Short Communication: Impact of pH and Temperature on the Acidifying Activity of *Carnobacterium maltaromaticum*

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ABSTRACT

The acidifying activity of *Carnobacterium maltaromaticum* LMA28, a strain isolated from French soft cheese, was studied in trypticase soy broth with yeast extract (TSB-YE) medium and in milk. In TSB-YE supplemented with lactose, glucose, or galactose, lactose and glucose were metabolized with a maximum growth rate of 0.32 h⁻¹ and galactose was not metabolized. During hydrolysis of lactose, the galactose moiety was not excreted. The major product was L(+) lactic acid, with no significant difference in the lactic acid yield. Glucose was not completely metabolized because cell growth stopped when pH values reached an average of 5.0. In sterilized UHT milk, the addition of 1 g/L of YE enhanced its coagulation. Compared with commercial starter lactic acid bacteria such as *Lactococcus lactis* DSMZ 20481 or *Streptococcus thermophilus* INRA 302, *Carnobacterium maltaromaticum* LMA 28 was shown to be a slow acidifying strain. However, in spite of this weak acidifying ability, *C. maltaromaticum* LMA 28 can sustain low pH values in coculture with *Lc. lactis* DSMZ 20481 or *S. thermophilus* INRA 302. The individual and interactive effects of initial pH values (5.2 to 8.0) and incubation temperatures (23 to 37°C) on acidifying activity were studied by response surface methodology. The 3 strains displayed different behaviors depending on pH and temperature. The psychrotrophic lactic acid strain *C. maltaromaticum* LMA 28 was able to grow at alkaline pH values and during storage conditions. It could be used as a potential ripening flora in soft cheese.

Key words: *Carnobacterium*, acidifying activity, cheese manufacturing, response surface methodology

In ripened cheese varieties, lactic acid bacteria (LAB) are the major contributors to flavor development. The

LAB can be divided into 2 groups: starter and non-starter lactic acid bacteria (Caplice and Fitzgerald, 1999; El Soda et al., 2000). Presumably originating from milk, ingredients used for cheese-making, or the dairy environment, NSLAB could actively participate in the ripening process (Peterson and Marshall, 1990). Knowledge of the microbiology and biochemistry of cheeses is fundamental to the enforcement of high quality of “Appellation d’Origine Protégée” products in Europe (Freitas and Malcata, 2000). Diversity of LAB is important to enhance proteolysis and lipolysis and can aid in the development of consistent cheese flavor (e.g., via peptide hydrolase system or esterase activities; Macedo et al., 2000; Macedo et al., 2003). In France, the *Carnobacterium* genus, especially *C. maltaromaticum*, was isolated from dairy products (Miller et al., 1974; Millière et al., 1994). The population level of *C. maltaromaticum* increases from low to high in ripened cheese because the species tolerates modifications of the environment during ripening (Edima et al., 2007); as such, it could be considered as a nonstarter LAB. This atypical LAB displays advantageous properties such as growth at low temperatures (psychrotrophy) and at alkaline pH values (up to 9.6), and some strains are able to produce bacteriocins (Mathieu et al., 1993; Quadri et al., 1994). After food processing, this species can potentially grow in chilled products stored for extended periods. The anti-*Listeria* property of some isolates of *Carnobacterium* should be an advantage for industrial cheeses (O’Sullivan et al., 2002), and bacteriocins from *Carnobacterium* could reduce the viable counts of *Listeria monocytogenes* (Wan et al., 1997). Moreover, because it has the same psychrotrophic property as *Listeria*, *Carnobacterium* sp. could prevent the development of *Listeria* in cheese because its growth rate is faster than that of *Listeria* in cold conditions (Buchanan and Klawitter, 1992; Mathieu et al., 1994). Assuming that no product defect is related to the presence of *Carnobacterium*, it could be useful in the manufacture of soft cheeses to investigate its positive influence in the preservation of cheese (Cailliez-Grimal et al., 2007). In

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French ripened “thermophilic” cheese technology with *Streptococcus thermophilus* and *Lactococcus lactis* as starters, the adjunct of *C. maltaromaticum* could give various characteristics to the product. There are few data available on *Carnobacterium* metabolism in milk. The aim of this work was therefore to study the acidifying properties of *C. maltaromaticum* compared with the conventional starters *S. thermophilus* and *Lc. lactis* with a view to integrating such a strain in the soft-cheese ripening process.

