0273-2289/96/5903—0245$08.25

Effect of pH on Growth and Fatty Acid Composition of *Lactobacillus büchneri* and *Lactobacillus fermentum*

PEKKA NIKKILÄ,* TOM JOHNSSON, HEIKKI ROSENQVIST, AND LEENA TOIVONEN

Helsinki Department of Technology, Department of Chemical Engineering, Laboratory of Biochemistry and Microbiology, Kemistintie 1, 02150 Espoo, Finland

Received January 30, 1995; Accepted May 2, 1995

ABSTRACT

Growth and fatty acid composition of two lactobacilli, *Lactobacillus büchneri* and *Lactobacillus fermentum*, were studied at different pHs of the media in a small-scale fermenter with particular interest in lactobacillic acid production of the cultures. Generally, the total fatty acid content of the bacterial cells increased with increasing culture age. The production of lactobacillic acid was affected in the two lactobacilli by both culture age and pH of the media, but in a very different manner. In *L. büchneri* cultures, the relative proportion of lactobacillic acid was highest when the pH was lowest (pH 4.5), whereas in *L. fermentum* cultures, the proportion of lactobacillic acid was highest at pH 7.0. The pH of the medium affected not only the relative proportion of lactobacillic acid, but also biomass production and total fatty acid accumulation of the cultures. Thus, by controlling the pH of the cultures, the volumetric yield of lactobacillic acid could be improved considerably compared to cultures without pH control.

Index Entries: Lactobacilli; fermentation; fatty acid composition; cyclopropane fatty acids; effect of pH.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Cyclopropane fatty acids have for several decades been known to occur as phospholipid and glycolipid esters in membranes of many different eubacteria. These bacteria include members of *Enterobacteriaceae, Pseudomonadaceae, Rhizobiaceae,* and *Lactobacillaceae* (*1*).

The main cyclopropane fatty acids of lactobacilli, lactobacillic acid (11,12-methyleneoctadecanoic acid; cy19:0[11c]) and dihydrosterculic acid (9,10-methyleneoctadecanoic acid; cy19:0[9c]), are formed by methylation of cis-vaccenic (18:1[11c]) and oleic acid (18:1[9c]), respectively. However, dihydrosterculic acid has generally been found only if oleic acid is added into the medium (*2*). The reaction is catalyzed by cyclopropane fatty acid (CFA) synthase, a soluble enzyme found in the cell cytoplasm, and it is known to require *S*-adenosyl-L-methionine (SAM) as the alkylating agent. A free monounsaturated fatty acid cannot act as lipid substrate, but it must be in an acylated form, bound to membrane lipids, which means that the enzymatic reaction takes place in a hydrophobic environment (*2,3*).

In spite of many investigations, the physiological significance of the synthesis of CFAs as well as the factors controlling the onset of their accumulation, still remain obscure (*4*). The regulatory and physiological aspects of CFA formation have been most thoroughly studied in *Escherichia coli* (*5*). In addition, the effects of cultural conditions on cyclopropane fatty acid formation have been studied to some extent, e.g., in cultures of other *Enterobacteriaceae* as well as in *Lactobacillaceae,* and *Pseudomonales* (*4,6–13*). Unfortunately, the regulatory mechanisms controlling the CFA production seem to differ from species to species, and no general conclusions can be made.

CFA formation has generally increased with increasing culture age in different bacterial strains studied (e.g., *6,7,12,14,15*). In lactobacilli, the pH of the culture also decreases during growth owing to lactic acid production, and therefore, it was concluded that the CFA formation might be dependent on pH. In *Lactobacillus plantarum* cultures, substantial cyclopropane fatty acid formation was shown by Buist and Findlay (*10*) to occur at all pH values (4.1–7.0). However, lowering the pH of the medium enhanced lactobacillic acid production (*4,10*).

The purpose of the work presented here was to study the effect of pH on growth and fatty acid composition of two different *Lactobacillus* strains, *Lactobacillus büchneri* TKK B-1059 and *Lactobacillus fermentum* ATCC 14931. These strains were previously shown to produce appreciable amounts of CFAs, especially lactobacillic acid if a medium free of oleic acid was used (*16*). Our final goal was to achieve a high volumetric production of lactobacillic acid, which we consider a commercially interesting compound because of its biological activity: Lactobacillic acid among other cyclopropane fatty acids is claimed to affect the properties of cell membranes. In bacterial cultures, the conversion of unsaturated fatty acids into CFAs

Table 1
Composition of MRS Medium (*18*)

