Growth of Nonproteolytic *Lactococcus lactis* in Culture Medium Supplemented with Different Casein Hydrolyzates[1]

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ABSTRACT

The growth and lactic acid production of nonproteolytic variants of *Lactococcus lactis*, three *Lactococcus lactis* ssp. *cremoris* strains (E8, Wg2, and HP) and one *Lactococcus lactis* ssp. *lactis* strain (1076), were compared with those of their parent strains in Garches medium supplemented with different casein hydrolyzates. Molecular weight distribution and AA composition of casein hydrolyzates were different. Two fractions of alcalase casein hydrolyzates separated and concentrated by a two-step ultrafiltration process were compared with two commercial casein hydrolyzates. Proteinase-negative variants of lactococci exhibited the same specific growth rate and production of lactic acid as proteinase-positive strains in all enriched Garches media. Cell growth was affected by molecular weight distribution of peptides in hydrolyzates, but not by their AA composition. *Lactococcus lactis* ssp. *lactis* grew better than *L. lactis* ssp. *cremoris*, but its lactic acid production was similar to that of E8 strains. Among *L. lactis* ssp. *cremoris*, Wg2 strains grew better in Garches medium supplemented with casein hydrolyzates with molecular weight <2000 Da, but growth and lactic acid production of HP strains were better in Garches medium enriched with casein hydrolyzates with molecular weight >2000 Da. Different casein hydrolyzate fractions could be used to supplement culture medium and to standardize milk cultures; however, choice of casein hydrolyzates depends on subspecies of lactococci.

(**Key words**: *Lactococcus*, alcalase, casein hydrolyzates, culture media)

Abbreviation key: **AL-AA** = alcalase AA fractions, **AL-MP** = alcalase mixture of polypeptides, **BCA** = Bacto casamino acid, **MWD** = molecular weight distribution, **Prt$^+$** = proteinase-positive, **Prt$^-$** = proteinase-negative, **RSM** = reconstituted skim milk, **TP** = trypticase peptone, μ_{max} = maximum specific growth rate.

INTRODUCTION

Mixed cultures of *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* are used as starters to produce numerous cultured dairy products (24). Lactococci acidify milk by formation of lactic acid from lactose and modify texture and flavor of dairy products. However, concentrations of free AA and peptides in milk are insufficient to support extended growth of lactococci (7). These lactic acid bacteria grow better in rich synthetic media than in milk (9, 19, 26). Additional AA and

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peptides needed for the growth of starters can be obtained from proteolysis of caseins. The process of casein degradation and utilization requires the combined action of cell wall-associated caseolytic proteinase, extracellular peptidase, AA transport systems, peptide transport systems, and intracellular peptidases (12, 13, 25), which form the complex proteolytic system of lactococci.

Some lactococci stop growing after depletion of all free AA and peptides from milk. These strains are nonproteolytic, proteinase-negative variants (**Prt⁻**) and cannot hydrolyze milk casein to obtain additional AA as opposed to proteolytic, proteinase-positive strains (**Prt⁺**) (27, 28). Specific growth rate of Prt⁻ variants and Prt⁺ strains is apparently associated with the composition of the growth medium (3, 8). In milk, the specific growth rate of Prt⁻ variants was lower than that of Prt⁺ strains (26), but both were similar in rich synthetic medium (19).

The objective of this study was to compare growth and lactic acid production of Prt⁻ and Prt⁺ strains of *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* in synthetic Garches medium. The synthetic medium was supplemented with different casein hydrolyzates to estimate the effect of peptide length and AA composition on the specific growth rate of lactococci.

MATERIALS AND METHODS

Casein Hydrolyzates

Hydrolyzates used to supplement Garches synthetic medium were casein pancreatic digest, trypticase peptone (**TP**) (BBL, Becton Dickinson, Cockeysville, MD), casein acid hydrolyzate, Bacto casamino acids, (**BCA**) (Difco Laboratories, Detroit, MI), alcalase mixture of polypeptides (**AL-MP**), and alcalase AA fraction (**AL-AA**) obtained from alcalase casein hydrolyzates according to the method described by Turgeon and Gauthier (29).

Sodium caseinate (ICN Pharmaceuticals Inc., Irvine, CA) was suspended in distilled water to obtain a solution containing 3.5% (wt/vol) of protein. This solution was hydrolyzed with an alcalase solution (5% wt/vol in .001*N* HCl) obtained from Novo Industri, Enzymes Division, Bagsvaerd, Denmark. The enzyme: substrate ratio was adjusted to 1:200. The hydrolysis was performed at 40°C for 45 min, and pH was maintained at 8 by addition of 4*N* NaOH. The proteolytic products were concentrated three times and diafiltered twice by UF using a membrane with a molecular cutoff of 30,000 Da (HF1-43-PM30; Romicon Inc., Woburn, MA). The permeate of the first UF was further partitioned by a second UF concentrated four times) with a smaller cutoff membrane of 1000 Da (two HF1-43-PM1; Romicon Inc.) to remove AA and small peptides. The retentate of the second UF was the AL- MP. The permeate fraction, composed of AA and small peptides, was the AL-AA. All fractions were lyophilized and stored at room temperature (23°C).

