

Production of conjugated linoleic acid by *Propionibacterium freudenreichii* ssp. *shermanii*

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Abstract — The kinetics of linoleic acid isomerization to conjugated linoleic acid (CLA) by *Propionibacterium freudenreichii* ssp. *shermanii* JS was studied in pH-controlled batch and fed-batch fermentations. In batch fermentations supplemented with 600–2 000 µg·mL⁻¹ linoleic acid, the rate of CLA formation was highest during the exponential phase of growth. By the end of the batch fermentations, high amounts of CLA corresponding to 80–87% conversion were accumulated with initial linoleic acid concentrations up to 2 000 µg·mL⁻¹. In fed-batch fermentations, where 600 µg·mL⁻¹ linoleic acid was added into the culture after exponential growth, a rapid but short conversion period occurred. Thus, both actively growing and non-growing cultures were able to effectively form CLA. However, the production rates per cell were significantly higher in growing cultures. This isomerization reaction could be a detoxification system for linoleic acid in the propionibacterium strain studied.

conjugated linoleic acid / linoleic acid isomerization / *Propionibacterium freudenreichii* ssp. *shermanii*

Résumé — Production d'acide linoléique conjugué par *Propionibacterium freudenreichii* ssp. *shermanii*. La cinétique d'isomérisation de l'acide linoléique en acide linoléique conjugué (ALC) par *Propionibacterium freudenreichii* ssp. *shermanii* JS a été étudiée au cours de fermentations batch et batch alimenté, à pH contrôlé. En fermentations batch supplémentées avec 600–2 000 µg·mL⁻¹ d'acide linoléique, le taux de formation d'ALC était plus élevé au cours de la phase exponentielle de croissance. À la fin des fermentations batch, des quantités élevées d'ALC correspondant à une conversion de 80–87 % étaient accumulées, avec des concentrations initiales d'acide linoléique allant jusqu'à 2 000 µg·mL⁻¹. En fermentations batch alimenté, où 600 µg·mL⁻¹ d'acide linoléique étaient ajoutés dans la culture après la phase exponentielle de croissance, une période de croissance

Oral communication at the 3rd International Symposium on Propionibacteria, Zurich, Switzerland, July 8–11, 2001.

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rapide mais courte avait lieu. Ainsi, les deux cultures, celle croissant activement et celle ne croissant pas, étaient capables de former effectivement de l'ALC. Cependant, les taux de production par cellule étaient significativement plus élevés dans les cultures croissantes. Cette réaction d'isomérisation pourrait être un système de détoxification pour l'acide linoléique dans la souche de bactérie propionique étudiée.

acide linoléique conjugué / isomérisation de l'acide linoléique / *Propionibacterium freudenreichii* ssp. *shermanii*

1. INTRODUCTION

Conjugated linoleic acid (CLA) has raised extensive interest because of its potentially beneficial physiological effects and anticarcinogenic properties [2, 3, 10, 11]. The positive effects of CLA originating from studies in animal models are currently being tested in clinical human trials [1, 4, 6].

Commercially available CLA products are manufactured by alkaline isomerization from vegetable oils [4]. These synthetic mixtures consist of several isomers of CLA of which the *cis*-9, *trans*-11 configuration is considered to have the highest biological activity [3]. This isomer was first found as an intermediate in the biohydrogenation of linoleic acid (LA) by a ruminal bacterium *Butyrivibrio fibrisolvens* [8]. Also other bacteria have been shown to be capable of converting LA to its conjugated form, including some lactic acid bacteria and propionibacteria used as dairy starter cultures. In these cases a major proportion of CLA formed has been in the *cis*-9, *trans*-11 configuration. The conversion reaction has been studied both in bacterial growing cultures and washed-cell suspensions [5, 9, 12, 13, 17, 20]. From these small-scale microbiological isomerization studies one can conclude that the toxicity of LA and its low solubility in aqueous solutions may have severe complications in the production of CLA. However, both of these problems can, at least to a great extent, be overcome by using detergent-stabilized micellar substrate systems [17]. By proper optimizing of reactant concentrations, LA concentrations that

otherwise would be lethal to organisms could be used and isomerized to CLA. This study describes such a conversion system. By using *Propionibacterium freudenreichii* ssp. *shermanii* JS as the isomerizing organism, the kinetics of growth and CLA formation was studied with various initial LA concentrations in pH-controlled batch and fed-batch fermentations.