Amount, g/L	Reagent[a]
20	Glucose
10	Proteose peptone
10	Beef extract
5.0	Yeast extract
2.6	$K_2HPO_4*3H_2O$
5.0	Sodium acetate
1.7	Diammonium citrate
0.2	$MgSO_4*7H_2O$
0.005	$MnSO_4*4H_2O$
1.0	Tween 80

[a]All reagents used were pro-analysis-grade.

in the stationary phase is considered to allow the cells to maintain adequate cell membranes, since CFAs are less sensitive to oxidation, but still preserve the membrane fluidity (*17*). Furthermore, lactobacillic acid methyl esters are known to promote the lateral mobility through cell membranes, which gives rise to interesting pharmaceutical applications (*18,19*).

MATERIALS AND METHODS

Bacterial Strains and Growth Media

L. büchneri TKK B-1059 and *L. fermentum* ATCC 14931 were used for these studies. The bacteria were maintained in MRS agar medium (*20*) at 4°C and subcultured every 4 wk. The composition of MRS medium is given in Table 1.

For preparation of inocula for fermenter experiments, a modified MRS medium (here designated as MRS50-T) was used (MRS medium containing 50 g glucose/L, but no Tween 80). The media employed in fermenter cultivations contained (per 1 L of tap water): 50 g of glucose, 20 g of yeast extract, 20 g of tryptone, 1 g of diammoniumcitrate, 0.05 g of $MnSO_4*4H_2O$, 0.1 g of $MgSO_4*7H_2O$, and either 1 g (medium A) or 10 g (medium B) of $CH_3COONa*3H_2O$.

Fermenter Cultivations

To study the effect of pH on growth and fatty acid composition of *L. büchneri* and *L. fermentum*, fermenter experiments were carried out in a 2-L Braun Biostat MD fermenter (B. Braun Melsungen, Germany) with a working volume of 1 L.

The inoculum was cultivated in two stages: First 0.2 mL of bacterial culture grown at 37°C in MRS50-T medium for 6 h was transferred into 5 mL of MRS50-T medium and allowed to grow to the exponential phase (150–200 Klett units). This culture (0.5 mL) was used to inoculate an Erlenmeyer flask containing 50 mL of MRS50-T medium. The flask was shaken at 60 rpm in a Certomat orbital shaker/incubator (type R/HK) at 37°C until the culture reached exponential phase (150–200 Klett units) after which it was used to inoculate the fermenter containing either 1 L of medium A (*L. büchneri*) or 1 L of medium B (*L. fermentum*). During the preparation of inocula, growth was monitored with a Klett-Summerson colorimeter (filter no. 66).

The temperature in all fermenter cultivations was 37°C and stirring speed 100 rpm. The aeration rate was 0.16 L/min. The pH of the cultures (4.5–7.0) was controlled automatically by adding 10% NH_4OH. During the cultivations, samples (2 × 5 mL) were withdrawn for the analyses of growth, fatty acid composition, and glucose consumption of the bacteria until the stationary phase of growth was reached.

Analyses

The samples (5 mL) taken during the fermenter cultivations were centrifuged for 15 min (6000*g*). The glucose content of the growth medium (supernatant) was analyzed using the DNS-method of Fischer and Stein (*21*). The cells were washed with tap water, freeze-dried, and weighed to estimate the growth of the cultures as dry weight. The dried cells were stored in −20°C for 1–5 d before fatty acid analysis.

To analyze the fatty acid composition, the freeze-dried cells were suspended in excess of saponification reagent and analyzed as described by Suutari et al. (*22*). GC analysis of fatty acid methyl esters was carried out by Hewlett-Packard model 5890A gas chromatograph equipped with a flame ionization detector, a capillary liquid system, and a model 7673A automatic liquid sampler. The GC conditions were HP-FFAP WCOT (25 m × 0.2 mm × 0.3 μm) column; carrier gas He at 1 mL/min; split ratio 1:20; inj. vol. 1 μL; column inlet pressure 150 kPa; inj. temp. 250°C; det. temp. 250°C; temp. program from 70 to 200°C at 25°C/min. Data analysis was performed with HP 3365 ChemStation software. The compounds were identified by GC peak retention times relative to fatty acid methyl ester standards (Sigma, St. Louis, MO) and verified with a mass-selective detector (Hewlett-Packard model 5971A) as described by Johnsson et al. (*16*). The absolute amounts of fatty acids were calculated by using heptadecanoic acid methyl ester (Sigma) as an internal standard.

Results of all the analyses are mean values of two parallel samples analyzed separately.