Chemical Analysis

Molecular weight distribution (**MWD**) profiles of all casein hydrolyzates were determined by high performance size exclusion chromatography (HPSEC, LKB Systems; Rockville, MD) using a TSK-2000SW column according to method reported by Turgeon and Gauthier (29). Protein standards were ovalbumin (42,950 Da), ribonuclease A (13,710 Da), polymyxin-β-sulfate (1447 Da), β-chain of insulin (3480 Da), and L-leucyl-L-leucyl-L-leucine (358 Da). Surface integration of the chromatograms was performed to compare the MWD of protein components from casein hydrolyzates. Total surface was separated into three ranges (<2000, 2000 to 5000, and >5000 Da) and expressed as a percentage of the total surface.

The AA composition of all casein hydrolyzates was determined with a High Performance Amino Acid System (6300; Beckman, Palo Alto, CA), following acid hydrolysis (HCl 6*N*, 100°C, 24 h) (29).

Total protein concentration of different casein hydrolyzates was measured using the macro-Kjeldahl method (2). The nitrogen to protein conversion factor was 6.38.

Starters

A Prt⁺ strain of *L. lactis* ssp. *lactis*, CNRZ 1076 (1076S), and its Prt⁻ variant, CNRZ 1075 (1075L), were purchased from the Centre de Recherche de Jouy-en-Josas (Institut national de recherche agronomique, Domaine de Vil-

vert, France). Three Prt$^+$ strains of *L. lactis* ssp. *cremoris* (Wg2S, HPS, and E8S) and their Prt$^-$ variants (Wg2L, HPL, and E8L) were obtained from the Netherlands Institute for Dairy Research (NIZO, Ede, The Netherlands). Each lactococcal culture was kept in reconstituted skim milk (RSM; 20% DM) containing 5% sucrose and .17% ascorbic acid, frozen, and stored at –60°C.

Plasmid Profile

The strains were thawed at room temperature (23°C), and two transfers were performed at 22°C for 16 h in M17 broth (Difco Laboratories) supplemented with .05% glucose (3% inoculum). The last transfer was performed in modified Elliker broth (Difco Laboratories) (10). The plasmid profile was determined for all strains of lactococci according to the method described by Anderson and McKay (1).

Cultivation and Growth Experiments

Milk was sterilized at 110°C for 10 min. The first transfer (for all strains of lactococci) was performed in RSM (12% DM) supplemented with .2% yeast extract (Difco Laboratories), and incubation was performed for 16 h at 22°C. For the second transfer, growth was in RSM without yeast extract for Prt$^+$ strains and with .2% yeast extract for Prt$^-$ strains for 6 h at 30°C. The last transfer was in M17 broth and incubated for 16 h at 22°C. Cultures were then centrifuged at 5000 rpm for 5 min at 23°C (Sorvall® RC-5B Centrifuge; Du Pont Co., Wilmington, DE). The pellet was washed with sterile saline solution (.9% NaCl). Bacteria were diluted in a saline solution and adjusted to an optical density of 1.25 (λ = 635 nm) using a Beckman spectrophotometer (DU® Series 60 Spectrophotometer; Beckman Instruments Inc., Fullerton, CA). The concentration of the inoculum was approximately 10^7 cfu/ml.

Garches medium (20) without BCA was used to test casein hydrolyzates. This medium contained 5 g/L of Asn, .2 g/L of Cys, 5 g/L of anhydrous sodium acetate, 10 g/L of lactose, .9 g/L of dibasic potassium phosphate, 2.33 g/L of dibasic sodium phosphate, .5 g/L of magnesium sulfate, 1 mg/L of *p*-aminobenzoic acid, 10 μg/L of biotin, and 1 mg/L of calcium pantothenate. Casein hydrolyzates (BCA, TP, AL-MP, and AL-AA) were added in concentration of 2.0% (wt/wt). All media, adjusted to pH 6.5, were sterilized using a 1000-ml presterilized Nalgene® disposable filter of .2 μ (Nalge Company, Rochester, NY). Each medium was distributed in 15-ml aliquots in 30-ml assay tubes. All media were inoculated with bacterial suspension and incubated at 30°C for 12 h. Each fermentation was conducted in triplicate except for fermentations in Garches medium supplemented with the BCA fraction, which were performed in duplicate.

Viable colony-forming units per milliliter were enumerated on M17 agar plates (Difco Laboratories), which were incubated anaerobically (gas pak system; BBL, Becton Dickinson) at 30°C for 48 h. Sample dilutions were in .1% peptone water. However, because some lactococci grow in long chains (5), approximately 3 g of glass beads (4 mm) were added in each dilution bottle and shaken vigorously 40 times before plating on M17 agar. Maximum specific growth rates (μ_{max}) were calculated during exponential growth using the following equation:

$$\ln X = \ln X_0 + \mu_{max} T \qquad [1]$$

where X_0 (intercept) is the biomass when time T = 0. The plot of ln X against time is a straight line with slope, μ_{max}.