2. MATERIALS AND METHODS

2.1. Microbial strain and growth medium

Propionibacterium freudenreichii ssp. *shermanii* strain JS (DSM 7067) was from the culture collection of Valio Ltd. (Helsinki, Finland). The inoculum was grown in a medium described by Suomalainen and Mäyrä-Mäkinen [18] at 30 °C for 72 h. In the CLA formation experiments, the strain was cultivated in whey permeate medium (WPM) containing 2% (w/v) whey permeate (Valio), 1% (w/v) yeast extract (LabM, Bury, England) and 0.5% (w/v) tryptone (LabM) supplemented with linoleic acid (LA; 99% pure, Sigma, St. Louis, MO, USA) in a concentration range of 0–2 000 µg·mL⁻¹. In the presence of LA, the fermentation medium was also supplemented with polyoxyethylene sorbitan monooleate (SO; Kotilen-O/1 VL, Chemische Fabrik Dr. W. Kolb Ag, Switzerland) at a concentration of 9 or 15 mg·mL⁻¹. LA was added as micellar LA stock solution into the medium before sterilization. Micellar LA was prepared by weighting 300 mg LA into 5 mL deoxygenated deionized water

containing 0.36 mL SO. The mixture was blended and 0.56 mL of 2 mol·L⁻¹ sodium hydroxide solution was added in order to clarify the mixture. The solution was transferred to a 50 mL volumetric flask and filled up with deoxygenated water. To facilitate addition of the highest LA concentrations without excess dilution of the growth medium, concentrated stock solutions were also prepared by keeping the ratio of LA, SO and sodium hydroxide constant.

2.2. Fermentations

The fermentations were carried out in a Biostat MD 2 fermentor (B. Braun, Melsungen, Germany) with a working volume of 2 L at 30 °C. The pH of the medium was controlled at pH 6.3 ± 0.2 with 8% ammonia solution. The inoculum was 2% of the fermentation volume.

In the fed-batch fermentations, the cultivations consisted of a primary growth phase followed by a separate production phase. The organism was grown to a late exponential phase (viable count 2 × 10¹⁰ cfu·mL⁻¹). In fed-batch fermentation A (FBA), the medium contained 9 mg·mL⁻¹ of SO. After the growth phase, LA stock was added to obtain a concentration of 600 µg·mL⁻¹. In fed-batch fermentation B (FBB), aqueous SO was added to a concentration of 9 mg·mL⁻¹ just prior to the addition of the LA stock in the late exponential phase.

Growth was monitored as optical density (OD) at 600 nm (U-2000 Spectrophotometer, Hitachi, Japan) and as viable counts. The viable counts were determined by pour-plating on sodium lactate agar containing 1% (w/v) yeast extract (LabM), 0.5% (w/v) tryptone (LabM), 1% (w/v) β-glycerophosphate (Merck, Darmstadt, Germany), 1.7% (v/v) sodium lactate (Merck) and 1.2% (w/v) agar (LabM). The plates were incubated anaerobically at 30 °C for 6-7 d.

Samples for fatty acid analysis and viable counts were taken after inoculation and

at intervals of 24 h. At LA concentration 600 µg·mL⁻¹, the samples were taken every two hours within fermentation time 0 to 10 h. At LA concentrations 930 and 1 400 µg·mL⁻¹, the samples were taken every three hours within fermentation time of 13.5 to 22 h, and 10 to 22 h, respectively. After that samples were taken at intervals of 24 h.

