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Phage-induced lysis of *Lactococcus lactis* during Saint-Paulin cheese ripening and its impact on proteolysis

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Summary — The autolysis of starter bacteria during cheese ripening results in the release of intracellular enzymes, especially peptidases, into the curd and in consequence is expected to accelerate and enhance the development of cheese flavour. In the course of a series of cheese assays with the non-autolytic *Lactococcus lactis* NCDO763 strain, the presence of a homologous bacteriophage was encountered. The phage was present at different levels which allowed us to evaluate the impact of the extent of starter bacteria lysis on Saint-Paulin-type cheese proteolysis during ripening. We estimated bacterial lysis by viable cell enumeration and electron microscopy observations over a 2-month ripening period. On the same cheeses, we have estimated proteolysis by measuring soluble nitrogen (SN) and phosphotungstic acid-soluble nitrogen (PTA-N) and by quantifying free amino acids. For phage levels up to 10^{10} pfu/g of cheese, the pH and dry matter values were in the standard range, whereas for a higher phage level incorrect acidification was observed. The extent of starter lysis increased with the phage level and it was also correlated with the rate of formation of small peptides and free amino acids. After 2 months of ripening, the PTA-N value was three-fold higher and the total free amino acid content was seven-fold higher in the cheese with a phage level of 10^{10} pfu/g than in the control cheeses. These results confirm that the early lysis of starter bacteria allows the release of intracellular peptidases which are active in cheese, and that the extent of starter lysis can be correlated with the proteolysis rate.

***Lactococcus lactis* / cheese ripening / lysis / phage / proteolysis**

Résumé — Lyse de *Lactococcus lactis* induite par des phages au cours de l'affinage de fromages de type Saint-Paulin et impact sur la protéolyse. L'autolyse des bactéries lactiques du levain résulte en la libération des enzymes intracellulaires, en particulier des peptidases, dans le

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caillé et en conséquence est susceptible de conduire à une accélération et une intensification du développement de l'arôme du fromage. Au cours d'une série de fabrications fromagères avec la souche non autolytique NCDO763, la présence d'un phage homologue a été rencontrée. Le phage était présent à des niveaux différents, ce qui nous a permis d'évaluer l'impact du degré de lyse des bactéries sur la protéolyse des fromages de type Saint-Paulin au cours de l'affinage. Les phages ont été titrés dans le fromage à 48 h et leur niveau variait entre 10^3 ufp/g et 10^{11} ufp/g de fromage. Nous avons estimé la lyse bactérienne par dénombrement des bactéries viables et par observation en microscopie électronique au cours des 2 mois d'affinage. Sur les mêmes fromages, nous avons estimé la protéolyse en mesurant la quantité d'azote soluble (NS) et d'azote soluble dans l'acide phosphotungstique (NSAPT) et par quantification des acides aminés libres. Pour des niveaux de phages allant jusqu'à 10^{10} ufp/g de fromage, les valeurs de pH et extrait sec étaient dans une gamme normale, alors que pour un niveau plus élevé de phages, l'acidification du lait était incorrecte. Le degré de lyse des bactéries augmentait avec le niveau de phages et était également corrélé avec la vitesse de formation des petits peptides et acides aminés libres. Après 2 mois d'affinage, la valeur de NSAPT était trois fois supérieure et la quantité d'acides aminés libres sept fois supérieure dans les fromages, avec un haut niveau de phages (10^{10} ufp/g) que dans les fromages témoins sans phages. Ces résultats confirment que la lyse précoce des bactéries permet le relargage de peptidases intracellulaires qui sont actives dans le fromage et le degré de lyse peut être corrélé avec la vitesse de protéolyse.