RESULTS AND DISCUSSION

Effect of pH on Growth of *L. büchneri* and *L. fermentum*

During the cultivation of lactic acid bacteria, acid production causes a dramatic decrease in pH of the medium, and finally the growth ceases at a pH characteristic of the bacterial strain. Consequently, the biomass yields of lactobacilli are relatively low in cultures with uncontrolled pH. If lactic acid is neutralized by base addition during cultivation, the growth can continue longer and thus the biomass yields can be improved (*23*). In this work, we studied the growth and fatty acid composition of *L. fermentum* and *L. büchneri* in media of varying pHs. The pH values for the cultivations were chosen according to preliminary experiments. We could not perform cultivations where the pH value of the cultures was significantly outside the chosen limits, since the bacteria could not grow in those conditions.

The results of all the analyses performed during the fermenter cultivations are collected in Tables 2 and 3. Figures 1 and 2 further represent the growth pattern of *L. büchneri* and *L. fermentum* at different pH values. *L. büchneri* gave a slightly better biomass yield when the pH of the medium was kept at 4.5 than when cultivated at pH 5.5. Instead, in *L. fermentum* cultures, the final dry weight was bigger at pH 6.0 than at pH 5.0. If the pH of the medium was adjusted to 7.0 by base addition, the growth was clearly restricted in both cases. As can be seen from Tables 2 and 3, the biomass increase of the cultures ceased when glucose was used up, thus suggesting that the glucose concentration was the growth-limiting factor.

Effect of pH on Fatty Acid Composition of the Bacteria

According to the fatty acid analyses, myristic (14:0), palmitic (16:0), hexadecenoic (16:1), stearic (18:0), cis-vaccenic (18:1[11c]), and lactobacillic (cy19:0[11c]) acid accounted for more than 95% of the total amount of cellular fatty acids in both bacterial strains studied (Tables 2 and 3). Furthermore, oleic acid (18:1[9c]) and dihydrosterculic acid (cy19:0[9c]) could be detected in traces.

The pH of the medium affected the fatty acid composition of both *Lactobacillus* strains studied. For comparison, the fatty acid compositions of *L. büchneri* cells in stationary phase and *L. fermentum* cells at the end of exponential phase when grown at different pHs are illustrated in Figs. 3 and 4, respectively.

In *L. büchneri* cultures, the effect of pH on the relative amounts of fatty acids was quite clear: The proportion of lactobacillic acid increased from 3.4 to 42.9% when lowering the pH of the medium (Fig. 3). Moreover, the relative proportion of cis-vaccenic acid was much higher at pH

Table 2
Effect of pH on Growth and Fatty Acid Composition of *L. büchneri* During Cultivation in a Small-Scale Fermenter

pH	Culture time	Dry wt g/L	Glu used g/L	Fatty acids, %/mg/g dry wt						FAC mg/ g/dry wt	Vac/ cy	Vcy mg/L
				14:0	16:0	16:1	18:0	18:1 (11c)	cy19:0 (11c)			
4.5	5.9 h	0.41	9.0	0.7/0.1	30.5/4.2	5.0/0.7	5.7/0.8	51.5/ 7.0	4.5/ 0.6	13.6	11.4	0.3
	11.8 h	1.44	18.5	0.6/0.1	36.2/5.6	4.7/0.7	5.9/0.9	36.0/ 5.6	15.8/ 2.4	15.4	2.3	3.5
	21.9 h	3.64	42.8	0.4/0.1	37.6/6.8	3.0/0.5	9.5/1.7	8.6/ 1.6	40.7/ 7.4	18.1	0.2	26.8
	24.9 h	3.98	50.0	0.3/0.1	37.2/6.9	2.7/0.5	11.2/2.1	5.6/ 1.1	42.7/ 7.9	18.6	0.1	31.6
	29.6 h	3.96	50.0	0.4/0.1	37.5/6.9	2.8/0.5	11.2/2.1	5.0/ 1.0	42.9/ 8.1	18.7	0.1	32.1
5.5	14.8 h	0.48	8.1	0.7/0.2	21.5/6.8	6.1/1.9	4.1/1.3	64.6/20.6	2.2/ 0.7	31.8	29.4	0.3
	17.3 h	1.06	11.1	0.4/0.1	22.0/6.9	5.4/1.7	3.7/1.2	63.2/20.0	4.5/ 1.4	31.4	14.3	1.5
	20.0 h	1.60	19.3	0.4/0.1	23.6/7.5	5.0/1.6	3.7/1.2	58.0/18.5	9.2/ 3.0	31.9	6.3	4.7
	21.3 h	2.03	23.5	0.4/0.1	24.3/7.5	4.9/1.5	3.5/1.1	54.1/16.8	12.7/ 3.9	31.1	4.3	8.0
	30.5 h	3.26	47.8	0.5/0.1	27.8/8.4	5.3/1.6	3.3/1.0	31.8/ 9.4	31.8/ 9.6	30.2	1.0	31.2
	37.3 h	3.02	50.0	0.5/0.2	27.5/9.0	5.2/1.7	3.2/1.1	26.7/ 8.8	36.7/12.1	32.8	0.7	36.4
7.0	13.0 h	0.40	8.4	2.1/0.1	28.9/1.9	9.4/0.6	5.3/0.4	50.6/ 3.4	0 / 0	6.7	—	0
	21.1 h	0.85	17.7	1.4/0.2	24.9/2.9	7.0/0.8	4.0/0.5	60.3/ 7.3	1.3/ 0.2	11.7	47.9	0.1
	25.0 h	1.11	24.1	0.7/0.1	24.4/3.4	6.6/1.0	3.4/0.5	61.8/ 9.0	1.4/ 0.2	14.6	42.9	0.2
	36.0 h	1.60	40.6	0.6/0.1	26.1/5.0	8.1/1.5	2.6/0.5	59.2/11.2	3.1/ 0.6	19.0	19.7	0.9
	39.5 h	1.62	46.5	0.8/0.1	26.7/5.1	8.7/1.7	2.5/0.5	57.7/10.9	3.3/ 0.6	19.0	17.4	1.0
	46.3 h	1.59	50	0.8/0.2	27.5/5.8	9.4/2.0	2.5/0.5	56.1/11.7	3.4/ 0.7	20.9	16.5	1.1