Titratable acidity, at the color change of phenolphthalein, was measured using a pH meter (Radiometer model PHM84 and titrator model TTT80; Copenhagen, Denmark) by addition of .11 *M* NaOH until the end point was reached (pH 8.6). Results were reported as grams of lactic acid per liter; values at 0 h were subtracted from the total. Growth and acidity were measured every 2 h.

Statistical Methods

Analysis of variance according to a split-plot design was used to determine the effects of different casein hydrolyzates and different strains of lactococci. The main plots were different Garches media and their replicates; the subplots were the strains. All of the main plots were conducted three times, except for Garches medium BCA, which was only replicated twice and was accounted for in the analysis of variance. Special attention was given to the computation of adjusted means, *F* tests, and standard errors (18). These statistical ana-

lyses were performed with the general linear models procedure of SAS (23).

RESULTS

Lactococci

Agarose gel electrophoresis of plasmid DNA, isolated from Prt$^+$ and Prt$^-$ strains of lactococci (Figure 1), shows that all Prt$^-$ variants lost a plasmid, except strain 1075L. The plasmid profile of variant 1075L was identical to the plasmid profile of strain 1076S.

The Prt$^+$ strains coagulated milk rapidly at 22°C, but Prt$^-$ variants took several days to coagulate milk at this temperature. No Prt$^-$ variants used in this study, including variant 1075L, could coagulate RSM (12% DM) without yeast extract, as opposed to Prt$^+$ strains (data not shown). Moreover, all Prt$^-$ variants produced small colonies on buffered milk agar compared with Prt$^+$ strains, which produced large colonies (16) (data not shown). Strain 1075L seemed to be a true Prt$^-$ variant in spite of its plasmid profile, which was identical to that of its parent strain, 1076S.

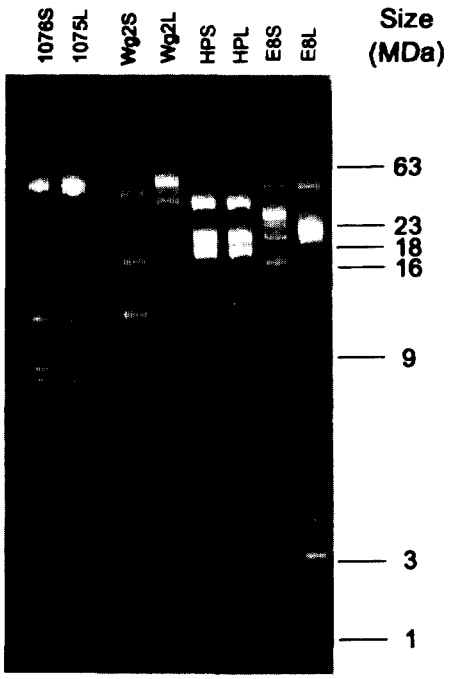

Figure 1. Agarose gel electrophoresis of plasmid DNA from proteinase-positive (1076S, Wg2S, HPS, and E8S) and proteinase-negative (1075L, Wg2L, HPL, and E8L) strains of lactococci. Sizes were estimated using *Escherichia coli* V517 as size standard.

Casein Hydrolyzates

The MWD of peptides contained in BCA, TP, AL-MP, and AL-AA casein hydrolyzates are given in Table 1. The BCA was essentially composed of small peptides (<2000 Da) and AA, whereas other casein hydrolyzates also contained larger peptides. The TP had a higher content of larger peptides than did other casein hydrolyzates, especially peptides with a molecular weight between 5000 and 2000 Da. The MWD of peptides for AL-MP and AL-AA fractions was intermediate between those of TP and BCA casein hydrolyzates.

The AA composition of different casein hydrolyzates is presented in Table 2. The concentration of AA in TP fractions was balanced (about 2.24 mg of each AA/100 mg of the TP

TABLE 1. Molecular weight distribution (MWD) of peptides contained in different hydrolyzates.

MWD[1]	Hydrolyzate fraction[2]			
	TP	BCA	Al-MP	AL-AA
	(%)			
MWD > 5000 Da	.5	0	.2	.1
5000 Da > MWD > 2000 Da	3.0	0	1.7	.8
MWD < 2000 Da	96.5	100.0	98.1	99.1

[1]The MWD was calculated from the integration of the total surface of the chromatogram. The total surface was separated in three ranges of molecular weight and expressed in percentage of the surface.

[2]TP = Trypticase peptone, BCA = Bacto casamino acids, AL-MP = alcalase mixtures of polypeptide, AL-AA = alcalase AA fractions.

TABLE 2. The AA composition of different hydrolyzates.