2.3. Determination of fatty acids

To determine the fatty acid composition in cell mass and medium, culture samples (4–10 mL) were centrifuged at 4 300 g for 15 min at 15–20 °C. The supernatant was filtered with a 0.2 µm filter and when needed, diluted with deionized water. The cell pellets were washed with 8 mL of tap water. Samples were frozen and lyophilized. The fatty acids were determined according to the methylation method described by Suutari et al. [19]. The fatty acids were saponified with 1–2 mL 3.7 mol·L⁻¹ NaOH in 49% (v/v) methanol at 100 °C for 30 min. The saponified fatty acids were methylated with 4–8 mL of 2.2 mol·L⁻¹ HCl in 64% (v/v) methanol at 80 °C for 10 min. Fatty acid methyl esters were extracted in 1.5 mL hexane/methyl tert-butyl ether solution (1:1, v/v) by shaking vigorously for 10 min. The organic phase was washed by shaking for 5 min with 3 mL 0.3 mol·L⁻¹ NaOH. The sample was dried with anhydrous Na₂SO₄ and analyzed by gas chromatograph (GC). GC analysis was carried out by using a Hewlett-Packard Model 5890A gas chromatograph equipped with a flame ionization detector, a capillary inlet system and a model 7673A high-speed automatic liquid sampler with a 10 µL syringe. The GC-conditions were as follows: HP-FFAP (25 m × 0.2 mm × 0.3 µm) column; carrier gas helium, column flow-rate approx. 1.0 mL·min⁻¹; total hydrogen flow-rate to the detector 40 mL·min⁻¹; make-up gas helium, flow-rate 30 mL·min⁻¹; septum purge flow-rate 1–2 mL·min⁻¹; split ratio

1:20; column inlet pressure 150 kPa; injector and detector temperatures 250 °C; oven temperature was programmed from 70 °C to 200 °C at the rate of 25 °C·min⁻¹. Peak areas were measured using a Hewlett-Packard model 3396A integrator. Nonadecanoic acid methyl ester (Sigma) was used as an internal standard. The analysis at Valio Ltd. confirmed that the strain used in this study did not form *trans*-9, *cis*-11 isomer of CLA.

2.4. Calculations

The specific growth rate ($\mu\text{-h}^{-1}$) for culture in the exponential growth phase was calculated by dividing the change in natural logarithms of optical density values by the length of the observation time. The amount of CLA formed by the bacterium was calculated by subtracting the initial concentration of CLA in the growth medium (CLA in SO) from the total CLA assayed. The yield of CLA (%) was defined as [CLA formed ($\text{g}\cdot\text{L}^{-1}$) divided by initial LA in the medium ($\text{g}\cdot\text{L}^{-1}$)] $\times 100\%$. The productivity in the fermentations was expressed as the volumetric production rate of CLA (VPR, $\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) and the specific production rate of CLA (SPR, $\mu\text{g}\cdot 10^{-9}\text{cfu}^{-1}\cdot\text{h}^{-1}$). VPR

was calculated by dividing the amount of CLA formed ($\mu\text{g}\cdot\text{mL}^{-1}$) by the length of the observation period (h). SPR was calculated by dividing VPR by the viable count ($\text{cfu}\cdot\text{mL}^{-1}$) at the end of the observation period. The fatty acid content of the cells ($\text{mg}\cdot\text{g}^{-1}$) was calculated as the amount of fatty acid per dry cell weight. The results in the figures and tables are presented as means \pm standard deviation ($n = 3\text{--}4$).

3. RESULTS

3.1. Batch fermentations

The conversion of LA to CLA during growth of *Propionibacterium freudenreichii* ssp. *shermanii* JS was studied in 2-litre batch and fed-batch fermentations equipped with pH control. Initial LA concentrations in WPM ranged from 600 to 2 000 $\mu\text{g}\cdot\text{mL}^{-1}$. The growth-preventing effect of LA was overcome by adding micellar LA stock solution into SO-supplemented WPM so that the final LA:SO ratio was 1:7.5–1:15 (w/w). With these ratios simultaneous growth and conversion of LA to CLA has been found possible without pH control [17].

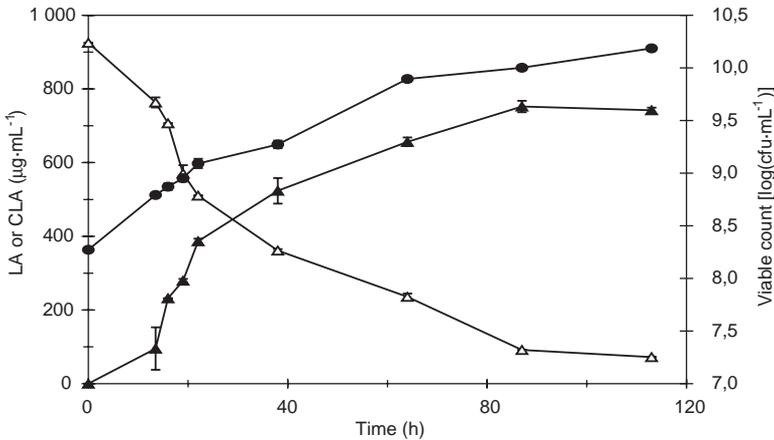


Figure 1. Time course of a typical batch fermentation of *P. freudenreichii* ssp. *shermanii* JS in WPM in the presence of LA and SO. Symbols: \blacktriangle formation of CLA; \triangle consumption of LA; and \bullet viable count.