FAC = total fatty acid content of the cells (mg/g dry wt), Vac/cy = content of 18:1(11c) per content of cy19:0(11c), Vcy = volumetric concentration of cy19(11c) (mg/L medium).

Table 3
Effect of pH on Growth and Fatty Acid Composition of *L. fermentum* During Cultivation in a Small-Scale Fermenter

pH	Culture time	Dry wt g/L	Glu used g/L	Fatty acids, mol%/mg/g dry wt						FAC mg/ g/dry wt	Vac/ cy	Vcy mg/L
				14:0	16:0	16:1	18:0	18:1 (11c)	cy19:0 (11c)			
5.0	5.2 h	0.83	6.2	1.8/0.2	41.8/ 5.1	7.7/0.9	2.7/0.3	24.3/ 3.0	20.6/ 2.5	12.2	1.2	2.1
	6.3 h	1.58	10.9	1.6/0.3	42.0/ 7.0	7.1/1.2	2.8/0.5	24.4/ 4.1	21.8/ 3.6	16.7	1.1	5.8
	10.0 h	3.21	22.5	1.4/0.2	40.8/ 6.8	5.5/0.9	2.5/0.4	20.7/ 3.4	28.8/ 4.8	16.5	0.7	15.3
	12.5 h	4.42	38.0	1.4/0.2	40.1/ 6.9	5.0/0.9	2.3/0.4	19.7/ 3.4	31.3/ 5.4	17.2	0.6	23.9
	15.8 h	4.01	50.0	1.2/0.4	29.3/ 9.4	5.3/1.7	1.9/0.6	38.8/12.4	23.4/ 7.5	32.0	1.7	30.1
6.0	2.8 h	0.52	2.0	2.0/0.5	41.6/10.5	7.9/2.0	3.1/0.8	34.3/ 8.7	11.2/ 2.8	28.1	3.1	1.5
	3.9 h	1.14	9.8	1.7/0.5	41.0/11.1	7.6/2.0	2.7/0.7	34.4/ 9.3	12.6/ 3.4	30.9	2.7	3.9
	5.3 h	2.28	16.2	1.7/0.5	39.5/11.9	6.9/2.1	2.5/0.8	31.5/ 9.5	17.9/ 5.4	35.5	1.8	12.3
	8.0 h	5.80	48.0	1.5/0.5	35.3/12.6	5.4/1.9	2.5/0.9	37.5/13.4	17.9/ 6.4	43.7	2.1	37.0
	10.2 h	1.90	50.0	1.3/0.5	33.5/12.1	4.1/1.5	2.8/1.0	45.3/16.4	12.9/ 4.7	46.4	3.5	8.9
7.0	3.3 h	0.47	2.0	2.8/0.5	35.9/ 6.5	8.6/1.6	2.7/0.5	39.8/ 7.2	10.1/ 1.8	18.0	3.9	0.9
	6.7 h	1.51	16	1.8/0.5	32.1/ 8.6	6.7/1.8	2.0/0.5	42.1/11.3	15.0/ 4.1	26.9	2.8	6.1
	10.7 h	2.36	36.1	2.1/0.6	33.7/ 9.8	5.3/1.6	1.9/0.6	30.3/ 8.8	26.6/ 7.7	29.1	1.1	18.2
	12.5 h	2.40	42.7	2.3/0.7	33.2/10.2	7.6/2.3	1.7/0.5	25.7/ 7.9	29.4/ 9.0	30.6	0.9	21.6
	23.2 h	2.35	50.0	2.6/0.8	33.1/10.6	7.8/2.5	1.6/0.5	21.5/ 6.9	33.3/10.7	32.0	0.6	25.1

