-1} yogurt of *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS, respectively. The strains of the protective culture did not grow in the yogurt during the manufacture and the cell numbers remained at ~10^{7} cfu g^{-1} yogurt during storage for 4 weeks at 6 °C (data not shown).

3.2. Physico-chemical and sensory properties of fermented milks

No difference in the concentrations of lactic acid was obtained in either the quark or the yogurt, manufactured with or without the protective culture after storage at 6 °C for 3 weeks. In yogurt, the concentration of propionic acid manufactured with the protective culture was 2 mg·100g^{-1} whereas the concentration of propionic acid in the control yogurt was less than the detection limit (< 0.5 mg·100g^{-1}). In quark manufactured with the protective culture, no propionic acid was formed by *P. freudenreichii* ssp. *shermanii* JS as the concentration of propionic acid was less than the detection limit. The concentration of acetic acid was higher in quark and in yogurt to which the protective culture had been added than in the controls. The concentration of diacetyl was higher in both quark and yogurt containing the protective culture than in the controls. A high amount of ethanol was found in control yogurt, which was probably produced by *P. quilermondii* PQ (*table I* and *figure 2*).

3.3. Effective process for wheat bread

The initial levels of *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS were of 1×10^{8} and 3×10^{8} cells·g^{-1} of sourdough, respectively. Their numbers did not increase during the fermentation of the sour dough for 4 or 10 h but after fermentation for 20 h, the numbers of *L. rhamnosus* LC705 increased by one log-unit while those of

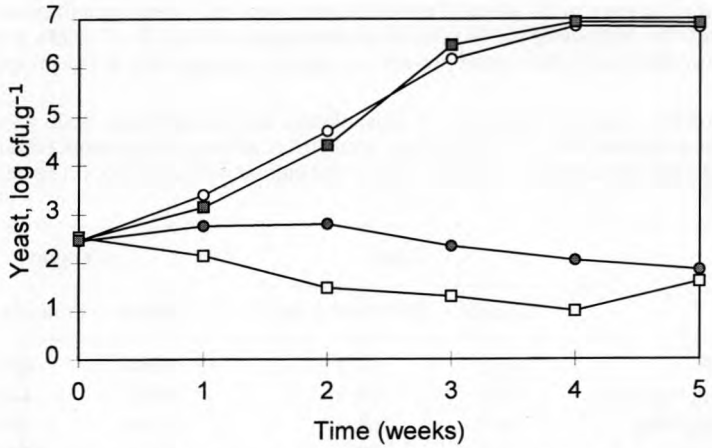

Figure 1. Growth of *Rhodotorula rubra* RHO at 6 °C in quark to which 0.01 % (v/v) protective culture (○), 0.1 % (v/v) protective culture (●), or 0.06 % (w/v) calcium sorbate (□) was added after manufacture, and control without any additive (■).

Figure 1. Croissance de *Rhodotorula rubra* RHO à 6 °C dans du fromage blanc additionné après fabrication de (○) 0,01 % (v/v) de culture protectrice ; (●) 0,1 % (v/v) de culture protectrice ; (□) 0,06 % (w/v) sorbate de calcium ; (■) contrôle sans additif.

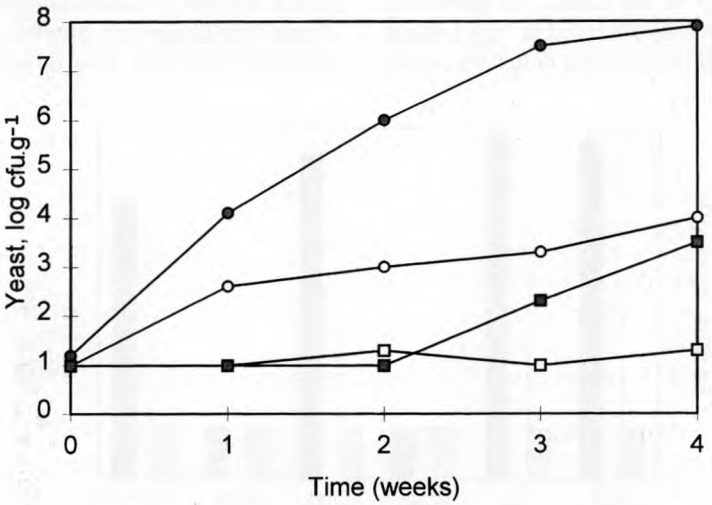

Figure 2. Growth of *Pichia quiliermondii* PQ at 6 °C in yogurt fermented without (●) or with (○) 0.1 % (v/v) of the protective culture and of *Rhodotorula rubra* RHO in yogurt fermented without (■) or with (□) 0.1 % (v/v) of the protective culture.