	Hydrolyzate fraction[1]			
	TP	BCA	AL-MP	AL-AA
	(mg/100 mg of hydrolyzates)			
Protein	82.06	64.18	82.99	83.44
AA				
Asp	2.26	4.28	4.35	4.92
Thr	2.04	2.59	3.18	3.20
Ser	1.80	3.04	3.19	3.40
Glu	2.50	3.23	3.45	3.47
Pro	1.94	7.83	8.01	8.90
Gly	1.28	1.36	1.64	1.63
Ala	1.51	3.22	2.51	2.95
Val	1.97	4.03	5.59	5.24
Met	2.57	1.03	.71	1.54
Ile	2.19	3.03	3.01	3.37
Leu	2.21	5.06	3.66	4.86
Tyr	3.05	1.03	4.77	.58
Phe	2.76	2.91	4.67	2.50
His	2.60	1.70	2.41	2.30
Lys	2.41	3.74	4.07	4.39
Arg	2.82	2.70	2.16	2.22
Total AA	35.91	50.78	57.38	55.47

[1]TP = Trypticase peptone, BCA = Bacto casamino acids. AL-MP = alcalase mixtures of polypeptide, AL-AA = alcalase AA fractions.

hydrolyzate fraction), but AA concentrations in BCA, AL-MP, and AL-AA fractions were more varied.

The AA concentrations in BCA and AL-AA fractions were very similar. In the AL-MP fraction, the concentrations of Met and Leu were lower, and concentrations of Tyr and Phe were higher, than those estimated in BCA and AL-AA fractions (Table 2). The use of two-step UF to separate and to concentrate alcalase hydrolyzates resulted in higher concentrations of Tyr and Phe, but lower concentrations of Met and Leu, in the AL-MP fraction than in the AL-AA fraction.

Growth and Lactic Acid Production

Growth and lactic acid production of lactococci in Garches medium supplemented with different casein hydrolyzates are presented in Figures 2 and 3, respectively. All lactococci grew in all media, except strains HPS and HPL, which did not grow in Garches medium enriched with BCA fraction (Figure 2). The growth and lactic acid production of all Prt^- variants were similar to those of Prt^+ strains. An uncoupling between bacterial growth and lactic acid production was observed (i.e., lactococci stopped growing, but production of lactic acid continued) for the 1076S and 1075L, E8S and E8L, and HPS and HPL strains in Garches medium supplemented with the casein hydrolyzates tested. However, for strains Wg2S and Wg2L, an uncoupling was only observed in Garches medium supplemented with TP and BCA fractions. Lactococcal strains 1076S, 1075L, E8S, and E8L in Garches medium enriched with TP fraction reached the stationary phase of growth after 4 h, but, with other casein hydrolyzates, the stationary phase was reached after 6 h. Strains HP and Wg2 and their Prt^- variants also reached the stationary phase after 6 h in all hydrolyzates.

Analysis of variance (Table 3) shows significant ($P < .01$) interaction between Garches medium supplemented with different casein hydrolyzates and different strains of lactococci on μ_{max} and lactic acid production. Growth of lactococci was significantly different ($P < .01$) in culture media enriched with different fractions that have different MWD, especially in