***Lactococcus lactis* / affinage / lyse / bactériophage / protéolyse**

INTRODUCTION

During cheese ripening, the starter bacteria enzymes play an essential role in the development of cheese flavour and texture (Olson, 1990). The best characterized of these enzymes are peptidases which take part in the hydrolysis of caseins by producing small peptides and free amino acids which are aroma precursors (Engels and Visser, 1996). They also decrease bitterness by degrading small hydrophobic peptides responsible for the bitter taste (Visser, 1977, 1993). In *Lactococcus lactis*, all the peptidases have been found to be located in the bacterial cytoplasm (Monnet et al, 1993; Kok and Venema, 1995). As a consequence, the autolysis of starter bacteria is considered to be a necessary step to release the intracytoplasmic peptidases into the curd and in addition it could be a limiting step in their action. In the same way, the efficient release of other intracellular enzymes potentially involved in the development of the organoleptic properties of cheese such as esterases, lipases or amino acid degradation enzymes should intensify their action. Thus, controlling or triggering the lysis of starter bacteria in the course of cheese ripening appears as an attractive way to accelerate and/or enhance the development of cheese flavour. In order to attain this, it is necessary both to study the autolytic system of starter lactic acid bacteria and to evaluate the influence of bacterial lysis on cheese ripening.

The ability of lactic acid bacteria to autolyse has been studied in vitro in buffer solutions and appears to be strain-dependent (Vegarud et al, 1983; Langsrud et al, 1987; Lortal et al, 1989; Niskasaari, 1989; El Soda et al, 1995). Recent studies have focussed on the molecular characterization of the endogenous bacterial enzymes responsible for cell autolysis named autolysins (Schockman and Höltje, 1994). These enzymes are peptidoglycan hydrolases, the characterization of which has begun in lactococci and lactobacilli (Buist et al, 1995; Østlie et al, 1995; Valence and Lortal, 1995; Chapot-Chartier, 1996). In the future, better knowledge of the autolytic system should help in selecting autolytic strains or constructing by genetic engineering strains with an inducible autolytic system.

The influence of starter bacteria autolysis on cheese ripening has already been studied (Bie and Sjörstrom, 1975 a,b; Crow et al, 1995a); more recently, a few studies have evaluated more precisely the natural ability of starter bacteria to autolyse in cheese and the impact of bacterial lysis on ripening in the case of lactococci in hard and semi-hard cheese such as Cheddar (Wilkinson et al, 1994) or Saint-Paulin (Chapot-Chartier et al, 1994). It was found that the ability of *L lactis* to autolyse is strain-dependent and that the early lysis of bacteria leads to a higher rate of proteolysis during ripening. In addition, sensory analysis data indicated a tendency to a concomitant decrease in bitterness. Another approach has also been used to demonstrate the impact of starter bacteria lysis on cheese ripening which overcomes the problem of the variability in enzymatic content when different strains are compared. Bacterial lysis was induced by addition of a homologous bacteriophage during cheese manufacture at a level which allows the starter bacteria to grow and acidify milk, but which further induces its lysis at the beginning of ripening. It was initially proposed by Lowrie et al (1974) who showed that the presence of bacteriophages in Cheddar cheese made with a bitter strain limits the development of bitterness and improves the flavour. Recently, Crow et al (1995b) have studied more precisely the influence of phage-assisted lysis of a bitter *L lactis* strain on proteolysis and organoleptic properties of Cheddar cheese. They observed that when the level of phages increased, the degree of starter lysis increased and was associated with an augmented rate of formation of amino acids and ammonia and a decrease in bitterness.

During a study involving Saint-Paulin cheesemaking experiments with *L lactis* subsp *lactis* NCDO763 and a derivative of this strain (TIL61) overexpressing the general aminopeptidase PepC (Neviani et al, 1990; Chapot-Chartier et al, 1993), we encountered a fortuitous contamination by a homologous bacteriophage. In most cases, the phage level was low enough to allow manufacture of cheese with pH and moisture values in the standard range. In this paper, we report the results obtained with different phage levels on the extent of lysis of lactococci and the rate of proteolysis in Saint-Paulin cheese. We estimated the starter bacteria lysis by viable bacteria enumeration and electron microscopy observations. Using the same cheeses, we measured proteolysis by nitrogen fraction analysis and free amino acid quantification. Since we have previously shown that the NCDO763 strain was non-autolytic in Saint-Paulin-type cheese (Chapot-Chartier et al, 1994), we can interpret the effects observed on cheese proteolysis in the presence of different levels of phages as the result of different degrees of lysis of the starter strain.