Figure 2. Croissance de *Pichia quiliermondii* PQ à 6 °C dans un yaourt fermenté sans (●) ou avec addition (○) de 0,1 % (v/v) de culture protectrice et de *Rhodotorula rubra* RHO dans un yaourt fermenté sans (■) ou avec addition (□) de 0,1 (v/v) de culture protectrice.

Table I. Levels of organic acids, diacetyl and ethanol in quark and yogurt manufactured with basic starter and with the addition of 0.1 % protective culture after storage for 3 weeks at 6 °C. (Control = product without protective culture; protective culture = product with 0.1 % protective culture [v/v]).

Tableau I. Acides organiques, diacetyle et éthanol dans du fromage blanc et du yaourt produits avec le levain additionné de 0,1 % de culture protectrice, après stockage trois semaines à 6 °C. (Contrôle = produit sans culture protectrice ; protective culture = produit avec 0,1 % de culture protectrice [v/v]).

	Quark		Yogurt	
	Control	Protective culture	Control	Protective culture
Lactic acid, %	0.72	0.73	0.94	0.94
Propionic acid, $mg \cdot 100g^{-1}$	< 0.5	< 0.5	< 0.5	2.0
Acetic acid, $mg \cdot 100g^{-1}$	38.0	64.0	5.0	10.0
Diacetyl, $mg \cdot kg^{-1}$	0.6	49.0	< 0.5	24.0
Ethanol, $mg \cdot kg^{-1}$	10.0	15.0	220.0	7.0
Benzoic acid, $mg \cdot kg^{-1}$	nt	nt	< 10	< 10

nt: not tested / nt : non déterminé.

P. freudenreichii ssp. *shermanii* JS did not (data not shown). The duration of the fermentation of sour dough with the protective culture had an effect on the inhibition of *Bacillus* spp. in the bread. No growth of *Bacillus* spp. was observed in bread manufactured with 10 % of sour dough fermented for 10 or 20 h with the protective culture, whereas *Bacillus* spp. started to grow after 2 d of storage at 20–22 °C in bread manufactured with 10 % of sour dough fermented for 4 h with the protective culture and in the control bread (*figure 3*). Similar results were obtained with 20 % sour dough. The vol-

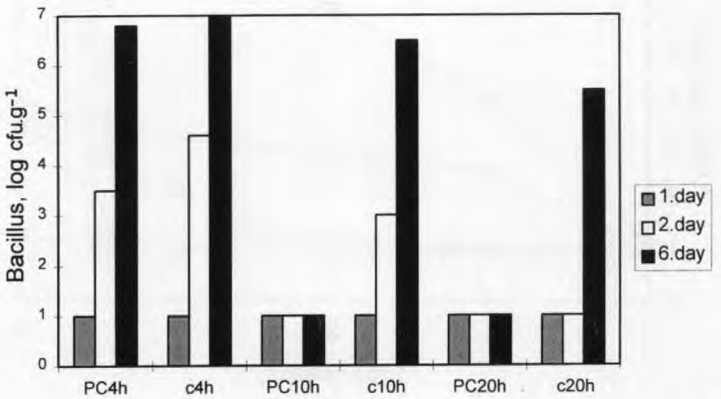

Figure 3. Growth of *Bacillus* spp. at 20–22 °C for 6 d in wheat bread made with 10 % of sour dough which was fermented with the protective culture for 4, 10 or 20 h. Control bread was made without the protective culture (PC= protective culture; c= control).