The 6 strains evaluated were *C. maltaromaticum* DSMZ 20730^T (Deutsche Sammlung von Mikro-Organismen und Zellkulturen, Braunschweig, Germany), *C. maltaromaticum* LMA 28, *C. maltaromaticum* LMA 29, *C. maltaromaticum* LMA 30 (Laboratoire de Microbiologie Alimentaire de l'ENSAIA-INPL, Vandoeuvre-lès-Nancy, France), *Lactococcus lactis* DSMZ 20481 and *Streptococcus thermophilus* INRA 302 (Institut National de la Recherche Agronomique, Jouy-en-Josas, France).

Strains were stored at -25°C in trypticase soy broth (TSB, bioMérieux, Craponne, France) supplemented with 0.6% (wt/vol) yeast extract (YE, Biokar Diagnostics, Beauvais, France) in 30% (vol/vol) glycerol. Strains were subcultured in TSB-YE and incubated at their optimal growth temperature.

For experimental use, the inoculated culture was thawed and incubated in TSB-YE at 30 or 37°C for 15 h (twice). The culture thus obtained was centrifuged 3 times at $10,000 \times g$ at 4°C for 15 min. At each stage, the pellet was washed in sterile tryptone salt (TS, 0.95% wt/vol; bioMérieux); subsequently, serial decimal dilutions were prepared in sterile TS. Bacterial enumerations were carried out using a spiral plating (Whitley Automatic Spiral Plater, WASP 2, AES Laboratoire France, Combourg, France). Based on the TSB-YE agar, the selective CM medium [3.5 mg/L of vancomycin, 5.0 mg/L of gentamicin, and 20 mg/L of nalidixic acid (Sigma-Aldrich, St-Quentin Fallavier, France)] was used for *C. maltaromaticum* (Edima et al., 2007) and TS-YE supplemented with 15 g/L agar for *S. thermophilus* and *Lc. lactis*. Strains were incubated following the required temperatures.

The TSB medium (150 mL) was modified by addition of YE (Biokar) and by substitution, or not, of 4.0 g/L lactose, glucose, or galactose (Fisher Scientific Labosi, Elancourt, France). Solutions of carbohydrates (10% wt/vol) were sterilized by filtration through pores of 0.22 μm diameter (Fisher). Strains were inoculated and cultures were incubated in a water bath at 30 or 37°C depending on the strain. Samples were collected every 5 h during 25 h of incubation to evaluate the variations of pH value and growth of the bacterial population to quantify organic acid production and the consumption

Figure 1. Kinetics of acidification of milk by the 4 *Carnobacterium maltaromaticum* strains at 30°C. Δ = *C. maltaromaticum* LMA 28; $\circ$ = *C. maltaromaticum* LMA 29; $\square$ = *C. maltaromaticum* LMA 30; $\diamond$ = *C. maltaromaticum* DSM 20730^T.

of carbohydrates by *C. maltaromaticum* LMA 28 and *S. thermophilus* INRA 302. Carbohydrates, lactic acid, ethanol, and acetic acid concentration were measured by using enzymatic kits (Biosentec, Toulouse, France) as described by the manufacturer. Absorbance was measured at 340 nm (Spectrophotometer UV 160 A, Shimadzu, Kyoto, Japan).

Acidification kinetics were followed by pH variation during fermentation continuously monitored by means of a glass electrode pH meter (Consort D230, Neuilly-sur-Seine, France) according to the Cinac system of Spinnler and Corrieu (1989). The pH was automatically recorded at 30-min intervals. Parameters were considered to characterize the kinetics of the process: maximum acidification rates ($V_{\max}$, pH unit/h), time necessary to reach $V_{\max}$ ($T_{\max}$, h), and final pH value (pH_f) (Spinnler and Corrieu, 1989).

Commercial sterilized UHT semi-skimmed milk (Scrib Cvba Block, Brussels, Belgium) supplemented with 0 or 1 g/L of yeast extract was distributed (150 mL) in sterilized (120°C, 15 min) glass bottles. Strains were inoculated and incubated at the required temperature in a water bath for the fermentation experiments. Acidifying kinetics were monitored as described previously.

