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Impact of pH on Changing the Fatty Acid Composition and Growth of *Lactobacillus plantarum* and *Lactobacillus casei*

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Abstract: ***Fatty acid composition and growth of Lactobacillus plantarum and Lactobacillus casei, were studied at different pHs of the culture media in a fermenter with according interest in lactobacillic acid production of the cultures. In this study, we notice, the increasing of total fatty acid content of the bacterial cells with increasing culture age. The production of lactobacillic acid was affected in lactobacillus by culture age and pH of the media, but in a very different manner. In Lb. casei cultures, the relative proportion of lactobacillic acid was highest when the pH was lowest (pH 4.5), whereas in Lb. plantarum 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.***

Keywords: pH, fatty acid, lactobacillic acid, *Lactobacillus plantarum*.

1. Introduction

Cyclopropane fatty acid (CFA) formation is a postsynthetic modification of the lipid bilayer that occurs as cultures of *Escherichia coli* and many other bacteria enter stationary phase. We report the first distinct phenotype for this membrane modification; early stationary phase cultures of strains lacking CFA[1].

The main cyclopropane fatty acids of lactobacilli, lactobacillic acid (11,12-methyleneoctadecanoic acid; cyl9:0[llc]) and dihydrosterculic acid (9,10-methyleneoctadecanoic acid; cyl9:0[9c]), are formed by méthylation of cis-vaccenic (18:l[llc]) and oleic acid (18:l[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 hydrophobie 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 hâve been most thoroughly studied in *Escherichia coli*[5]. In addition, the effects of cultural conditions on cyclopropane fatty acid formation hâve 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.

Early studies of CFA-producing bacteria found that these modified fatty acids first appear in the late exponential or early stationary phase of growth. In *Azotobacter vinlandii*, CFAs are made only during encystment [14]. The basis of the timed appearance of CFAs is reported to be the induction of CFA synthase in several bacteria, including *Pseudomonas* spp. [15,16], *Proteus vulgaris*[17],and *Lb. plantarum*[18], but growth- phase-specific induction of the enzyme was not obvious in early studies of *E. coli*. It has more recently been shown that *E. coli* produces a sharp peak of CFA synthase activity, which is easily missed, in the transition from exponential growth to stasis [19]. 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 plantarum* G100. These strains were previously shown to produce appréciable amounts of CFAs, especially lactobacillic acid if a medium free of oleic acid was used [20]. 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.

It is unanimously agreed that the extent of the cyclopropanation of the monounsaturated fatty acids represents one of the major adaptive responses of the bacterial cells in order to stabilize the membrane fluidity known as "homeoviscous adaptation" [21].However, the role of CFAs in membrane fluidity adjustments remains unclear. According to the hypothesis of Härtig et al. [22], the presence of CFAs could make the membrane more rigid because of their higher lipid melting points and their poorer ability to pack into the acyl chain array of the phospholipid bilayer in comparison those of with unsaturated fatty acids [23].But contrary effects were obtained for measurements of membrane physical changes due to cyclopropane formation.

2. Materials and Methods

2.1 Strain and growth media

Lb. plantarum G100 was used for these studies. The bacteria were maintained in MRS agar medium[24] at 4°C and subcultured every 4 week. The composition of MRS medium is given in Table 1.

Raegent[a]	Amount g/L
Glucose	20
Peptone casein	10
Beef extract	10
Yeast extract	5.0
K_2HPO_4, $3H_2O$	2.6
Sodium acetate	5.0
Diammonium citrate	1.7
$MgSO_4$ $7H_2O$	0.2
$MnSO_4$, $4H_2O$	0.05
Tween 80	1.0

[a]*All reagents used were given by wvr and were pro-analysis grade*

For préparation of inocula for fermenter experiments, a modified MRS medium according to ……(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 $MnS0_4$*$4H_20$, 0.1 g of $MgS0_4$*$7H_20$, and either 1 g (medium A) or 10 g (medium B) of CH_3COONa*$3H_20$.