MATERIALS AND METHODS

Bacterial strains

Lactococcus lactis subsp *lactis* NCDO763 (CNRZ 1260) (also named ML3) (Davies et al, 1981) was obtained from the CNRZ culture collection (INRA, Jouy-en-Josas, France). The TIL61 strain is a derivative of NCDO763 overexpressing the PepC aminopeptidase (Neviani et al, 1990). The *pep*C gene and its own promoter (Chapot-Chartier et al, 1993) were cloned in the multicopy plasmid pIL253 (Simon and Chopin, 1988) and the resulting plasmid was electroporated in NCDO763. During the work, the strains were stored on autoclaved reconstituted skim milk at –20 °C.

Cheese manufacture

Cheese was manufactured in an isolator (La Calhène, France) fitted with HEPA filters and sanitized by peracetic acid vapour before each run. All the equipment in contact with pasteurized milk and curd was autoclaved at 120 °C for 20 min. Three small Saint-Paulin cheeses (250 g) were manufactured in a small cheese vat from 6 L of milk according to the previously described procedure (Alkhalaf et al, 1988; Chapot-Chartier

et al, 1994). Milk was pasteurized at 72 °C for 15 s and inoculated with a culture of NCDO763 or its derivative TIL61 (2%) grown overnight in autoclaved reconstituted skim milk. Renneting was immediately carried out at 33 °C using 0.03% rennet (520 mg/L chymosin, SBI, France) and milk clotting was obtained after about 20 min. After 20 min of setting, the curd was cut and then stirred for 20 min. Lactose was partially removed from the curd by substituting 30% of the whey with water at 32 °C. The curd was stirred again for 20 min before moulding and pressing overnight in a room at 24 °C. One day after renneting, the cheeses were salted in freshly prepared saturated brine for 4 h at 13 °C, dried and waxed 2 days after renneting. They were stored for 60 days at 12 °C and 95% relative humidity.

Despite the care taken to work in aseptic conditions, the presence of an NCDO763 homologous phage was encountered at different levels in the first experimental cheeses. We then completely eliminated the phage by dismantling all the parts of the equipment before cleaning, then sanitizing with hypochlorite solution. This procedure was carried out after each assay and the completely fitted equipment was sanitized on the spot in the isolator before each new experiment.

Bacterial enumeration and bacteriophage titration

Cheese samples (10 g) were homogenized in 50 mL of trisodium citrate buffer (2%, w/v) with an Ultra-turrax homogenizer (20 000 rpm; twice for 30 s). Bacterial population in the cheese was estimated at different times throughout ripening: at 48 h, and at 15, 30 and 60 days. Viable bacterial counts were determined on M17 (Biokar, France) agar plates after 48 h incubation at 30 °C. Non-starter lactic acid bacteria (NSLAB) were enumerated on Rogosa medium (Merk, Socolab, France) agar plates after 48 h incubation at 30 °C in anaerobic jars. The starter counts were expressed as colony forming units per gram of cheese (cfu/g).

Phage plaque forming units (pfu) counts were determined on the same samples by plating appropriate cheese dilutions on M17 agar containing 10 mmol/L Ca^{2+} previously inoculated with NCDO763 as described by Limsowtin et al (1994) according to the method of Hull (1977).

Electron microscopy

Cheese samples were removed at different ripening times. They were cut into small cubes of about 1 mm per side, first fixed in glutaraldehyde, and then in osmium tetroxide as described previously (Chapot-Chartier et al, 1994). They were then dehydrated in acetone and embedded in Epon 810. Sections were made with a Reichert microtome, stained with uranyl acetate and lead citrate, and examined with a Zeiss-EM10 electron microscope. Numerous fields on several sections were examined to estimate the extent of starter lysis.

Chemical analysis of cheese

The cheese pH was measured by means of a penetration electrode (type 406, Ingold, Urfdorf, Switzerland). The dry matter was determined on a cheese sample dried at 102 °C for 16 h. Nitrogen analysis were performed after preparation of a cheese extract in 0.5 mol/L sodium citrate, pH 7.0. Total nitrogen (TN), soluble nitrogen (SN) (after precipitation at pH 4.4 with 1 N HCl) and 2.5% phosphotungstic acid-soluble nitrogen (PTA-N) were measured on cheese samples prepared according to Gripon et al (1975). The nitrogen content was determined after mineralization of the solutions by an automated Kjeldahl method.