To study the impact of the combination of pH and temperature on $V_{\max}$ (pH units/h) and $T_{\max}$ (h) factors, a Doehlert experimental design was retained (Doehlert, 1970); it included the initial pH of the milk (X_1) at 5 levels (5.2, 5.9, 6.6, 7.3, and 8.0) by addition of 1 N NaOH or 10 N lactic acid (Sigma-Aldrich Chimie S.A.R.L., l'Isle d'Abeau-Chesnes, France) and the incubation temperature (X_2) at 3 levels (23, 30, and 37°C). The response (R) expressed as $V_{\max}$ or $T_{\max}$ values can be predicted in all experimental regions according to the following equation: $R = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$, where X_1 represents the initial pH value; X_2 the incubation temperature; β_0 is the constant term; β_1 determines the influence of pH; β_2 the influence

Table 1. Acidifying kinetics parameters (mean $\pm$ SE) of *Carnobacterium maltaromaticum* LMA28 in trypticase soy broth with yeast extract (TSB-YE)¹

Item ²	TSB-YE with			
	No glucid	Lactose	Glucose	Galactose
V _{max} , pH/h	ND ³	0.24 $\pm$ 0.04	0.25 $\pm$ 0.04	ND
T _{max} , h	ND	25 $\pm$ 0.1	18 $\pm$ 0.1	ND
pH _f	6.60 $\pm$ 0.05	5.0 $\pm$ 0.1	5.0 $\pm$ 0.1	6.6 $\pm$ 0.1
log ₁₀ (N _f), cfu/mL	8.50 $\pm$ 0.02	9.50 $\pm$ 0.07	9.50 $\pm$ 0.06	8.50 $\pm$ 0.03
Glucids, mM	ND	11.0 $\pm$ 0.6	22.0 $\pm$ 0.6	22.0 $\pm$ 0.6
Remaining glucids, mM	ND	4.3 $\pm$ 0.9	10.0 $\pm$ 0.4	22.0 $\pm$ 0.6
Lactate produced, mM	ND	18.4 $\pm$ 1.7	17.7 $\pm$ 0.8	ND
Acetate, mM	ND	ND	ND	ND
Ethanol, mM	2.4 $\pm$ 0.1	2.5 $\pm$ 0.2	2.4 $\pm$ 0.2	2.5 $\pm$ 0.1

¹Inoculation of 3.50 $\pm$ 0.02 log₁₀ cfu/mL with *C. maltaromaticum* LMA 28 at 30°C.

²V_{max} = maximum acidification rate in pH unit per h; T_{max} = time corresponding to V_{max}; pH_{max} = pH corresponding to T_{max}; pH_f = pH obtained at the end of incubation; tpH 5.5 = time corresponding to pH value 5.5; log₁₀(N_f) = enumeration at the end of fermentation.

³ND = not detected.

of temperature; β_{12} the interaction effect between pH and temperature; β_{11} and β_{22} are “shape” parameters. Data analysis, ANOVA, and polynomial regressions were performed using the NEMROD software (LPRAI, Marseille, France; Mathieu and Phan-Tan-Luu, 1997).

Milk acidification profiles by the 3 isolated *C. maltaromaticum* strains from cheeses (LMA 28, LMA 29, LMA 30), and the type strain (DSMZ 20730^T) were analyzed. All strains were able to grow on lactose with β -galactosidase activity and produce L(+) lactic acid at various amounts (data not shown). For *C. maltaromaticum* LMA 28, the final pH of the culture was 4.7 after 125 h, and 6.0 $\pm$ 0.5 for the 3 other strains (Figure 1). Because of its greater acidifying properties, only *C. maltaromaticum* LMA 28 was retained for further study.

Acidifying kinetics of *C. maltaromaticum* LMA 28 were analyzed on TSB-YE without carbohydrate or supplemented with 4.0 g/L of lactose, glucose, or galactose (Table 1). Preadaptations of that strain on TSB-YE glucose or TSB-YE lactose by 2 successive cultures did

not lead to significant differences in the acidification kinetics (data not shown).

With an initial bacterial population of *C. maltaromaticum* LMA 28 of 3.5 log₁₀ cfu/mL, significant acidification appeared only after 14 to 16 h at 30°C under all experimental conditions (data not shown). In TSB-YE with or without galactose, the final population reached 8.5 log₁₀ cfu/mL with a pH value of 6.6. The presence of lactose or glucose allowed an increase of approximately 1 log₁₀ cfu/mL of population with a final pH of 5.0. The V_{max} value was similar on glucose and lactose, and the T_{max} value was lowest on glucose (Table 1). That strain was able to hydrolyse lactose and ferment glucose but not galactose, like *S. thermophilus*. To confirm these results, we quantified lactose, glucose, and galactose and the corresponding products (lactic acid, ethanol, acetic acid; Table 1). As expected, galactose was not metabolized. Glucose and lactose were consumed with a maximum growth rate (μ_{max}) of 0.32 $\pm$ 0.01 h⁻¹ and the major product was L(+) lactic acid. There was no significant difference in lactic acid yields (72 to 77%).