2.2 Fermentation and cultivations

To study the effect of pH on growth and fatty acid composition of *Lb. plantarum* G100, 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 1L of medium (*Lb. plantarum* G100*)*. During the préparation of inocula, growth was monitored with a Klett-Summerson colorimeter (filter no. 66).

The température in ail fermenter cultivations was 37°C and stirring speed 100 rpm. The aération 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 x 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.

2.3 Data analyses

The samples (5 mL) taken during the fermenter cultivations were centrifuged for 15 min (6000g). The glucose content of the growth medium (supernatant) was analyzed using the DNS-method of Fischer and Stein[25]. 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. [26]. 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 x 0.2 mm x 0.3 /un) column; carrier gas He at 1 mL/min; split ratio 1:20; inj. vol. 1 /mL; 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 rétention 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. [20]. The absolute amounts of fatty acids were calculated by using heptadecanoic acid methyl ester (Sigma) as an international standard. Results of ail the analyses are mean values of two parallel samples analyzed separately.

3. Results and Discussion

3.1 Impact of pH on growth of *Lb. plantarum*G100

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 *lactobacillus* 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 [27]. In this work, we studied the growth and fatty acid composition of *Lb. plantarum* 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.

Table 2. Effect of pH growth and fatty acid composition of *Lactobacillus casei*

pH	Culture time	Dry wt g/L	Glucose used g/L	Fatty acids, %mg/g dry wt						FAC mg/g/ dry wt	Vac/ cy	Vcy mg/L
				$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1(11c)}$	$Cy_{19:0}$			
4.5	6 h	0.41	9.0	0.7/0,1	30.5/4.2	5.0/0.8	5.7/0.8	51.5/7.0	4.5/0.6	13.6	11.4	0.3
	12 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
	18 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 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
	30 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	12 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
	15 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
	18 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
	24 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 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	31.8/9.636.	30.2	1.0	31.2
	36 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	7/12.	32.8	0.7	36.4
7.0	12 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	0.1/0.1	6.7	-	0
	24 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
	30 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 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
	42 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
		1.59	50.0	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 = fatty acid content of the cells (mg/g/dry wt) Vac/Cy = content of $C_{18:1(11c)}$ per content of $C_{y19:0(11c)}$, Vcy = volumetric concentration of cy19(11c) (mg/L medium)

Table 3 : Effect of pH growth and fatty acid composition of *Lactobacillus plantarum*G100

pH	Culture time	Dry wt g/L	Glucose used g/L	Fatty acids, %mg/g dry wt						FAC mg/g/ dry wt	Vac/ cy	Vcy mg/L
				$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1(11c)}$	$C_{y19:0}$			
5.0	6 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
	9 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
	12 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
	18 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
	24 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	3 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
	5 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
	8 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
	10 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
	15 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	6 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
	12 h	1.51	16.1	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
	18 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
	24 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
	30 h	2.37	46.6	2.4/0.7	33.3/10.5	7.7/2.4	1.7/0.6	24.6/7.8	33.1/10.6	31.2	0.7	22.3
	35 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	33.4/10.7	32.0	0.6	25.1

FAC = fatty acid content of the cells (mg/g/dry wt) Vac/Cy = content of $C_{18:1(11c)}$ per content of $C_{y19:0(11c)}$, Vcy = volumetric concentration of cy19(11c) (mg/L medium)

The results of ail the analyses performed during the fermenter cultivations are collected in Table 2 and Figure 1 further represent the growth pattern of *Lb.* plantarum at different pH values. *Lb. casei* 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 Lb. plantarum 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