FAC = total fatty acid content of the cells (mg/g dry wt),Vac/Cy = content of 18:1(11c) per content of cy19:0(11c), Vcy = volumetric concentration of cy19(11c) (mg/L medium).

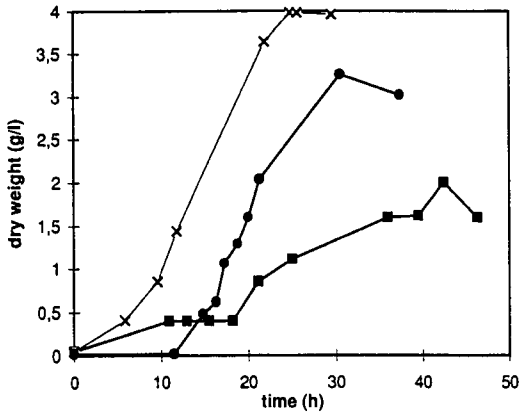

Fig. 1. Effect of pH of the medium of growth of *L. büchneri*, x = pH 4.5, ● = pH 5.5, and ■ = pH 7.0.

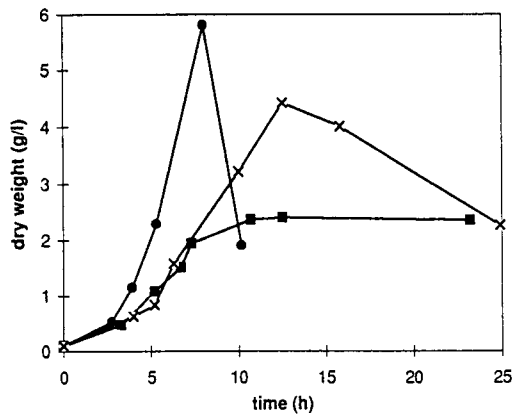

Fig. 2. Effect of pH of the medium of growth of *L. fermentum*, x = pH 5.0, ● = pH 6.0, and ■ = pH 7.0.

7.0 than at 4.5 (56.1 and 5.0%, respectively). The relative proportions of the saturated fatty acids, palmitic and stearic acid were in contrast lower at pH 7.0 than at pH 4.5. In *L. plantarum* cultures, lactobacillic acid biosynthesis was proposed by Smith and Norton (4) to be controlled by CFA synthase activity as well as by SAM and fatty acid substrate (cis-vaccenic acid) levels. Furthermore, *S*-adenosylhomocysteine (SAH) hydrolase activity of the cells might play an important role in the regulation of lactobacillic acid formation, since high activities of SAH hydrolase prevent product inhibition of CFA synthase by SAH (7). In *L. plantarum* cultures, it has previously been shown that lowering the pH of the medium caused an increase in the amount of lactobacillic acid in the bacterial cells and that this was mainly owing to an induction in CFA synthase activity (4). According to our results (Fig. 3), *L. büchneri* cultures responded to changes in pH of the medium in a similar manner. However, it has to be pointed out that in the studies with *L. plantarum* (4) and also with *E. coli* (14), only the