Table 2. Acidifying kinetics parameters (mean $\pm$ SE) of milk¹

Culture medium	Bacterial strain	Parameter ²		
		V _{max} , pH/h	T _{max} , h	pH _f
Milk	<i>Carnobacterium maltaromaticum</i> LMA 28	ND ³	ND	6.3 $\pm$ 0.1
	<i>Streptococcus thermophilus</i> INRA 302	0.28 $\pm$ 0.04	13.0 $\pm$ 0.1	4.7 $\pm$ 0.1
	<i>Lactococcus lactis</i> DSMZ 20481	0.23 $\pm$ 0.04	17.0 $\pm$ 0.1	4.4 $\pm$ 0.1
Milk-YE	<i>C. maltaromaticum</i> LMA 28	0.12 $\pm$ 0.04	18.0 $\pm$ 0.1	5.0 $\pm$ 0.1
	<i>S. thermophilus</i> INRA 302	0.57 $\pm$ 0.02	7.0 $\pm$ 0.1	4.3 $\pm$ 0.1
	<i>Lc. lactis</i> DSMZ 20481	0.46 $\pm$ 0.01	12.0 $\pm$ 0.1	4.4 $\pm$ 0.1

¹Sterilized half-skim milk UHT was supplemented with 1 g/L of yeast extract (YE) and acidified by *C. maltaromaticum* LMA 28, *S. thermophilus* INRA 302 at 37°C, and *Lc. lactis* DSMZ 20481 at 30°C.

²V_{max} = maximum acidification rates in pH unit per h; T_{max} = time corresponding to V_{max}; pH_f = pH obtained at the end of incubation.

³ND = not detected.

Figure 2. Kinetics of acidification of milk supplemented with yeast extract (1 g/L) by *Carnobacterium maltaromaticum* LMA 28 and *Lactococcus lactis* DSMZ 20481 at 30°C. ● = *C. maltaromaticum* LMA 28; ◇ = *Lc. lactis* DSMZ 20481; □ = *C. maltaromaticum* LMA 28 + *Lc. lactis* DSMZ 20481.

Glucose was not completely metabolized because growth stopped when the pH value reached 5.0. Acetic acid and D(–) lactic acid were not detected; ethanol was produced from yeast extract metabolism (2.5 mM). During the hydrolysis of lactose, the galactose moiety not metabolized was not found in the culture medium. To confirm that result, the galactose-negative strain *S. thermophilus* INRA 302 was used. As expected, galactose, which is not fermented during lactose hydrolysis, was excreted. So, hydrolysis of 9 mM of lactose produced 8.7 mM galactose and 12 mM of L(+) lactic acid. Glucose was immediately metabolized according to the glycolysis pathway (Embden-Meyerhoff-Parnas) like other homofermenters *Carnobacterium* (De Bruyn et al., 1988) and was never detected during the growth of *S. thermophilus* INRA 302 and *C. maltaromaticum* LMA 28 in culture.

The absence of fermentation of galactose by some lactic bacteria is an important parameter in cheese manu-

Figure 3. Kinetics of acidification of milk supplemented with yeast extract (1 g/L) by *Carnobacterium maltaromaticum* LMA 28 and *Streptococcus thermophilus* INRA 302 at 30°C. ● = *C. maltaromaticum* LMA 28; ◇ = *S. thermophilus* INRA 302; □ = *C. maltaromaticum* LMA 28 + *S. thermophilus* INRA 302.

facturing because of postacidification. Indeed, galactose accumulated in the milk by galactose-negative strains such as *Lactobacillus bulgaricus* and *S. thermophilus* (Turner and Martley, 1983; Thomas and Crow, 1984) can be the substrate for those microorganisms able to metabolize it.