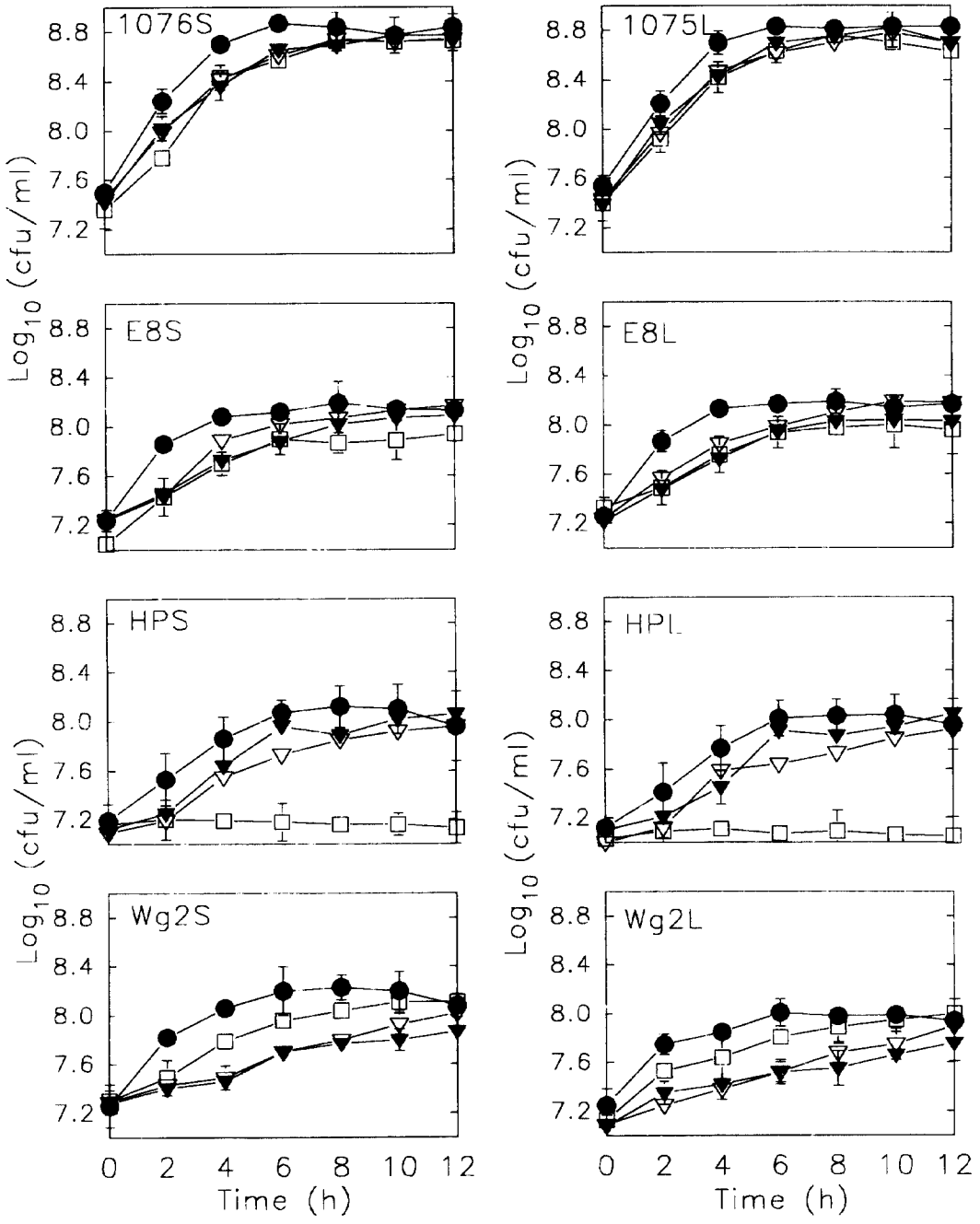

Figure 2. Growth of proteinase-positive (1076S, E8S, HPS, and Wg2S) and proteinase-negative (1075L, E8L, HPL, and Wg2L) strains of lactococci in Garches medium supplemented with trypticase peptone (●), alcalase mixtures of polypeptide (▼), alcalase AA fractions (▽), and Bacto casamino acids (□). Error bars represent the standard errors of the means.