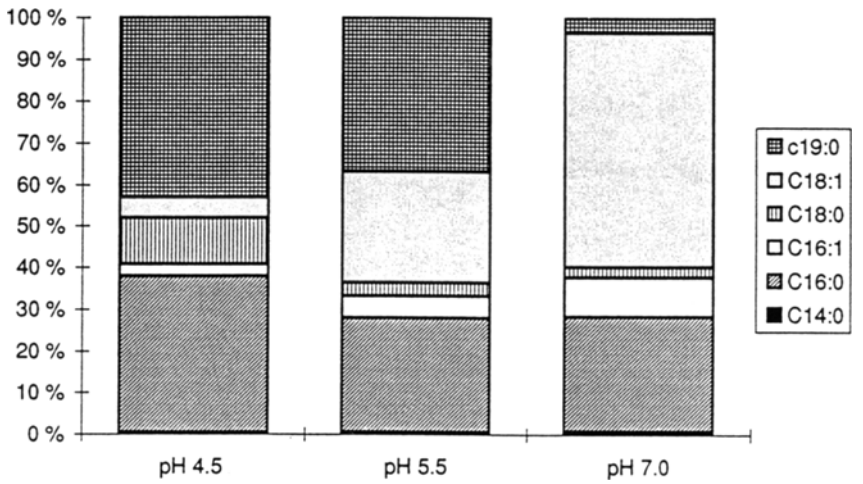

Fig. 3. Effect of pH of culture medium on fatty acid composition of *L. büchneri* cells harvested in stationary phase of growth.

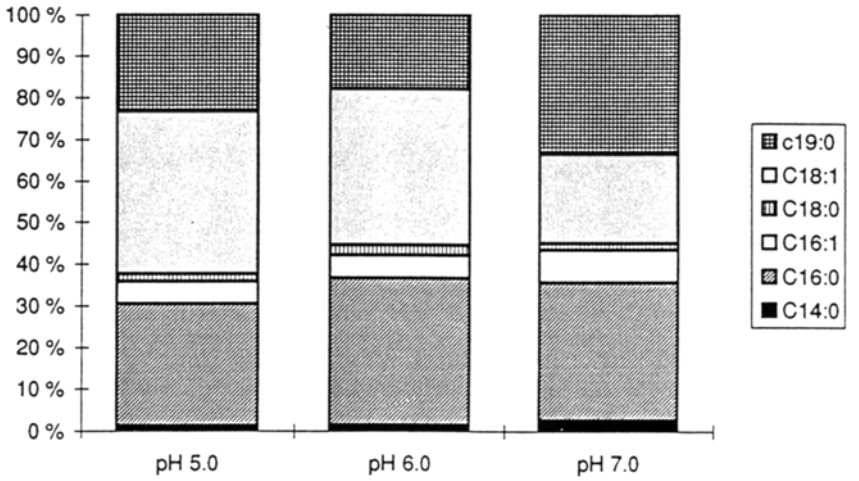

Fig. 4. Effect of pH of culture medium on fatty acid composition of *L. fermentum* cells harvested at the end of the exponential phase of growth.

relative proportions of cyclopropane fatty acids at different pHs were compared. At least in *L. büchneri* cultures, the absolute amount of lactobacillic acid was bigger at pH 5.5 than at 4.5 in the stationary growth phase, although the relative proportion was smaller (Table 2). This might be owing to higher fatty acid substrate (cis-vaccenic acid) levels at pH 5.5, since the pH of the medium seemed to affect also the total fatty acid accumulation, the fatty acid content of the cells being much lower at pH 4.5 than at pH 5.5 (Fig. 5). In conclusion, the volumetric production of lactobacillic acid, which we here wanted to maximize, was in *L. büchneri* cultures best at pH 5.5 (36.4 mg/L, Fig. 5). This was over 2.5 times more than in shake flasks at uncontrolled pH (13.8 mg/L, unpublished results).

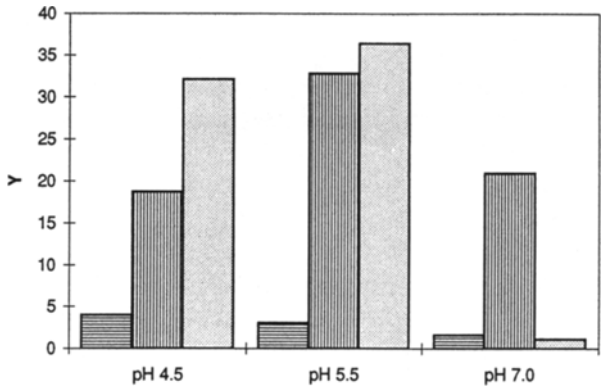

Fig. 5. Effect of pH on dry wt (g/L), total fatty acid content of the cells (mg/g dry wt), and volumetric production of lactobacillic acid (mg/L medium) of *L. büchneri* cultures in the stationary phase of growth. ▤ = dry wt, ▥ = total fatty acids and ▨ = volumetric production of lactobacillic acid.