The acidification kinetic parameters of milk by *C. maltaromaticum* LMA 28, *S. thermophilus* INRA 302, and *Lc. lactis* DSMZ 20481 are reported in Table 2. With *C. maltaromaticum* LMA 28, in unsupplemented milks, the pH was 6.3 after 45 h of incubation, and the milk was not coagulated. As expected, the addition of YE in milk improved the acidification performances of the 3 strains. The optimal concentration to improve their acidifying performances was 1 g/L. Greater concentrations (1 to 6 g/L) gave similar results (data not shown). *Carnobacterium maltaromaticum* LMA 28 was a slow

Table 3. Coefficient estimations of the different quadratic models for the 2 variables, pH ($V_{\max}$) and temperature ($T_{\max}$), of the Doehlert's matrix¹

Parameter ²	$V_{\max}$			$T_{\max}$		
	<i>C. m</i>	<i>S. t</i>	<i>Lc. l</i>	<i>C. m</i>	<i>S. t</i>	<i>Lc. l</i>
β_0	0.130*	0.360*	0.455**	19.000**	16.000	9.000***
β_1	0.067	0.272*	0.197*	7.667	9.667	0.167
β_2	0.006	0.274*	0.121	19.630	27.135*	4.330**
β_{11}	–0.020	0.080	0.070	7.500	28.000	1.000
β_{22}	–0.120	0.103	0.037	28.166**	10.000	4.667**
β_{12}	0.069	0.179	0.058	6.928	1.155	0.577
R^2	0.905	0.975	0.965	0.998	0.967	0.998
R^2A	0.669	0.912	0.878	0.993	0.884	0.993

¹*C. m* = *Carnobacterium maltaromaticum* LMA 28; *S. t* = *Streptococcus thermophilus* INRA 302; *Lc. l* = *Lactococcus lactis* DSMZ 20481.

² β_0 = constant coefficient; β_1 = initial pH coefficient; β_2 = incubation temperature coefficient; β_{12} = interaction effect between initial pH and incubation temperature; β_{11} , β_{22} = “shape” parameter; R^2 = correlation coefficient; R^2A = adjusted correlation coefficient.

*** ≤ 1‰; 1‰ < ** ≤ 1%; 1% < * ≤ 5%.

Figure 4. Isoresponse curves of acidification $V_{\max}$ of a sterilized partially UHT skimmed milk in function of pH and temperature. X1 axis = pH values at fixed temperature (30°C, center of the domain); X2 axis = temperature values at fixed pH (6.6, center of the domain). a) *Carnobacterium maltaromaticum* LMA 28; b) *Streptococcus thermophilus* INRA 302; c) *Lactococcus lactis* DSMZ 20481.

acidifying strain, with the weakest $V_{\max}$ and greatest $T_{\max}$ values. Increasing the initial biomass from 3 log₁₀ to 8 log₁₀ cfu/mL significantly reduced the $T_{\max}$ of *C. maltaromaticum* LMA 28 (data not shown). At the end of milk-YE fermentation by *S. thermophilus* INRA 302 or *Lc. lactis* DSMZ 20481, the pH value reached 4.4 or 4.3, respectively.

To analyze the behavior of *C. maltaromaticum* LMA 28 in such conditions of pH, cocultures were tested with *S. thermophilus* and *Lc. lactis*, starters usually used in French cheese-making thermophilic technology.

In coculture with *C. maltaromaticum* LMA 28, different levels of inoculation were tested with *Lc. lactis* DSMZ 20481. In all cases, the acidification curve was

similar to that of *Lc. lactis* in pure culture (Figure 2). With *S. thermophilus* INRA 302, at 30 (Figure 3), 35, or 39°C, the acidification rate curve of coculture also followed the curve of *S. thermophilus* INRA 302.

These coculture experiments showed that the growth of *C. maltaromaticum* LMA 28 was not inhibited by the production of lactic acid, with a final population of 8.9 log₁₀ cfu/mL. Even if the growth stopped when the pH value reached 5.0 (as shown previously), *C. maltaromaticum* LMA 28 can support the low pH values produced by the 2 other strains.

To study the impact of combinations of pH and temperature on acidification rate, a Doehlert experimental design was used. Polynomial equations obtained with

Figure 5. Isoresponse curves of acidification $T_{\max}$ of a sterilized partially UHT skimmed milk in function of pH and temperature. X1 axis = pH values at fixed temperature (30°C, center of the domain); X2 axis = temperature values at fixed pH (6.6, center of the domain). a) *Carnobacterium maltaromaticum* LMA 28; b) *Streptococcus thermophilus* INRA 302; c) *Lactococcus lactis* DSMZ 20481.