Table I. Growth and formation of CLA in batch fermentation of *P. freudenreichii* ssp. *shermanii* JS at different initial LA concentrations in WPM supplemented with SO. Specific growth rate (μ_m), viable count, amount of CLA formed, volumetric and specific production rate of CLA (VPR and SPR, respectively) are maximal values obtained. The maximal VPR and SPR were obtained in observation range indicated in parentheses.

LA	SO	μ_m	Viable count	CLA	<i>cis</i> -9, <i>trans</i> -11 isomer from total CLA	VPR	SPR
($\mu\text{g}\cdot\text{mL}^{-1}$)	($\text{mg}\cdot\text{mL}^{-1}$)	(h^{-1})	($\log[\text{cfu}\cdot\text{mL}^{-1}]$)	($\mu\text{g}\cdot\text{mL}^{-1}$)	%	($\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$)	($\mu\text{g}\cdot 10^{-9}\text{cfu}^{-1}\cdot\text{h}^{-1}$)
0	0	0.044 ± 0.007	10.49 ± 0.02	0	-	-	-
600 ± 13	9	0.034 ± 0.001	10.29 ± 0.02	490 ± 21	85 ± 3	22 ± 2 (10–25 h)	36 ± 4 (8–10 h)
930 ± 38	15	0.031 ± 0.001	10.19 ± 0.01	812 ± 7	93 ± 1	57 ± 5 (13.5–16 h)	76 ± 7 (13.5–16 h)
$1\ 400 \pm 46$	15	0.022 ± 0.001	10.11 ± 0.01	$1\ 140 \pm 51$	95 ± 1	34 ± 3 (13–16 h)	36 ± 4 (13–16 h)
$2\ 000 \pm 28$	15	0.014 ± 0.001	10.04 ± 0.02	$1\ 600 \pm 87$	95 ± 1	19 ± 2 (0–15 h)	51 ± 5 (0–15 h)

Figure 1 shows the growth kinetics of a batch fermentation at an initial LA level of $930 \mu\text{g}\cdot\text{mL}^{-1}$. The formation of CLA correlated well with growth, with a concomitant decrease in LA. Fermentations at various initial LA concentrations showed that increments in the initial level of LA decreased the specific growth rate (Tab. I) and also decreased the final cell densities slightly. In spite of this, the final amount of CLA increased as the initial concentration of LA was increased and even with $2\,000 \mu\text{g}\cdot\text{mL}^{-1}$ LA the yield of CLA was 80%. Less than 10% of the initial LA was left in the medium at the end of the fermentations. In all batch fermentations, *cis*-9, *trans*-11 isomer of CLA comprised 85–95% of CLA formed and over 98% of CLA formed was extracellular.

The volumetric and specific production rates of CLA were highest during the first 24 h of fermentation (Tab. I). The values were, however, strongly influenced by the length of the observation range. In fermentations with a LA:SO ratio of 1:15, almost 70% of the total amount of CLA was formed within the first 40 h. At initial LA

concentrations exceeding $930 \mu\text{g}\cdot\text{mL}^{-1}$, there was a slight decreasing trend in maximal volumetric production rates. However, the specific production rate values indicate that cultures growing more slowly due to high initial LA concentrations still converted LA effectively to CLA per cell (Tab. I).

According to the present results, it seemed that a major part of CLA was formed at early stages of growth. Therefore, the first ten hours of the cultivation were studied more closely. After inoculation, division of cells began immediately (Fig. 2), and LA isomerization initiated after the first four hours from inoculation. After that there was a positive correlation between the increments in viable counts and amounts of CLA.