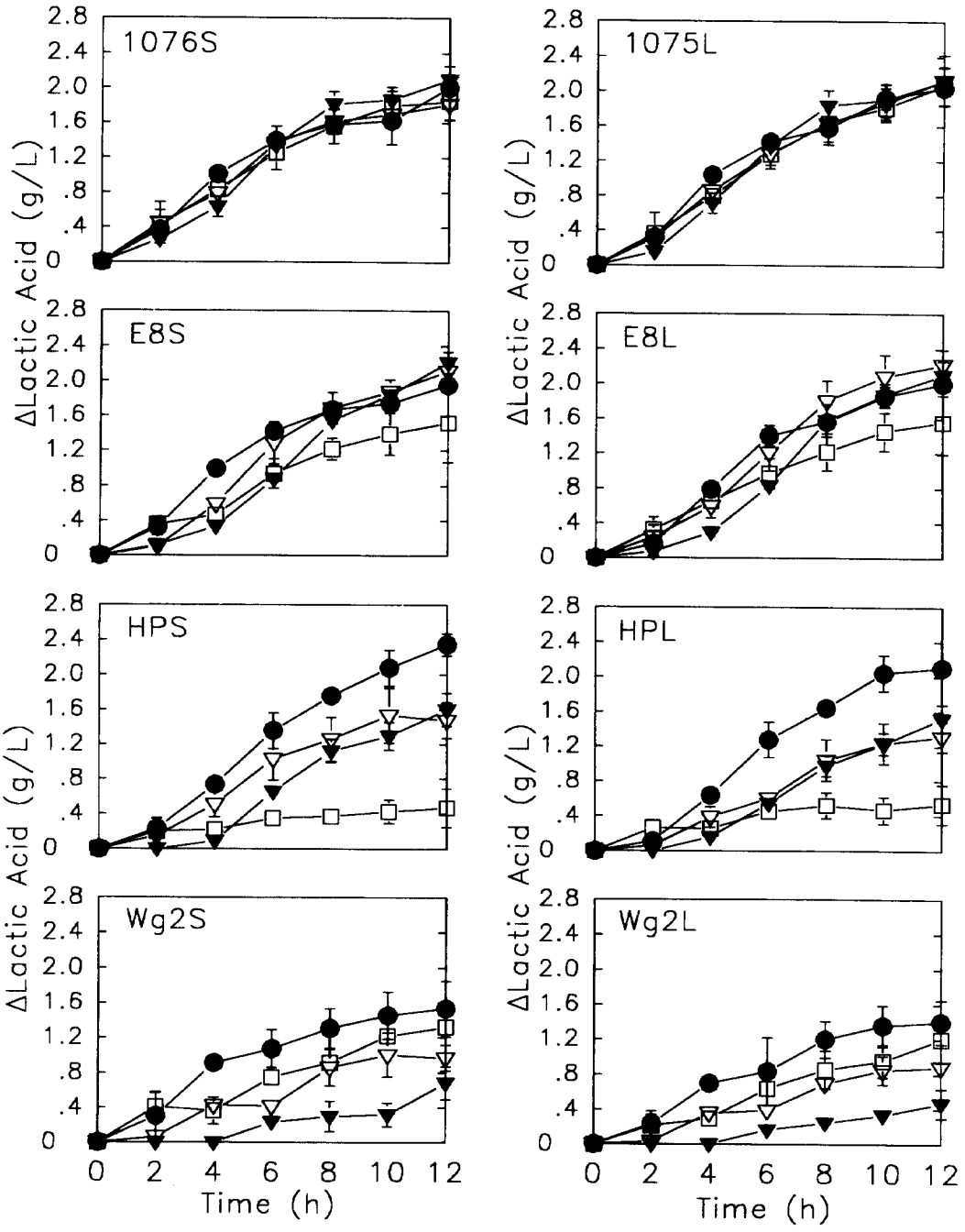

Figure 3. Lactic acid production (ΔLactic Acid) of proteinase-positive (1076S, E8S, HPS, and Wg2S) and proteinase-negative (1075L, E8L, HPL, and Wg2L) strains of lactococci in Garches medium supplemented with trypticase peptone (●), alcalase mixtures of polypeptide (▼), alcalase AA fractions (▽), and Bacto casamino acids (□). Error bars represent the standard errors of the means.

TABLE 3. Effect of Garches medium supplemented with different casein hydrolyzates on specific growth rate and lactic acid production of lactococcal strains.

Factors	df	MS	
		μ_{max}	ΔLactic acid[1]
			(g/L)
Replications	2	.00042	11.643
Culture media	3	.08649**	11.874
Error A	4	.00412	6.409
Strains	7	.016943**	16.764**
Media × strains	21	.01814**	3.264**
Error B	42	.00101	.243

[1]ΔLactic acid = Lactic acid production.

**$P < .01$.

Garches medium supplemented with BCA fraction (Figures 2 and 3).

The μ_{max} for Prt^+ and Prt^- strains in Garches medium supplemented with TP, AL-MP, Al-AA, and BCA casein hydrolyzates are presented in Table 4. Lactococcal Prt^- had μ_{max} similar ($P > .05$) to that of Prt^+ strains.

In Garches medium supplemented with TP and AL-AA fractions, *L. lactis* ssp. *lactis* 1076S and 1075L had significantly higher μ_{max} ($P < .01$) than *L. lactis* ssp. *cremoris*. Among *L. lactis* ssp. *cremoris* strains, μ_{max} of Wg2S and Wg2L were significantly lower ($P < .01$) than that of other strains, but μ_{max} of strains E8S, E8L, HPS, and HPL were similar ($P > .05$).

In Garches medium supplemented with AL-MP hydrolyzate, μ_{max} of strains Wg2S and Wg2L was also significantly lower ($P < .01$) than that of other strains. No significant ($P > .05$) difference was observed between other strains, except for HPS, for which μ_{max} was similar to those of Wg2S and Wg2L.

In Garches medium supplemented with BCA hydrolyzate, μ_{max} of strains 1076S and 1075L was significantly higher ($P < .01$) than that of *L. lactis* ssp. *cremoris* strains. However, as opposed to other casein hydrolyzates, strains Wg2S and Wg2L had significantly higher ($P < .01$) μ_{max} than strains HPS and HPL, but similar ($P > .05$) to those obtained for strains E8S and E8L.

The μ_{max} of strains 1076S, 1075L, E8S, and E8L were significantly higher ($P < .01$) in Garches medium supplemented with TP fraction than with other casein hydrolyzates. However, μ_{max} of strains HPS and HPL were significantly lower ($P < .01$) in Garches medium enriched with BCA hydrolyzate than in other casein hydrolyzates, but μ_{max} of strains Wg2S and Wg2L were significantly higher ($P < .01$) in BCA fraction than in other fractions. In addition, μ_{max} of lactococcal strains estimated in the presence of the AL-MP fraction were similar ($P > .05$) to those estimated with the AL-AA fraction.

TABLE 4. Maximum specific growth rate (μ_{max}) of proteinase-positive (Prt^+) and proteinase-negative (Prt^-) lactococci in Garches medium supplemented with different casein hydrolyzates.[1]

Type	Strain	Specific growth rate[2]			
		TP	AL-MP	AL-AA	BCA
Prt^+	1076S	$.69^{a,A}$	$.40^{ab,B}$	$.45^{a,B}$	$.50^{a,B}$
	E8S	$.51^{b,A}$	$.37^{ab,B}$	$.25^{bc,B}$	$.35^{b,B}$
	HPS	$.41^{b,A}$	$.27^{bc,A}$	$.34^{b,A}$	$.05^{c,B}$
	Wg2S	$.17^{c,B}$	$.15^{c,B}$	$.16^{c,B}$	$.25^{b,A}$
Prt^-	1075L	$.67^{a,A}$	$.49^{a,B}$	$.47^{a,B}$	$.50^{a,B}$
	E8L	$.47^{b,A}$	$.29^{bc,B}$	$.26^{bc,B}$	$.27^{b,B}$
	HPL	$.38^{b,A}$	$.33^{ab,A}$	$.32^{b,A}$	$.04^{c,B}$
	Wg2L	$.24^{c,A}$	$.16^{c,B}$	$.12^{c,B}$	$.27^{b,A}$

^{a,b,c}Means with the same superscripts in the same column do not differ ($P \geq .01$).