Free amino acid quantification

Grated cheese (5 g) was mixed with 40 mL of 0.2 mol/L sodium citrate buffer (pH 2.2) containing 5 mg/L pentachlorophenol as preservative and 200 mg/L EDTA. The mixture was stirred for 15 min, homogenized with an Ultra-turrax homogenizer (five times for 1 min at 11 000 rpm) and then rapidly filtered through filter paper. The proteins and large peptides present in the filtrate were subsequently removed by 3% sulfosalicylic acid precipitation. Ten ml of the filtrate were mixed with 10 mL of 7.5% sulfosalicylic acid solution, and the final volume adjusted to 25 mL with citrate buffer. The precipitate was filtered out on filter paper. The cleared solution was diluted twice with 0.1 N lithium citrate buffer (pH 2.2). Amino acids were separated by ion exchange chromatography on a Biotronik LC 5000 amino acid analyzer programmed for

physiological fluids with ninhydrin post-column derivatization and colorimetric detection at 570 and 440 nm. The total free amino acid amount was the sum of the amounts of every amino acid. The results were expressed in g/kg of cheese.

RESULTS AND DISCUSSION

Cheese manufacture

The *L lactis* subsp *lactis* NCDO763 strain or the TIL61 strain, a derivative of NCDO763 overexpressing the PepC aminopeptidase, were used as starters to manufacture Saint-Paulin-type cheese in an isolator equipped with a 6-L small cheese vat. This system was initially designed to operate under aseptic conditions and in a confined space with genetically modified strains. Nevertheless, in the first experimental cheeses, the presence of a homologous bacteriophage was encountered in a number of experiments, probably due to insufficient sterilization of some parts of the isolator (see Materials and Methods). Phages were titrated in the cheeses at 48 h after manufacture and their number did not increase later on. The phage level ranged from 10^3 to 10^{11} pfu/g in the different cheese assays (table I) carried out with NCDO763 (four assays) or TIL61 (five assays). After eradication of the bacteriophage, control cheeses (< 10 pfu/g) were also made with both strains. When the level of phages in the young cheese was below 10^{11} pfu/g, their presence did not affect cheese manufacture. Indeed, the cheese pH and dry matter values at 48 h were in the standard range (table I). Only when the phage level reached 10^{11} pfu/g did incorrect acidification occur (pH 5.45). In a previous study (Chapot-Chartier et al, 1994), we showed that the NCDO763 strain did not autolyse during Saint-Paulin cheese ripening. Thus we had at our disposal a set of assays in which starter bacteria lysis was induced by different phage levels which were susceptible to result in different degrees of bacterial lysis.

Estimation of starter bacteria lysis

The viable bacterial counts were determined over the 2 months of ripening. We checked

Table I. Data on cheese assays at 48 h.
Données sur les fabrications fromagères à 48 h d'affinage.

Strain	*Phages (pfu/g)*	*pH*	*Dry matter (%)*	*Viability (cfu/g)*
NCDO763	<10	4.94	58.3	$4.6\ 10^9$
	10^8	5.05	55.9	$8.1\ 10^8$
	10^9	4.95	56.1	$1.2\ 10^9$
	10^{10}	5.10	56.9	$9.4\ 10^8$
	10^{10}	5.14	56.5	$2.3\ 10^8$
TIL61	<10	4.97	56.5	$5.9\ 10^9$
	10^3	4.93	55.9	$4.2\ 10^9$
	10^7	4.97	54.8	$3.0\ 10^9$
	10^{10}	5.06	56.8	$4.1\ 10^8$
	10^{10}	5.04	55.8	$4.6\ 10^8$
	10^{11}	5.48	55.2	$7.1\ 10^7$