3.2. Fed-batch fermentations

The kinetics of CLA formation and its connection to growth was investigated further in fed-batch fermentations. The fermentations consisted of a growth phase to obtain high cell concentrations in the

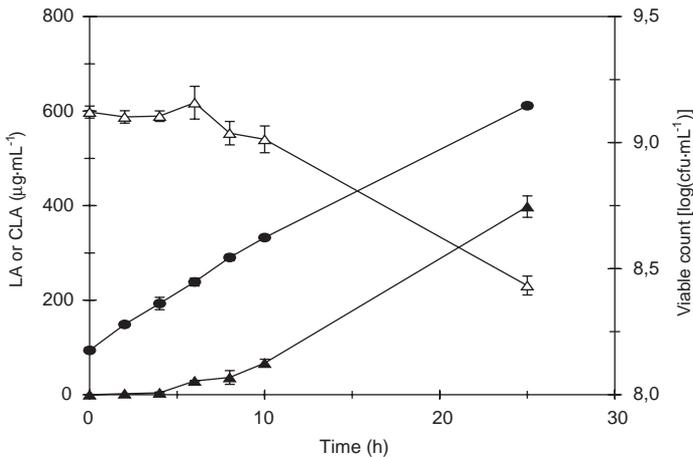


Figure 2. The onset of CLA formation in a batch fermentation of *P. freudenreichii* ssp. *shermanii* JS in WPM in the presence of LA and SO. Symbols: \blacktriangle formation of CLA; \triangle consumption of LA; and \bullet viable count.

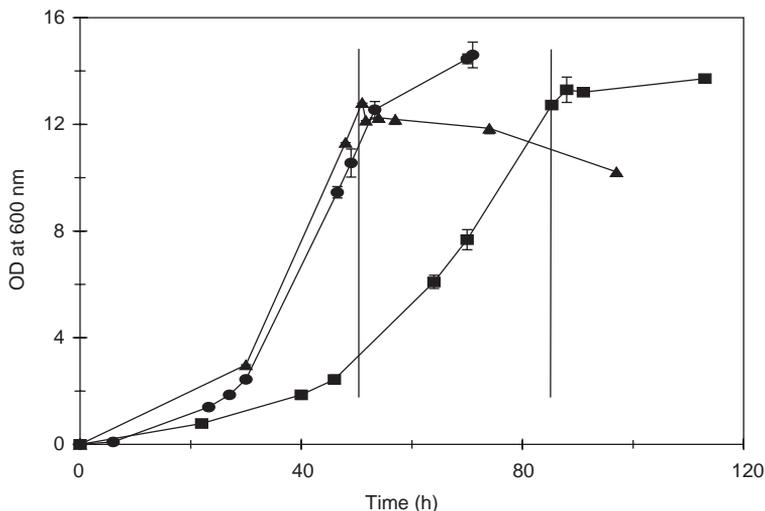


Figure 3. Growth of *P. freudenreichii* ssp. *shermanii* JS in fed-batch fermentations in WPM. The vertical lines indicate the time of $600 \mu\text{g}\cdot\text{mL}^{-1}$ LA (FBA) (■) and $15 \text{ mg}\cdot\text{mL}^{-1}$ SO and $600 \mu\text{g}\cdot\text{mL}^{-1}$ LA additions (FBB) (▲). Fermentation without LA and SO (●).

absence of LA, followed by a conversion phase. The growth phase was carried out in WPM with or without SO until a late exponential phase (viable count $2 \times 10^{10} \text{ cfu}\cdot\text{mL}^{-1}$) before the addition of the micellar LA stock solution to a LA concentration of $600 \mu\text{g}\cdot\text{mL}^{-1}$. In fed-batch fermentation A (FBA), the culture was developed in the presence of $9 \text{ mg}\cdot\text{mL}^{-1}$ SO and in fermentation B (FBB) SO was added just before LA addition.

The presence of SO in WPM from the beginning of the growth phase (FBA) decreased the growth rate and the late exponential growth phase was reached about 30 h later than in FBB (Fig. 3). In both fed-batch fermentations (A and B), the addition of LA stopped the growth immediately or within 3 h. In spite of this, CLA was formed rapidly in both fermentations after addition of LA. In FBB, the conversion of LA to CLA initiated almost instantaneously after addition of SO and LA, and maximum volumetric and specific production rates were obtained already within 0.5–3 h (Tab. II). In FBA, formation of CLA was fastest within 3–6 h following the addition of LA.