Figure 3. Dénombrement de *Bacillus* à 20–22 °C pendant 6 j dans du pain réalisé avec 10 % de pâte acide fermentée par la culture protectrice pendant 4, 10 ou 20 h. Le pain de contrôle était réalisé sans culture protectrice (PC = culture protectrice ; c = contrôle).

ume of sour dough added to the dough had an effect on the *Bacillus* spp. counts especially in warm and moist conditions. Bread with 10 to 20 % of sour dough fermented with the protective culture for 10 h inhibited the growth of *Bacillus* spp. totally, whereas in the control bread, the number of *Bacillus* reached a level of 10^4 g^{-1} after 6 d of storage at 20–22 °C at an RH of < 20 % (*figure 4a*). In warm and moist conditions (28–30 °C; RH of 70 %) *Bacillus* spp. were inhibited for at least 6 d in bread made with 15 to 20 % of sour dough fermented for 10 h with the protective culture, whereas in the control bread, *Bacillus* spp. reached a level of 10^4 cells·g^{-1} after 2 d. Bread made with 10 % of sour dough showed high counts of *Bacillus* spp. after 3 d storage (*figure 4b*).

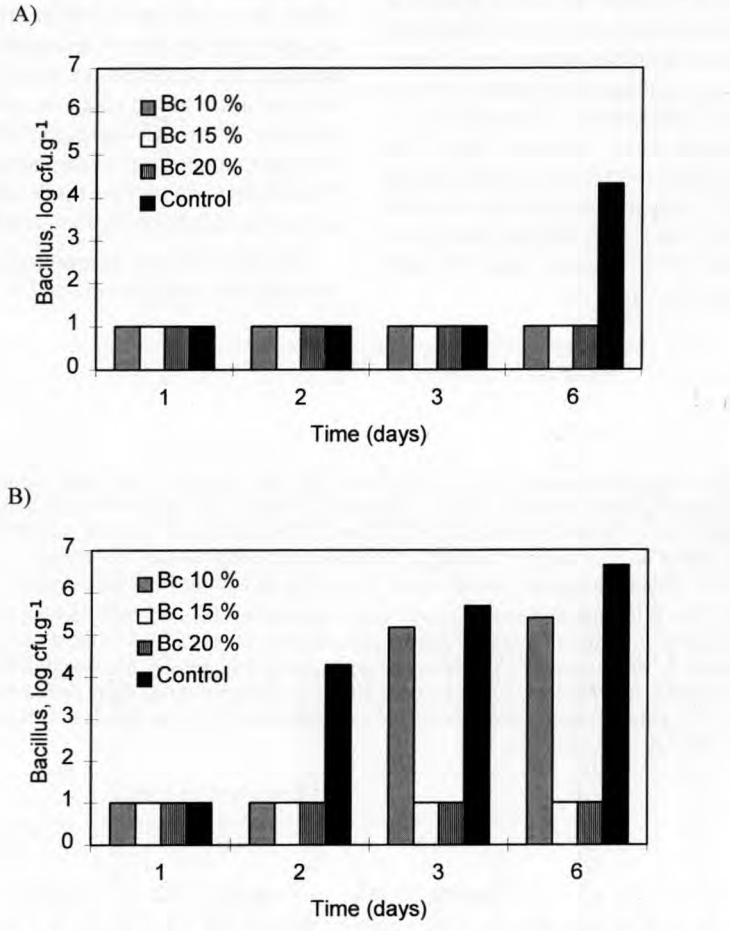

Figure 4. Growth of *Bacillus* spp. in wheat bread during (**A**) storage for 6 d at 20–22 °C at an RH of < 20 % and (**B**) at 28–30 °C at an RH of 70 % for 6 d. Breads were made by adding 10, 15 or 20 % of sour dough fermented with or without the protective culture for 10 h. Control bread was made without the protective culture (PC = protective culture).

Figure 4. Dénombrement de *Bacillus* dans du pain conservé : (**A**) 6 j à 20–22 °C à une humidité relative < 20 % ; (**B**) 6 j à 28–30 °C à une humidité relative de 70 %. Les pains étaient réalisés avec addition à la pâte de 10, 15 ou 20 % de pâte acide fermentée avec ou sans la culture protectrice pendant 10 h. Le pain de contrôle était réalisé sans culture protectrice (PC = culture protectrice).

3.4. Physico-chemical quality of bread

The duration of the fermentation time of the sour dough had an effect on the pH and on the lactic and propionic acid but not the acetic acid concentrations of bread manufactured with the protective culture (*table II*). It was observed that the longer the fermentation time, the higher the concentration of lactic acid and the lower the pH of the bread manufactured with the protective culture. The level of propionic acid did not increase beyond 10 h fermentation. No differences in the concentrations of acetic acid were obtained in either test bread or control bread, except for the lower concentration of acetic acid (15 mg·100g^{-1}) in the test bread fermented for 20 h than in other breads (18–19 mg·100g^{-1}).