^{A,B}Means with the same superscripts in the same row do not differ ($P \geq .01$).

[1]TP = Trypticase peptone, AL-MP = alcalase mixture of polypeptides, AL-AA = alcalase AA fractions, BCA = Bacto casamino acids.

[2]Standard error of means for Garches medium supplemented with TP, AL-MP, and AL-AA hydrolyzates was .03, and for Garches medium supplemented with BCA hydrolyzate was .04.

Lactic acid production for Prt^+ and Prt^- strains in Garches medium supplemented with TP, AL-MP, AL-AA, and BCA casein hydrolyzates are presented in Table 5. Lactic acid production of lactococci after 12 h was similar ($P > .05$) in all media, except for strains HPS and HPL in BCA fraction and for strains Wg2S and Wg2L in AL-MP and AL-AA fractions. Moreover, no significant ($P > .05$) difference was observed between production of lactic acid of Prt^- variants and Prt^+ strains.

In Garches medium supplemented with TP fraction, all lactococci produced similar ($P > .05$) quantities of lactic acid. In Garches medium supplemented with AL-MP and AL-AA fractions, production of lactic acid was significantly lower ($P < .01$) for strains Wg2S and Wg2L than for other strains of lactococci. However, in Garches medium supplemented with BCA fraction, lactic acid production of strains HPS and HPL was lower than for other lactococci. The difference between strains of lactococci for lactic acid production was less pronounced than for μ_{max} because of an uncoupling effect between growth and lactic acid production. Production of lactic acid of most strains continued even if bacterial growth stopped after 4 or 6 h of incubation (Figures 1 and 2).

DISCUSSION

The Prt^- strains are genetic variants of lactococci that cannot hydrolyze casein because they do not have cell-wall protease (27, 28). In some strains of lactococci, plasmids are suspected to encode for the proteolytic systems (11, 17, 22). In this study, plasmid profile supports evidence that genetic changes occurred between Prt^+ and Prt^- strains of *L. lactis* ssp. *cremoris*. However, plasmid profile of *L. lactis* ssp. *lactis* did not support genetic changes between variant 1075L Prt^- and its parent cell, strain 1076S, in spite of the fact that strain 1075L seemed to be a true Prt^-.

In milk, μ_{max} of Prt^- variants are generally lower than that of Prt^+ strains (26). However, the growth rate between Prt^- and Prt^+ strains is generally similar in rich synthetic medium (19), which is in agreement with results observed in this study. Different casein hydrolyzates used to supplement Garches medium had slightly different MWD. Three of these casein hydrolyzates were constituted of peptides with molecular weight >2000 Da (TP, AL-MP, and AL-AA), and BCA fraction had only peptides with molecular weight <2000 Da. None of these casein hydrolyzates inhibited growth or lactic acid production of Prt^- and Prt^+ strains. In these rich Garches media, Prt^- variants and Prt^+ strains did not have to hydrolyze proteins to obtain peptides and AA because casein hydrolyzates were rich in peptides and in AA. Peptide transport systems (15, 21) and peptidase activities (6) of Prt^- variants seemed to be similar to those of parent cells.

Cell growth and production of lactic acid differed between subspecies of *Lactococcus lactis*. In all Garches media, *L. lactis* ssp. *lactis* had higher μ_{max} than *L. lactis* ssp. *cremoris*. Lactococci have limited biosynthetic abilities; they need AA, such as Leu, Met, Glu, His, and Phe, to stimulate their growth (7, 27). However, *L. lactis* ssp. *lactis* has lower AA requirements than *L. lactis* ssp. *cremoris* (28).

Among strains of *L. lactis* ssp. *cremoris*, strains E8 had the highest μ_{max} in all supplemented Garches media, and strains Wg2 had the lowest μ_{max}, but only in Garches medium supplemented with casein hydrolyzates that have MWD >2000 Da (TP, AL-MP, and AL-AA). In Garches medium enriched with BCA fraction, which had MWD <2000 Da, strains Wg2 and E8 had similar μ_{max}. Contrary to strains Wg2, strains HP grew well in Garches medium enriched with casein hydrolyzates that have high MWD, but very slowly in Garches medium with BCA fraction. In milk, most strains of lactococci can satisfy their growth requirements with peptides and AA (14, 21, 30). The latter are obtained from hydrolysis of casein by a complex lactococcal proteolytic system (13). However, Hugenholtz et al. (7) have shown that, in chemically defined media, peptides seem to be better lactococcal growth factors than AA. In the present study, some lactococci (strains E8 and, especially, strains HP) grew better in Garches medium enriched with hydrolyzates that have higher MWD, whereas strains Wg2 preferred supplementation with BCA fraction, which had MWD <2000 Da.