for the absence of non-starter lactic acid bacteria (NSLAB) throughout this period by plating on Rogosa medium. In each assay, their number remained under 10 cfu/g cheese. Thus, the bacterial counts that were estimated on M17 medium measured the starter bacteria viability. The viable cell counts at 48 h are presented in table I. These values appear to vary according to the phage levels found in cheese, and four groups of cheeses could be defined independently of the starter strain. The first group comprised the control cheeses (< 10 pfu/g) and cheeses with a low number of phages (up to 10^7 pfu/g) in which the bacterial population at 48 h was the expected one ($3–5 \times 10^9$ cfu/g) with the NCDO763 strain (Chapot-Chartier et al, 1994). The second group contained cheeses with intermediate phage levels ($10^8–10^9$ pfu/g) in which the viable cell counts at 48 h were around 10^9 cfu/g. The third group contained cheeses with high phage levels (10^{10} pfu/g) in which the viable cell counts at 48 h reached $2–5 \times 10^8$ cfu/g. In the fourth group (cheese with 10^{11} pfu/g), the viable cell counts were even lower (7×10^7 cfu/g).

In each case, cell viability decreased from one to two orders of magnitude over the 2-month ripening period. However, the die-off rate was independent of the phage levels and could not be related to the presence or absence of phages (data not shown).

In order to examine whether the decrease in bacterial counts obtained at 48 h in the presence of phages resulted from low final cell density due to phage infection or rather from early lysis, the morphological state of bacteria in cheese was observed by electron microscopy on samples taken at different ripening times in some of the cheese assays made with NCDO763 or TIL61. We had previously observed that in the absence of phages, the NCDO763 strain was not autolytic and that bacterial integrity appeared well conserved at 48 h (Chapot-Chartier et al, 1994).

In the case of high phage levels (10^{10} pfu/g; NCDO763), few intact bacteria were found as early as 48 h, whereas much cellular debris resulting from cell lysis was present (fig 1A). These observations suggest that in presence of high phage levels, most of the bacteria are lysed very early, ie, during the first 48 h. As a result, the viable cell counts estimated at 48 h do not appear representative of the total bacterial population obtained during cheesemaking, but rather give an indication of the degree of early cell lysis. The fact that higher cell densities were obtained could thus explain that milk acidification was correct in this case. Too high a number of phages (10^{11} pfu/g) probably decreases the final cell density at the end of cell growth, which leads to incorrect acidification of milk.

In the case of intermediate phage level (10^9 pfu/g; NCDO763), the situation was more complex. According to electron microscopy observations, the starter lysis seems to be delayed. The majority of bacteria appeared well conserved at 48 h, whereas most of them were lysed between 15 and 30 days (data not shown).

For low phage levels (up to 10^7 pfu/g; TIL61), the cell envelope in most of the cells appeared well conserved at 48 h, and also after 30 or 60 days of ripening (fig 1B). The only morphological alteration that could be detected was the degradation of ribosomes leading to clearer regions in the cytoplasm, characteristic of bacteria under starvation (Thomas et al, 1969).

Estimation of proteolysis during ripening

Proteolysis was first estimated in the different cheeses by classical nitrogen determinations after fractionation. Whatever the starter strain, the SN was not significantly different between control cheeses and cheeses with low or intermediate phage lev-