The production rates in FBB were two-fold compared to those in FBA. On the other hand, the final concentration of CLA in FBB was significantly lower than in FBA where the final level of CLA was about the same as in the batch fermentation at the corresponding initial LA concentration of $600 \mu\text{g}\cdot\text{mL}^{-1}$. Maximum specific production rates in fed-batch fermentations were significantly lower than the values obtained in batch fermentations (Tabs. I and II). For example, the maximum specific production rate values in FBB and in a batch fermentation containing $600 \mu\text{g}\cdot\text{mL}^{-1}$ LA were 4.6 and $36 \mu\text{g}\cdot 10^{-9} \text{ cfu}^{-1}\cdot\text{h}^{-1}$, respectively. This indicates the importance of the physiological state of the culture in the efficiency of the conversion reaction.

3.3. Cellular fatty acid compositions

Although a major part of CLA formed was found in the growth medium, LA and CLA were also detected among the cellular fatty acids. The cells also contained oleic acid originating from SO. In batch

Table II. Productivity of *P. freudenreichii* ssp. *shermanii* JS in fed-batch fermentations in WPM. In FBA approximately 600 $\mu\text{m}\cdot\text{mL}^{-1}$ LA was added into culture containing 15 $\text{mg}\cdot\text{mL}^{-1}$ SO and in FBB similar amounts of SO and LA were added into the culture at late exponential growth phase. Reaction time was counted from the LA addition. Volumetric and specific production rates of CLA (VPR and SPR, respectively) were calculated with the reaction times shown in table.

	Reaction time (h)	CLA ($\mu\text{g}\cdot\text{mL}^{-1}$)	VPR ($\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$)	SPR ($\mu\text{g}\cdot 10^{-9}\text{ cfu}^{-1}\cdot\text{h}^{-1}$)
FBA	0	0	0	0
	3	58 ± 5	19 ± 3	1.0 ± 0.3
	6	170 ± 15	37 ± 7	2.2 ± 0.5
	28	518 ± 60	16 ± 4	0.6 ± 0.1
FBB	0	0	0	0
	0.5	6 ± 1	12 ± 2	0.6 ± 0.1
	3	246 ± 25	96 ± 10	4.6 ± 0.6
	6	322 ± 33	25 ± 3	0.8 ± 0.2
	22	338 ± 52	1 ± 0.2	<0.1

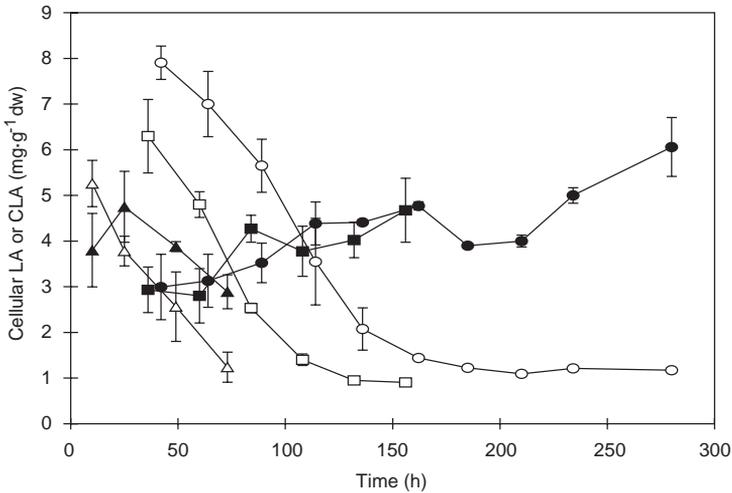


Figure 4. Cellular LA (open symbols) and CLA (closed symbols) in batch fermentations of *P. freudenreichii* ssp. *shermanii* JS in WPM supplemented with SO at initial LA concentrations of 600 (▲), 1 400 (■) and 2 000 (●) $\mu\text{g}\cdot\text{mL}^{-1}$.

fermentations, the amount of cellular LA was dependent on both the initial LA concentration in WPM and the age of the culture. The level of cellular LA was highest with high initial LA concentrations

(Fig. 4). In each culture, cellular LA decreased significantly as the fermentation progressed. On the other hand, the amount of cellular CLA representing less than 1.7% of the total amount of CLA formed was not

dependent on the initial LA concentration and it was only slightly influenced by the age of the culture. In fed-batch fermentations, the cell samples taken immediately after LA addition contained both LA and CLA (3–5 and 1 mg per g dry cell weight, respectively). This suggests that the isomerization of LA started very rapidly when the cells came into contact with LA.