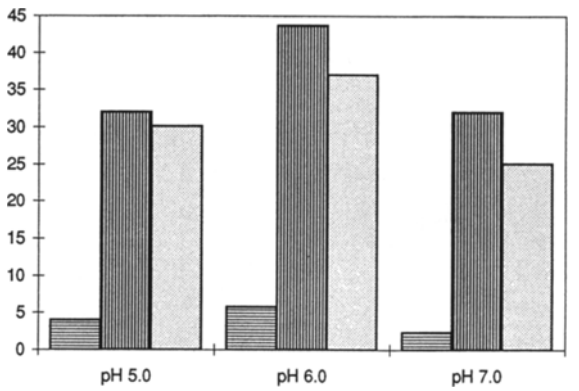

Fig. 6. Effect of pH on dry wt (g/L), total fatty acid content of the cells (mg/g dry wt), and volumetric production of lactobacillic acid (mg/L medium) of *L. fermentum* cultures at the end of exponential phase of growth. ▤ = dry wt, ▥ = total fatty acids and ▨ = volumetric production of lactobacillic acid.

In *L. fermentum* cultures, the effect of pH was more obscure. In contrast to *L. büchneri* cultures, both the absolute and relative amount of lactobacillic acid was highest and the proportion of cis-vaccenic acid lowest at pH 7.0 at the end of exponential growth phase (Table 3 and Fig. 4). Both at pH 5.0 and 6.0, the amount of cis-vaccenic acid increased considerably at the beginning at the stationary growth phase (Table 3), and a quick cell lysis occurred soon after the cessation of growth (Fig. 2). Instead, at pH 7.0, the growth was slow (Fig. 2); no cell lysis occurred and the cells were able to use cis-vaccenic acid for lactobacillic acid synthesis until the stationary phase of growth (Table 3). As illustrated in Fig. 6, the pH of the culture medium affected again not only the fatty acid pattern, but also the total fatty acid accumulation of the cultures, with cellular fatty acid content being highest at pH 6. However, in *L. fermentum* cultures, the CFA

synthase activity was not likely to be increased at low pH with culture aging, although the total fatty acid synthesis was enhanced, and therefore, cis-vaccenic acid content was not diminished at pH 5.0 and 6.0 during growth as in *L. büchneri* cultures (Tables 2 and 3). This indicates that the regulatory mechanisms controlling lactobacillic acid biosynthesis in *L. fermentum* were different from those in *L. büchneri* and *L. plantarum*. However, since the maximal dry weight was reached at pH 6.0, the best volumetric production of lactobacillic acid (37 mg/L) was achieved at pH 6.0 (Fig. 6) in spite of having a higher relative proportion of lactobacillic acid at pH 5.0 and at 7.0 (Fig. 4). As a result, the production was at pH 6.0 over five times higher than in shake flasks at uncontrolled pH (7 mg/L, unpublished results).

Effect of Culture Age on Fatty Acid Content of the Cells at Different pH

The total fatty acid content (mg/g cells) of both *Lactobacillus* strains increased with increasing culture age. The changes in the fatty acid patterns during cultivations can be seen from Tables 2 and 3. The major change in *L. büchneri* cultures at pH 4.5 and 5.5 was the increase in both absolute and relative amounts of lactobacillic acid and a concomitant decrease in cis-vaccenic acid with increasing culture age. This naturally led to a dramatic decrease in the cis-vaccenic acid/lactobacillic acid ratio during cultivation (Table 2). Cyclopropane fatty acid accumulation has previously been reported to occur with increasing culture age in some other lactobacilli as well. However, this phenomenon has been related to the naturally occurring acidification in cultures with uncontrolled pH (*10*). Here, we could detect substantial accumulation of lactobacillic acid with culture aging, although the pH was kept constant throughout the cultivation, thus indicating that the decrease in pH of the culture is not alone responsible for the enhancement of CFA production, but other factors also have to be involved in controlling the lactobacillic acid accumulation with culture aging.

When cultivated at pH 7.0, the ability of *L. büchneri* to produce lactobacillic acid from cis-vaccenic acid was clearly restricted, and thus, it was merely the accumulation of cis-vaccenic acid along with an increase in the absolute amount of palmitic acid that caused the increase in the total fatty acid content of the cells. Still, a decrease in the cis-vaccenic acid/lactobacillic acid ratio occurred with increasing culture age also at pH 7.0 (Table 2). Thus, the interchange of cis-vaccenic and lactobacillic acid occurred in *L. büchneri* cells with increasing culture age to some extent at all the pH values studied.