4. DISCUSSION

Sorbic, benzoic and acetic acids are used widely in the food industry as preservatives because of their antimicrobial activity against various bacteria, yeasts and molds [6]. Our studies focused on replacing these additives with an antagonistic culture containing *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS. The combined effect of strains of the protective culture against spoilage yeasts and molds [12] and *Bacillus* ssp. was stronger than the effect of either culture alone (data not shown) presumably due to a synergistic effect of the different metabolites of this mixed culture. The mechanism and the mode of inhibitory action has not been characterized.

The shelf life of fermented milks was prolonged by initial levels of 2×10^7 cells of

Table II. The effect of duration of fermentation of the sour dough on pH, total titratable acidity (TTA) and concentrations of organic acids in wheat bread. Bread was made with sour dough fermented with an initial level of both *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS $1–3 \times 10^8$ cells·g^{-1} dough for 4, 10 or 20 h by adding 20 % of the sour dough to the final dough. Control bread was fermented without protective culture for 4, 10 or 20 h by adding 10 % sour dough to the dough.

Tableau II. Effet de la durée de fermentation de la pâte à pain (levain) sur le pH, l'acidité titrable (TTA) et la concentration en acides organiques dans le pain. Le pain était réalisé à l'aide d'une pâte avec un niveau initial de *L. rhamnosus* LC705, ainsi que de *P. freudenreichii* ssp. *Shermanii* JS de $1–3 \times 10^8$ cellules·g^{-1} de pâte, fermentée pendant 4, 10 ou 20 h avec addition de 20 % de cette pâte acidifiée à la pâte finale. Le pain de contrôle était fermenté sans culture protectrice pendant 4, 10 ou 20 h avec addition de 10 % de pâte acidifiée.

	Fermentation time					
	4 h		10 h		20 h	
	Control	PC	Control	PC	Control	PC
Lactic acid, %	< 0.04	0.04	< 0.04	0.17	< 0.04	0.23
Propionic acid, mg·100g^{-1}	1.0	4.0	1.0	5.0	1.0	5.0
Acetic acid, mg·100g^{-1}	19.0	19.0	18.0	19.0	19.0	15.0
pH	5.5	5.5	5.5	4.8	5.5	4.6
TTA, mL	3.0	3.2	3.0	4.5	2.8	4.7

PC = protective culture / PC = culture protectrice.

both *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS·g^{-1} product. The cell numbers of *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* did not increase during the storage of fermented milks at 6 °C for 4 weeks, but the protective strains continued to metabolize as the concentrations of diacetyl and acetic acid in quark and the concentrations of diacetyl, propionic and acetic acids in yogurt increased during storage. In a production scale test of quark, the protective culture at a level of 2×10^7 cells·g^{-1} inhibited the growth of molds (data not shown). The sensory quality of this product was superior to the control product due to the production of diacetyl from citrate by the protective culture. The protective culture did not interfere with the basic starters in yogurt as the cell counts of *S. thermophilus* and *L. bulgaricus* were similar in both the control yogurt and in the yogurt manufactured with the protective culture.

Inhibition of yeasts and molds by the protective culture cannot be based on pH and acids alone. Propionic acid and its salts are primarily inhibitory to molds and *Bacillus* spp. at concentrations of 0.1–5 % [8]. In our studies, using the protective culture, the concentration of propionic acid reached 0.002 % (yogurt), < 0.005 % (quark) and 0.005 % (wheat bread). Acetic acid is effective against yeasts and bacteria at concentrations of 0.4–0.8 % (w/v) [8], while, the concentrations in the present study were 0.06 % (w/v) in quark and < 0.02 % (w/v) in bread. Concentrations of diacetyl in fermented milks made with the protective culture were below the inhibitory concentration of 300 mg·kg^{-1} [5]. Concentrations of lactic acid were 0.78–0.94 % in fermented milks prepared with or without the protective culture. Some reports [5, 11] suggest that the formation of benzoic acid by lactic acid bacteria may be partially responsible for inhibition of yeasts but according to our results, the level of benzoic acid in yogurt did not increase with the protective culture.