Interestingly, the supplementation of WPM with LA and SO in batch fermentations did not cause any significant increase in the total fatty acid content of the cells. In the presence of LA and SO, the cellular fatty acid content varied between 39 and 50 mg·g⁻¹ and in plain WPM the value was 44 mg·g⁻¹. This indicates that the cellular fatty acids characteristic of this organism were partly substituted by external LA, CLA, and oleic acid.

4. DISCUSSION

Propionibacterium freudenreichii ssp. *shermanii* JS efficiently converted LA to CLA during pH-controlled batch cultivations. As the conversion progressed the LA:SO-ratio also changed from that enabling optimum growth and so the growth rates and rate of CLA production were concomitantly lowered during the progress of the cultivations. This did not, however, affect the yield of CLA when the initial LA concentrations were in the range 600 to 2 000 µg·mL⁻¹. This is in contrast to previous cultivations without pH control. For example, with an initial LA concentration of 1 000 µg·mL⁻¹ the final pH was 4.8 and the yield of CLA was 57% [17]. The decreasing pH may have unfavorably influenced not only the enzyme activity but probably also the state of dispersion of the LA-SO mixture in WPM. Kepler and Tove [7] showed that the optimum pH for the linoleate isomerase of *B. fibrisolvans* was 7.0–7.2, although the pH range for enzyme activity was broad, from 5.5 to 8.5, as confirmed by

Kim et al. [9]. According to Pariza and Yang [15], the optimum pH range for CLA formation in cell suspensions of *Lactobacillus reuteri* was 7.4–8.8 even though the conversion still occurred at pH 5.5. This information together with the present data suggests that reduction in pH reduces the yield of CLA. The reduction in the yields is probably more prominent with high initial LA concentrations since a conversion inhibiting pH is reached while a significant portion of LA may still be unconverted.

Considerable amounts of CLA were also formed in the fed-batch fermentations. The high cell densities when LA was added enabled high volumetric production rates and yields during a relatively short reaction period. The conversion reaction started immediately when the cells came into contact with LA. However, by comparing the specific production rates of batch and fed-batch fermentations, it is evident that actively growing cultures were significantly more efficient than cultures approaching the stationary phase. The fed-batch fermentations with the present *Propionibacterium* strain and the earlier observations of Pariza and Yang [15] and Ogawa et al. [14] support the view that the presence of active LA isomerase enzyme also in non-growing cells may be a general property of a wide variety of bacteria.

The physiological basis for LA isomerization by propionibacteria is not known. It has been suggested that the isomerization reaction initiates a series of biohydrogenations to accept the metabolic hydrogen in *B. fibrisolvans* [16]. However, in the present organism such a reductive reaction sequence did not occur since the formed *cis*-9, *trans*-11 isomer of CLA was not converted further to other fatty acids. Instead, detoxification of LA via conversion to CLA as proposed by Jiang et al. [5] is a more probable physiological role for the isomerization in the present organism. Jiang et al. [5] observed a positive

correlation between CLA formation and the ability to tolerate LA among three CLA-forming strains of propionibacteria. Other studies have shown that the ability to tolerate LA depends on cell density and growth phase. Kim et al. [9] showed that an inoculum culture of *B. fibrisolvens* A38 was already inhibited at a LA level of 4 $\mu\text{g}\cdot\text{mL}^{-1}$, but the growing cultures tolerated ten-fold concentrations of LA. These pieces of information together with the present data favor the view of a detoxification mechanism.

5. CONCLUSION

In conclusion, both actively growing and stationary phase cultures of *P. freudenreichii* ssp. *shermanii* JS were able to convert LA to CLA with high efficiency. However, the production rates per cell were significantly higher in growing cultures. In fed-batch fermentations, where the conversion reaction occurred after exponential growth, the high initial rates of production decreased rapidly with time. This isomerization reaction could be a detoxification system for LA in the propionibacterium strain studied.

ACKNOWLEDGEMENTS

The work was supported by Valio Ltd. and the National Technology Agency of Finland (TEKES).