C. maltaromaticum LMA 28, *S. thermophilus* INRA 302, and *Lc. lactis* DSMZ 20481 are summarized in Table 3.

The $V_{\max}$ value compared at the domain center (Table 3) was least for *C. maltaromaticum* LMA 28 ($\beta_0 = 0.130$) and greatest for *Lc. lactis* DSMZ 20481 ($\beta_0 = 0.455$). For *C. maltaromaticum* LMA 28, coefficient terms of the polynomial equation showed that the initial pH (pH_i) positively influenced $V_{\max}$ ($\beta_1 = 0.067$); therefore, the increase of the pH value improved the acidifying performance of *C. maltaromaticum* LMA 28. Incubation temperature had no influence on $V_{\max}$, but the interactions between the pH_i and temperatures had an influence ($\beta_{12} = 0.069$, Figure 4a).

The $V_{\max}$ of milk acidified by *S. thermophilus* INRA 302 was greater than that of *C. maltaromaticum* LMA 28 ($\beta_0 = 0.360$). Moreover, this thermophilic strain was not tested at its optimal growth temperature (40 to 41°C) but at lower temperatures (23 to 37°C). In the range of pH tested, the acidification rate varied from 0.36 to -0.08 pH unit/h. The influence of the incubation temperature was as important as the initial pH of milk. As expected, the increase of the incubation temperature increased the acidification of milk, and the interaction between pH_i and temperature also had a notable influence on $V_{\max}$ (Figure 4b).

The $V_{\max}$ values of *Lc. lactis* DSMZ 20481 were influenced by pH values; lowering the pH_i reduced the

$V_{\max}$ of milk. The incubation temperature also had a positive impact on $V_{\max}$ when the pH was >6.6 (Figure 4c). Increasing pH and temperature optimized the $V_{\max}$ value for the 3 strains, with pH having a greater impact on *C. maltaromaticum* LMA 28, temperature having a greater impact on *S. thermophilus* INRA 302, and both pH and temperature having an impact on *Lc. lactis* DSMZ 20481.

The $T_{\max}$ values were compared at the domain center (Table 3). The lower $T_{\max}$ expressed the faster acidifying character of the strain. Therefore, *Lc. lactis* DSMZ 20481 was the fastest strain ($\beta_0 = 9.000$) and *C. maltaromaticum* LMA 28 the slowest ($\beta_0 = 19.000$). For *C. maltaromaticum* LMA 28, incubation temperature reduced the $T_{\max}$ value and pH increased it. For *S. thermophilus* INRA 302, under these experimental conditions, temperature negatively influenced $T_{\max}$ ($\beta_2 = -27.135$); the reduction of the incubation temperature decreased its acidifying performance. For *Lc. lactis* DSMZ 20481, $T_{\max}$ increased when temperature decreased ($\beta_2 = -4.33$), whereas pH had a weak impact on that parameter.

The optimized culture conditions for *C. maltaromaticum* LMA 28 were different from those of *S. thermophilus* INRA 302 and *Lc. lactis* DSMZ 20481 (Figure 5). For *C. maltaromaticum* LMA 28, the reduction of $T_{\max}$ was obtained with an increase of pH (until 8.0) and a decrease of temperature (28°C), unlike *S. thermophilus* INRA 302. For *Lc. lactis* DSMZ 20481, the reduction of $T_{\max}$ was obtained with an increase of temperature.

In soft-cheese manufacture, these 3 species could play a major role at different stages of the process. First, *S. thermophilus* rapidly acidified milk at 39°C. Then, *Lc. lactis* (the mesophilic species usually used as a starter) took over and could acidify when the curd temperature decreased (28 to 30°C). Then, *C. maltaromaticum*, a psychrotrophic bacterium able to growth at alkaline pH, could act as a ripening flora.

Carnobacterium maltaromaticum is compatible with a starter such as *Lc. lactis* or *S. thermophilus* with no modification of the acidifying curve. One strain of this species was first isolated from milk having developed a distinct malty aroma (Miller et al., 1974), which corresponded to aldehydes such as 3-methylbutanal (Larrouture-Thiveyrat and Montel, 2003). Such a flavor could be considered an off-flavor if too pronounced. On the other hand, it could modify the characteristics of the product. In fact, cheeses containing *Carnobacterium* do not exhibit off-flavors; for example, the Camembert Réo, which contained more than 7 log₁₀ cfu/g of *C. maltaromaticum* (Cailliez-Grimal et al., 2007). Further studies, including evaluation of conditions to produce such a malty aroma, are needed to achieve a

better understanding of the role of *C. maltaromaticum* in French soft cheeses.

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