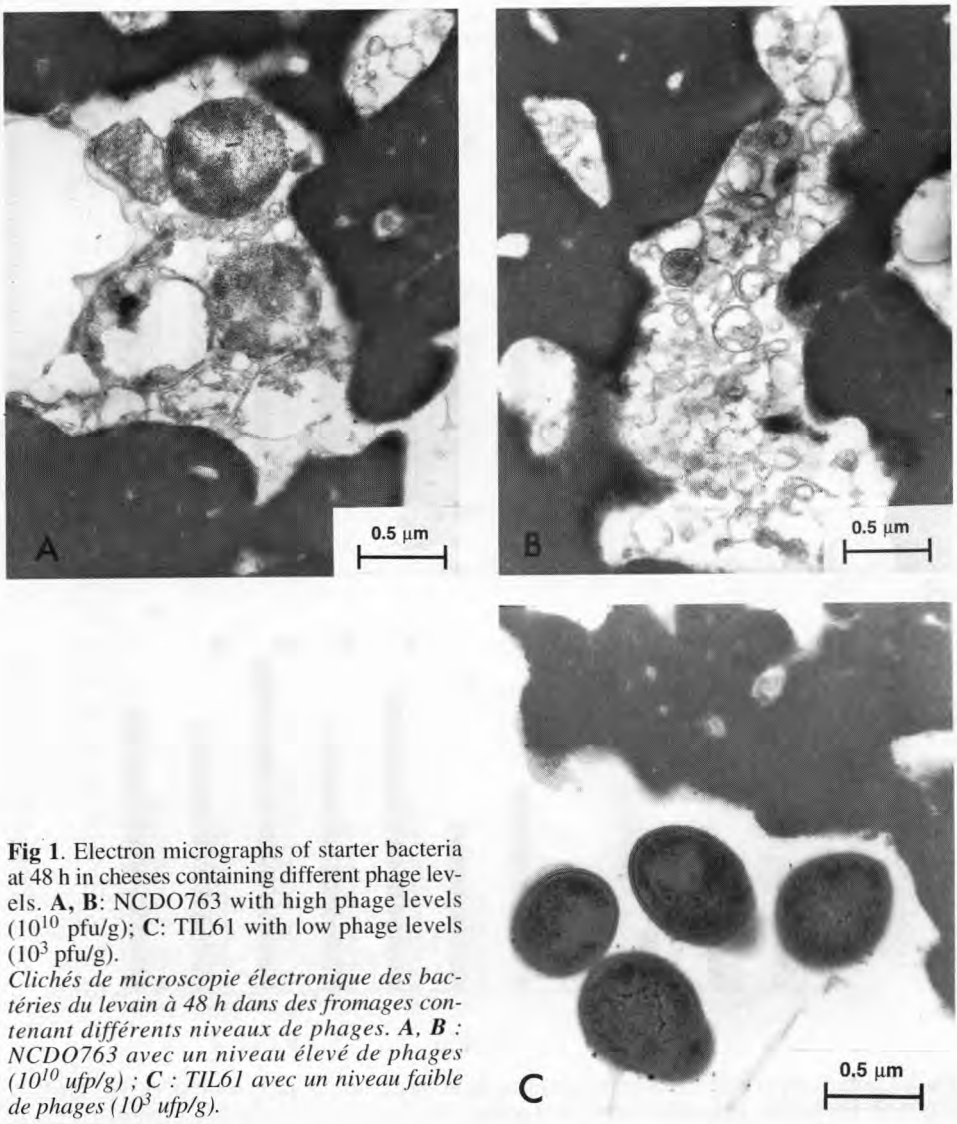

Fig 1. Electron micrographs of starter bacteria at 48 h in cheeses containing different phage levels. **A, B**: NCDO763 with high phage levels (10^{10} pfu/g); **C**: TIL61 with low phage levels (10^3 pfu/g).
*Clichés de microscopie électronique des bactéries du levain à 48 h dans des fromages contenant différents niveaux de phages. **A, B** : NCDO763 avec un niveau élevé de phages (10^{10} ufp/g) ; **C** : TIL61 avec un niveau faible de phages (10^3 ufp/g).*

els, ie, up to 10^9 pfu/g (18.7 ± 1.1% of total nitrogen after 2 months). In contrast, in the case of high phage levels, the SN value was significantly higher than in the control cheese (22.8 ± 2% of total nitrogen after 2 months).

Concerning PTA-N, its rate of increase varied according to the level of phages (fig 2A). For low levels (up to 1×10^7 pfu/g), PTA-N was not significantly different from the control. For higher phage levels, the formation rate of PTA-N increased with the