In *L. fermentum* cultures, the effect of culture age on fatty acid composition was not similar to that in *L. büchneri* cultures. The amount of lactobacillic acid did increase during the cultiavations, but at the end of exponential growth phase, severe cell lysis occurred at pH 5.0 and 6.0, thus causing dramatic changes in the fatty acid pattern of the cells (Table 3).

Instead, at pH 7.0, the absolute amount of cis-vaccenic acid and consequently the ratio of cis-vaccenic acid to lactobacillic acid decreased clearly with increasing culture age.

In conclusion, the production of lactobacillic acid in *L. büchneri* and *L. fermentum* clearly appeared to be affected by pH of the culture medium. No general conclusions about the effect of pH on CFA synthesis could be made, since the two lactobacilli responded to pH of the media in a very different manner. Furthermore, when maximizing the volumetric production of lactobacillic acid, the effect of pH was not straightforward: In addition to lactobacillic acid biosynthesis, also biomass production and total fatty acid accumulation were affected by pH of the medium. However, by controlling the pH of the cultures, lactobacillic acid yields could be improved considerably, which makes the production of lactobacillic acid technologically and economically more feasible. In addition, the high relative proportion of lactobacillic acid achieved in *L. büchneri* cultures and the absence of the other cyclopropane fatty acid isomer, dihydrosterculic acid, facilitate the extraction and purification of lactobacillic acid from the cell lipids. The effects of other environmental parameters on lactobacillic acid production in *L. büchneri* and *L. fermentum* are currently being investigated.

ACKNOWLEDGMENT

The financial support of the Technology Development Centre of Finland (TEKES) is gratefully acknowledged.

REFERENCES

1. Ratledge, C. and Wilkinson, S. G. (1988), *Microbial Lipids*, vol. 1. Academic, London.
2. Buist, P. H. and MacLean, D. B. (1981)), *Can. J. Chem.* **59,** 828–838.
3. Law, J. H. (1971), *Acc. Chem. Res.* **4,** 199–202.
4. Smith, D. D. and Norton, S. J. (1980), *Arch. Biochem. Biophys.* **205,** 564–570.
5. Wang, A.-Y., Grogan, D. W., and Cronan, J. E., Jr. (1992), *Biochemistry* **31,** 11020–11028.
6. Jungkind, D. L. and Wood, R. C. (1974), *Biochim. Biophys. Acta* **337,** 286–297.
7. Halper, L. A. and Norton, S. J. (1975), *Biophys. Res. Commun.* **62,** 683–688.
8. Jacques, N. A. and Hunt, A. L. (1980), *Biochim. Biophys. Acta* **619,** 453–470.
9. Jacques, N. A. (1982), *J. Gen. Microbiology* **128,** 177–184.
10. Buist, P. H. and Findlay, J. M. (1985), *Can. J. Chem.* **63,** 971–974.
11. Ordonez, J. A., de la Hoz, L., Azcona, J. I., and Sanz, B. (1985), *Can. J. Biochem.* **31,** 361–366.
12. Lonvaud-Funel, A. and Desens, C. (1990), *Sci. Aliments* **10,** 817–829.
13. Suutari, M. and Laakso, S. (1992), *J. Gen. Microbiol.* **138,** 445–450.
14. Knivett, V. A. and Cullen, J. (1965), *Biochem. J.* **96,** 771–776.
15. Law, J. H., Zalkin, H., and Kaneshiro, T. (1963), *Biochim. Biophys. Acta* **70,** 143–150.
16. Johnsson, T., Nikkilä, P., Rosenqvist, H., and Toivonen, L. (1995), *Appl. Environ. Microbiol.* **61,** 4497–4499.

17. Okuyama, H. and Lands, W. E. M. (1969), *J. Biol. Chem.* **244,** 6514–6519.
18. Kosower, E. M., Kosower, N. S., Faltin, Z., Diver, A., Saltoun, G., and Frensdorff, A. (1974), *Biochim. Biophys. Acta* **363,** 261–266.
19. Kosower, E. M. and Kosower, N. S. (1977), US Patent 4001428.
20. De Man, J. C., Rogosa, M., and Sharpe, M. E. (1960), *J. Appl. Bact.* **23,** 130–135.
21. Fischer, E. and Stein, E. (1961), *Biochem. Prep.* **8,** 27–33.
22. Suutari, M., Liukkonen, K., and Laasko, S. (1990), *J. Gen. Microbiol.* **136,** 1469–1476.
23. Pettersson, H.-E. (1975), *Appl. Microbiol.* **29,** 133–140.