REFERENCES

- [1] Berven G., Bye A., Hals O., Blankson H., Fagertun H., Thom E., Wadstein J., Gudmundsen O., Safety of conjugated linoleic acid (CLA) in overweight or obese human volunteers, *Eur. J. Lipid Sci. Technol.* 102 (2000) 455–462.
- [2] Gnädig S., Rickert R., Sébédio J.L., Steinhart H., Conjugated linoleic acid (CLA): physiological effects and production, *Eur. J. Lipid Sci. Technol.* 103 (2001) 56–61.
- [3] Ip C., Chin S.F., Scimeca J.A., Pariza M.W., Mammary cancer prevention by conjugated dienoic derivative of linoleic acid, *Cancer Res.* 51 (1991) 6118–6124.
- [4] Jahreis G., Kraft J., Tischendorf F., Schöne F., von Loeffelholz C., Conjugated linoleic acids: Physiological effects in animal and man with special regard to body composition, *Eur. J. Lipid Sci. Technol.* 102 (2000) 695–703.
- [5] Jiang J., Björck L., Fondén R., Production of conjugated linoleic acid by dairy starter cultures, *J. Appl. Microbiol.* 85 (1998) 95–102.
- [6] Kelley D.S., Taylor P.C., Rudolph I.L., Benito P., Nelson G.J., Mackey B.E., Erickson K.L., Dietary conjugated linoleic acid did not alter immune status in young healthy women, *Lipids* 35 (2000) 1065–1071.
- [7] Kepler C.R., Tove S.B., Biohydrogenation of unsaturated fatty acids, III. Purification and properties of a linoleate Δ^{12} -*cis*, Δ^{11} -*trans*-isomerase from *Butyrivibrio fibrisolvens*, *J. Biol. Chem.* 242 (1967) 5686–5692.
- [8] Kepler C.R., Hirons K.P., McNeill J.J., Tove S.B., Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*, *J. Biol. Chem.* 241 (1966) 1350–1354.
- [9] Kim Y.J., Liu R.H., Bond D.R., Russell J.B., Effect of linoleic acid concentration on conjugated linoleic acid production by *Butyrivibrio fibrisolvens* A38, *Appl. Environ. Microbiol.* 66 (2000) 5226–5230.
- [10] Kritchevsky D., Antimutagenic and some other effects of conjugated linoleic acid, *Br. J. Nutr.* 83 (2000) 459–465.
- [11] Lee K.N., Kritchevsky D., Pariza M.W., Conjugated linoleic acid and atherosclerosis in rabbits, *Atherosclerosis* 108 (1994) 19–25.
- [12] Lin T.Y., Conjugated linoleic acid concentrations as affected by lactic cultures and additives, *Food Chem.* 69 (2000) 27–31.
- [13] Lin T.Y., Lin C-W., Lee C-H., Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid, *Food Chem.* 67 (1999) 1–5.
- [14] Ogawa J., Matsumura K., Kishino S., Omura Y., Shimizu S., Conjugated linoleic acid accumulation via 10-hydroxy-12-octadecaenoic acid during microaerobic transformation of linoleic acid by *Lactobacillus acidophilus*, *Appl. Environ. Microbiol.* 67 (2001) 1246–1252.
- [15] Pariza M.W., Yang X.-Y., Method for producing conjugated fatty acids, United States Patent 5.856.149 (1999).
- [16] Polan C.E., McNeill J.J., Tove S.B., Biohydrogenation of unsaturated fatty acids by rumen bacteria, *J. Bacteriol.* 88 (1964) 1056–1064.

- [17] Rainio A., Vahvaselkä M., Suomalainen T., Laakso S., Reduction of linoleic acid inhibition in production of conjugated linoleic acid by *Propionibacterium freudenreichii* ssp. *shermanii*, *Can. J. Microbiol.* 47 (2001) 735–740.
- [18] Suomalainen T.H., Mäyrä-Mäkinen A.M., Propionic acid bacteria as protective cultures in fermented milks and breads, *Lait* 79 (1999) 165–174.
- [19] Suutari M., Liukkonen K., Laakso S., Temperature adaptation in yeasts: the role of fatty acids, *J. Gen. Microbiol.* 136 (1990) 1469–1474.
- [20] Verhulst A., Janssen G., Parmentier G., Eyssen H., Isomerization of polyunsaturated long chain fatty acids by Propionibacteria, *System. Appl. Microbiol.* 9 (1987) 12–15.