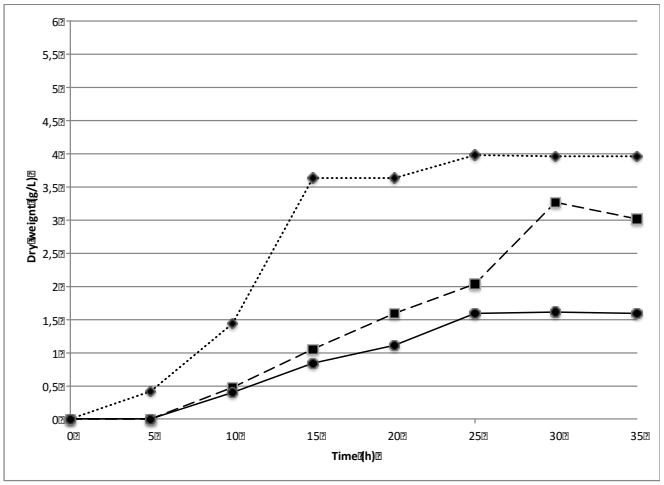

Figure 1: Impact of medium's pH on Lactobacillus casei growth, ◆= pH 4,5; □ ■= pH 5,5 and □ □•= pH 7,0

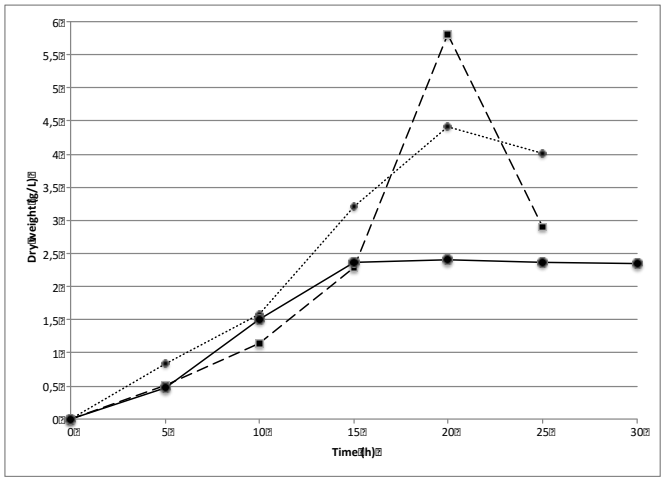

Figure 2: Impact of medium's pH on Lactobacillus plantarum growth, ◆= pH 5,0; ■= pH 6,0 and • = pH 7,0

growth was clearly resΔicted 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.

3.2 Impact of pH on fatty acid composition

According to the fatty acid analyses, myristic ($C_{14:0}$), palmitic ($C_{16:0}$), hexadecenoic ($C_{16:1}$), stearic ($C_{18:0}$), cis-vaccenic (18:l[llc]), and lactobacillic (cyl9:0[llc]) acid accounted for more than 95% of the total amount of cellular fatty acids in both bacterial strains studied (Tables 2 and 3). Further- more, oleic acid (18:l[9c]) and dihydrosterculic acid (cyl9:0[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 *Lb. Casei* cells in stationary phase and *Lb. plantarum* cells at the end of exponential phase when grown at different pHs are illustrated in figures 3 and 4, respectively.

In *Lb.casei* 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 (Table 2). Moreover, the relative proportion of cis-vaccenic acid was much higher at pH7.0 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 *Lb. plantarum* cultures, lactobacillic acid biosyn- thesis 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 rôle in the régulation of lactobacillic acid formation, since high activities of SAH hydrolase prevent product inhibition of CFA synthase by SAH [29]. In *Lb. plantarum* cultures, it has previously been shown that lowering the pH of the medium caused an in- crease in the amount of lactobacillic acid in the bacterial cells and that this was mainly owing to an induction in CFA synthase activity [30]. Accord- ing to our results (Fig. 3), Lb. casei 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 *Lb. Plantarum*[30] and also with E. *coli* [31], only the relative proportions of cyclopropane fatty acids at different pHs were compared. At least in *Lb. casei* cultures, the absolute amount of lacto- bacillic 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. 3). In conclusion, the volumétrie production of Iacto- bacillic acid, which we here wanted to maximize, was in *Lb. casei*cultures best at pH 5.5 (36.4 mg/L, Fig. 3). This was over 2.5 times more than in shake flasks at uncontrolled pH (13.8 mg/L, unpublished results).