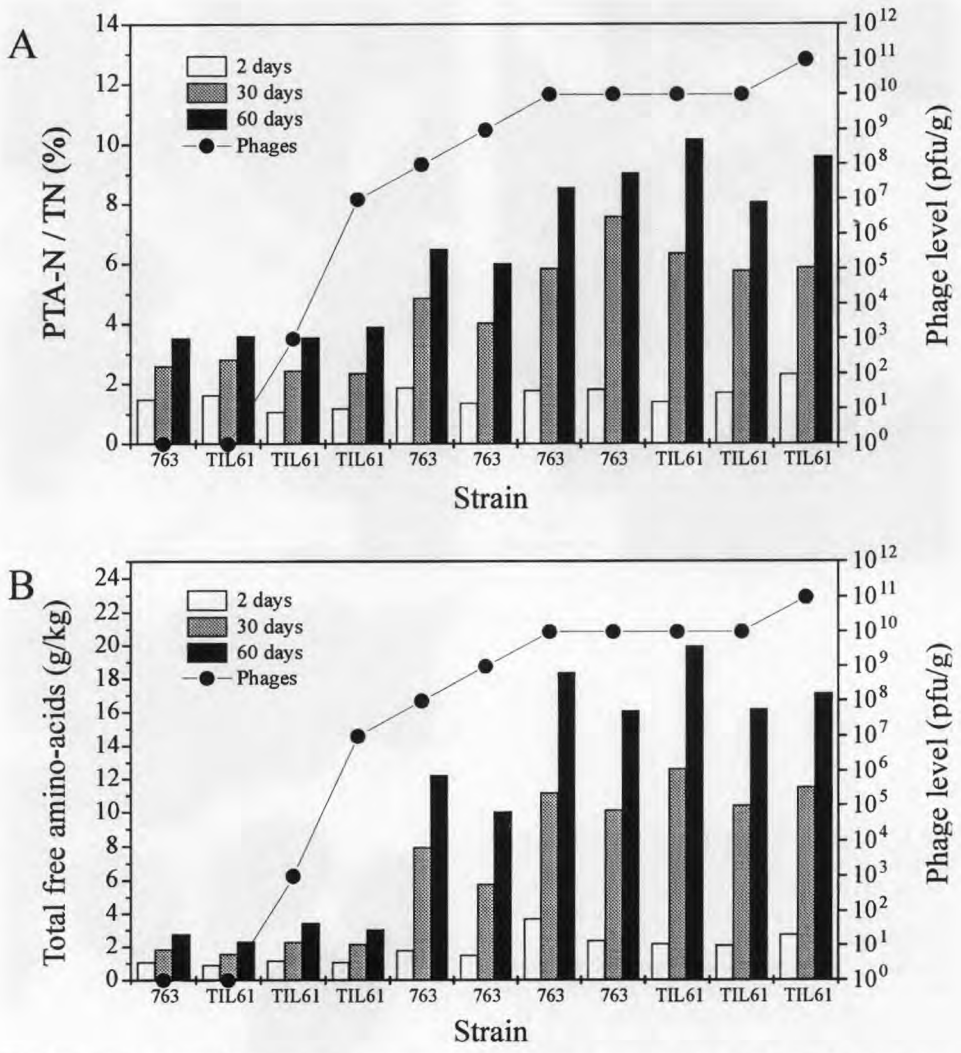

Fig 2. Quantification of proteolysis during ripening in cheese assays made with NCDO763 (763) or TIL61. **A**: Formation of phosphotungstic acid-soluble nitrogen (PTA-N). PTA-N is expressed as a percentage of total nitrogen (TN). **B**: Formation of total free amino acids. The concentration of total free amino acids in cheese is expressed in g/kg of cheese. PTA-N and free amino acid amounts were determined after 2, 30 and 60 days in each case. The phage levels (–●–) in each assay are indicated in the figure. The different assays are set out according to increasing phage levels and correspond to the different assays in table I.

*Quantification de la protéolyse au cours de l'affinage dans des fabrications fromagères réalisées avec NCDO763 (763) ou TIL61. **A** : Formation de l'azote soluble dans l'acide phosphotungstique (PTA-N). PTA-N est exprimé en pourcentage de l'azote total (TN). **B** : Formation des acides aminés libres. La concentration des acides aminés libres totaux est exprimée en g/kg de fromage. Les quantités de PTA-N et d'acides aminés libres ont été déterminées après 2, 30 et 60 jours dans chacun des cas. Le niveau de phages (–●–) dans chaque essai est indiqué sur la figure. Les différents essais sont rangés selon des niveaux de phages croissants et correspondent aux différents essais du tableau I.*

phage levels. After 2 months of ripening, the PTA-N value was three-fold higher in the cheeses with 1×10^{10} pfu/g than in the control cheeses.