Because of the uncoupling effect, differences between strains of lactococci for production of lactic acid were slight compared with those for μ_{max}. For *L. lactis* ssp. *cremoris*,

TABLE 5. Production of lactic acid by proteinase-positive (Prt+) and proteinase-negative (Prt−) lactococci in Garches medium supplemented with different casein hydrolyzates.[1]

Type	Strain	Lactic acid production[2]			
		TP	AL-MP	AL-AA	BCA
		(g/L)			
Prt+	1076S	2.04^{a,A}	1.70^{a,A}	2.10^{a,A}	1.76^{a,A}
	E8S	1.99^{a,A}	1.92^{a,A}	2.21^{a,A}	1.43^{a,A}
	HPS	2.38^{a,A}	1.48^{ab,A}	1.60^{a,A}	.38^{b,B}
	Wg2S	1.57^{a,A}	.83^{b,BC}	.68^{b,C}	1.23 ^{a,AB}
Prt−	1075L	2.08^{a,A}	1.89^{a,A}	2.12^{a,A}	1.98^{a,A}
	E8L	2.04^{a,A}	2.05^{a,A}	2.10^{a,A}	1.47^{a,A}
	HPL	2.14^{a,A}	1.05^{ab,A}	1.52^{a,A}	.45^{b,B}
	Wg2L	1.44^{a,A}	.82^{b,BC}	.47^{b,C}	1.11^{a,AB}

^{a,b,c}Means with the same superscripts in the same column do not differ ($P \geq .01$).

^{A,B,C}Means with the same superscripts in the same row do not differ ($P > .01$).

[1]TP = Trypticase peptone, AL-MP = alcalase mixture of polypeptides, AL-AA = alcalase AA fractions, BCA = Bacto casamino acids.

[2]Standard error of means for Garches medium supplemented with TP, AL-MP, and AL-AA hydrolyzates was .18 and for Garches medium supplemented with BCA hydrolyzate was .27.

lactic acid production was generally related to μ_{max}. Lactococcus lactis ssp. *cremoris* and *L. lactis* ssp. *lactis* are often used together in cheese making for their capacity to produce lactic acid (4). In the present study, lactic acid production of *L. lactis* ssp. *lactis* was similar to that of strains E8 and HP of *L. lactis* ssp. *cremoris*, in spite of the fact that strains of *L. lactis* ssp. *lactis* exhibited higher μ_{max} than strains of *L. lactis* ssp. *cremoris*.

The μ_{max}were generally higher in Garches medium supplemented with TP fraction. The AA composition of this fraction was balanced but did not correspond to normal concentrations of AA in casein (31). Moreover, this fraction contained 56% of unidentified non-AA N. As opposed to TP fraction, concentrations of AA in BCA, AL-MP, and AL-AA hydrolyzate fractions had an AA profile similar to that of casein (31) and only 21, 24, and 26% of unidentified non-AA N, respectively. The high MWD of peptides, balance between AA concentrations, and high concentration of non-AA N in TP fraction may explain higher cell growth obtained with TP fraction. For cell growth, concentrations of Met, Leu, Tyr, and Phe in the AL-MP fraction are less important than MWD.

The μ_{max} and lactic acid production did not differ between the AL-AA and AL-MP fractions. Permeate (the AL-AA fraction), obtained after the second UF with a cutoff membrane of 1000 Da, was considered to be a by-product (29). According to our results, this by-product could be used to supplement growth media for lactococci.

Medium supplementation with specific casein hydrolyzates may also depend on lactococci subspecies. Some strains, such as strains HP, grew better in media enriched with large peptides, but others, such as strains Wg2, preferred media with small peptides. Lactococci possess a number of proteinases, peptidases, and different transport systems of AA and peptides. The combined action of these enzymes and transport systems determines which AA become available for growth (28). Some subspecies of lactococci can proceed only with dipeptide and tripeptide transport systems, but others obtain essential AA from direct uptake of the free AA (12).

Alcalase casein hydrolyzates may be available to standardize milk cultures to grow some lactococci used as starters during cheese making. St-Gelais et al. (26) showed that strains E8 and HP could be used together in mixed cultures to produce Cheddar cheese. As presented in this study for *L. lactis* ssp. *cremoris*, the E8 and HP Prt− variants and their parent strains exhibited high μ_{max} and lactic acid production in Garches medium supplemented with AL-AA and AL-MP casein hydrolyzate fractions.

CONCLUSIONS

This study showed that different casein hydrolyzates rich in AA and peptides could be used to supplement culture medium and to promote the growth of Prt^+ strains of lactococci and their Prt^- variants. Moreover, some strains of lactococci grew better in culture media enriched with casein hydrolyzates that have high MWD, whereas other strains of lactococci preferred culture media supplemented with casein hydrolyzates that have low MWD.

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