The optimal sour dough process was developed with the protective culture of LC705 and JS. The initial level of $1–3 \times 10^8$ of both *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *shermanii* JS g^{-1} sour dough with a fermentation time of 10 h and with the addition of over 10 % to the dough improved the shelf life of the wheat bread. Inhibition of *Bacillus* spp. in wheat bread may be partially explained by lower pH and higher amounts of lactic acid in test bread compared to control bread. In addition, the sensory quality of the test bread was optimal. The pH of the wheat bread made with sour dough is optimal around pH 5 while, below pH 4.2, the product is considered too acidic [14]. The growth of *Bacillus* species causing ropiness in wheat bread was found to be dependent on temperature and moisture of the environment and was faster at an RH of 70 % at 28–30 °C than at an RH of < 20 % at 20–22 °C.

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REFERENCES

[1] Anon., Säuregradbestimmung von Brot, in: Standard-Methoden für Getreide, Mehl und Broth, 6th ed., Arbeitsgemeinshaft Getreideforschung, Verlag Moritz Schäfer, Detmold, 1978, pp. 106–107.

[2] Anon., Benzoic acid, sorbic acid and p–hydrokxybenzoic acid esters, Liquid chromatographic determination in foods, NMKL (Nordic Commitee on Food Analysis) method No 124, 2nd ed., 1997.

[3] Al-Zoreky N., Ayres J.W., Sandine W.E., Antimicrobial activity of Microgard™, J. Dairy Sci. 74 (1991) 758–763.

[4] Barefood S., Grinstead D.A., Bacteriocins from dairy propionibacteria and inducible bacteriocins of lactic acid bacteria, in: Hoover D.G., Steenson L.R. (Eds.), Bacteriocins of Lactic Acid Bacteria, Academic Press Inc., San Diego, 1993, pp. 219–231.

[5] Daeschel M.A., Antimicrobial substances from lactic acid bacteria for use as food preservatives, Food Technol. 43 (1989) 164–167.

[6] Davidson P.M., Branen A.L., Antimicrobials in Foods, Marcel Dekker Inc., New York, 1993.

[7] De Vuyst L., Vandamme E.J., Bacteriocins of Lactic Acid Bacteria. Microbiology, Genetics and Applications, Blackie Academic & Professional, Glasgow, 1994.

[8] Doores S., Organic Acids, in: Davidson P.M., Branen A.L. (Eds.), Antimicrobials in Foods, 2nd ed., Marcel Dekker Inc., New York, 1993, pp. 95–136.

[9] Florez-Galarza R.A., Glantz B.J., Bern C.J., Van Fossen L.D., Prevention of high moisture corn by microbiological fermentation, J. Food Prot. 48 (1985) 407–411.

[10] Hettinga D.H., Reinbold G.W., The propionic acid bacteria – a review II. Metabolism, J. Milk Food Technol. 35 (1972) 358–372.

[11] Holzapfel W.H., Geisen R., Schillinger U., Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes, Int. J. Food Microbiol. 24 (1995) 343–362.

[12] Mäyrä-Mäkinen A., Suomalainen T., *Lactobacillus casei* spp. *rhamnosus*, Bacterial Preparation Comprising Said Strain, and Use of Said Strain and Preparations for the Controlling of Yeast and Moulds, United States Patent US 5 378 458, 1995.

[13] Odame-Darkwah J.K., Marshall D.L., Interactive behavior of *Saccharomyces cerevisiae*, *Bacillus pumilus*, and *Propionibacterium freudenreichii* ssp. *shermanii*, Int. J. Food Microbiol. 19 (1993) 259–269.

[14] Pagenstedt B., Mechanisierung von Weizenmehl-Vorteig-Stufen, Getreide, Mehl u. Brot 37 (1983) 147–153.

[15] Spreer E., Cheese Manufacture, in: Spreer E. (Ed.), Milk and Dairy Product Technology, Marcel Dekker Inc., New York, 1998, pp. 243–334.

[16] Svensen A., Gassfraktometrisk metode til kvalitativ og kvantitativ bestemmelse av flyktige monokarbonsyrer i ost, Melding nr. 97 fra Meieriinstituttet, Norges Landbrukhogskole, 1961.

[17] Ulberth F., Headspace gas chromatographic estimation of some yogurt volatiles, J. Assoc. Off. Anal. Chem. 74 (1991) 630.