The quantification of free amino acids in cheese during ripening confirms the results obtained for PTA-N analysis. The higher the phage level, the higher was the rate of free amino acid formation (fig 2B). In this case, the differences observed were even larger: after 2 months of ripening, the total free amino acid content was seven-fold higher in the cheese with a phage level of 10^{10} pfu/g than in the control cheese. When a higher phage level (10^{11} pfu/g) was reached, which did not allow correct acidification of the milk, no further increase in the formation rate of free amino acids was observed.

Correlations between extent of starter bacteria lysis and proteolysis

In the presence of various phage levels, different levels and patterns of lysis of the NCDO763 strain or its derivative TIL61 were induced. We can distinguish three situations: a very early extensive lysis of most of the cells (high phage levels), more progressive and delayed lysis (intermediate phage levels) and absence of lysis (low level of phages and control).

For each strain, we observed a positive correlation between the phage levels in cheese and the rate of formation of PTA-N and free amino acids. Since we also observed that the extent of starter bacteria lysis was related to the phage levels, we can conclude that the higher the cell lysis, the higher was the rate of formation of small peptides and free amino acids. These results are in agreement with previous studies (Chapot-Chartier et al, 1994; Wilkinson et al, 1994; Crow et al, 1995b) which also established a relationship between the lysis of starter bacteria and the proteolysis rate. The accumulation of free amino acids suggests that some of the released peptidases are active after their release in cheese, as previously reported in the studies mentioned above. Moreover, our results indicate that the early lysis of bacteria appears very efficient in increasing the rate of free amino acid formation and that peptidases, or at least some of them, remain stable in cheese during 2 months of ripening. Sensory analysis would be necessary to determine whether the higher rate of proteolysis was accompanied by an improvement in organoleptic properties, as was previously found in the case of Cheddar cheese (Crow et al, 1995b).

These observations concerning the effect of phages can be compared with the fact that the introduction of highly acidifying strains of lactococci, which are in addition resistant to the phages encountered in industrial cheese plants, results in cheese graded as strongly acidic and bitter.

A moderate increase in the rate of SN formation was also observed. The SN content was 20% higher after 2 months of ripening in the cheeses with high phage levels than in the controls. This increase could result from a higher plasmin activity since the pH was slightly higher in the cheeses with high phage levels. Another possibility is that this increase results from the activity of the lactococci proteolytic system. After disruption of the cell envelope, the diffusion of cell wall proteinase through the curd and thus casein accessibility could be facilitated, or intracellular proteolytic activity could be liberated (Coolbear et al, 1994).

A ten-fold overexpression of the general aminopeptidase PepC did not lead to an increased rate of proteolysis whatever the extent of starter bacteria lysis. According to our results, the limiting factor in formation of free amino acids appears to be the amount of peptidases released in cheese rather than the availability of peptidic substrates. Therefore, aminopeptidase PepC

appears to contribute only to a low extent to the formation of free amino acids. This could be due to the inactivation or low catalytic activity of PepC under cheese physicochemical conditions. These observations suggest that the other general aminopeptidase PepN found in lactococci (Tan et al, 1992) might be the main enzyme with broad specificity which is active in cheese.

The results presented in this paper provide further experimental support to the hypothesis that the autolysis of starter lactococci can induce intensification of biochemical processes in cheese, likely to result in acceleration of cheese ripening or intensification of aroma. They are stimulating for further research into obtaining strains with a high autolytic capacity. Previous studies have already reported on the selection of strains whose lysis can be induced upon temperature shift during Cheddar cheesemaking (Feirtag and McKay, 1987a, b). Further research will aim at a better knowledge of the mechanism of autolysis in *L lactis* strains under cheese environmental conditions, in order to develop screening tests for the selection of autolytic strains. Another approach is based on the construction of autolytic strains via genetic engineering which was previously carried out by cloning a bacteriophage lysin gene in a *L lactis* strain (Shearman et al, 1992) or by cloning the major autolysin *acm*A gene (Buist et al, 1995) downstream of an inducible promoter/operator region of a temperate bacteriophage or a salt-inducible promoter (Buist et al, 1996). Future developments will aim at obtaining food-grade modified strains with an inducible autolytic system which could be triggered by physico-chemical factors compatible with cheese technology.

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