In *Lb. plantarum* cultures, the effect of pH was more obscure. In con- trast to Lb. casei 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). 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. 4, 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. Flowever, in *Lb. plantarum* 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 *Lb. casei* cultures (Tables 2 and 3). This indicates that the regulatory mechanisms controlling lactobacillic acid biosynthesis in *Lb. plantarum* were different from those in *Lb. casei* and *Lb.plantarum.* However, since the maximal dry weight was reached at pH 6.0, the best volumétrie production of lactobacillic acid (37 mg/L) was achieved at pH 6.0 (Fig. 4) in spite of having a higher relative proportion of lactobacillic acid at pH 5.0 and at 7.0. As a result, the production was at pH 6.0 over five times higher than in shake flasks at uncontrolled pH.

Impact 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 âge. The changes in the fatty acid patterns during cultivations can be seen from Tables 2 and 3. The major change in Lb. casei 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 âge. 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 âge 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 responsi- ble for the enhancement of CFA production, but other factors also hâve to be involved in controlling the lactobacillic acid accumulation with culture aging

When cultivated at pH 7.0, the ability of *Lb. casei* 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 âge also at pH 7.0 (Table 2). Thus, the interchange of cis-vaccenic and lactobacillic acid occurred in Lb. casei cells with increasing culture âge to some extent at ail the pH values studied.

In *Lb. plantarum* cultures, the effect of culture âge on fatty acid composition was not similar to that in Lb. casei 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 conse- quently the ratio of cis-vaccenic acid to lactobacillic acid decreased clearly with increasing culture âge.

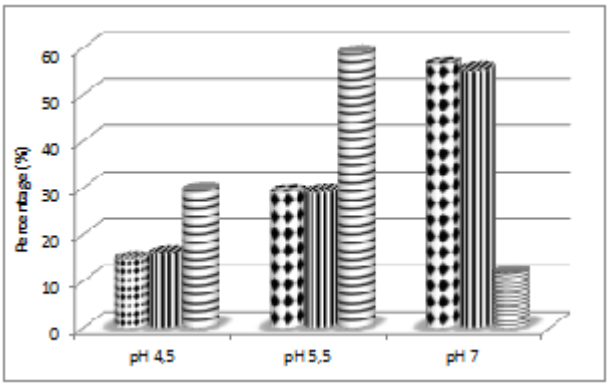

Figure 3 : Impact of pH on dry wt (g/L), total fatty acid content of the cells (mg/g dry wt), and volumetric production of lactobacillic acid (mg/L medium) of Lb. casei cultures in the stationary phase of growth. ◆= dry wt, III = total fatty acids and Ξ = volumetric production of lactobacillic acid.

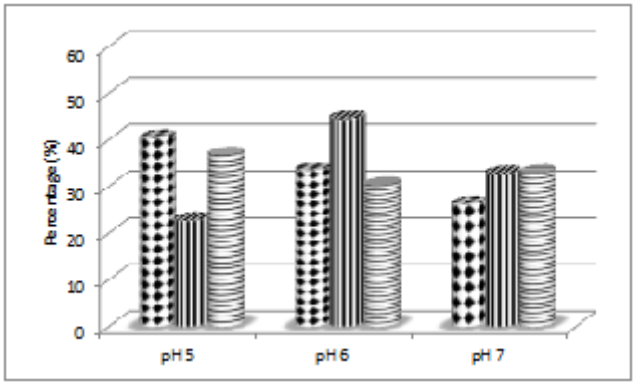

Figure 4 : Impact of pH on dry wt (g/L), total fatty acid content of the cells (mg/g dry wt), and volumetric production of lactobacillic acid (mg/L medium) of Lb. plantarum cultures in the stationary phase of growth. ◆= dry wt, III = total fatty acids and Ξ = volumetric production of lactobacillic acid.

In conclusion, the production of lactobacillic acid in *Lb. casei* and *Lb. plantarum* 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 volumétrie pro-duction 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. How- ever, 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 *Lb. casei* cultures and the absence of the other cyclopropane fatty acid isomer, dihydroster- culic acid, facilitate the extraction and purification of lactobacillic acid from the cell lipids. The effects of other environmental parameters on lactobacillic acid production in *Lb. casei* and *Lb. plantarum* are currently being investigated.

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