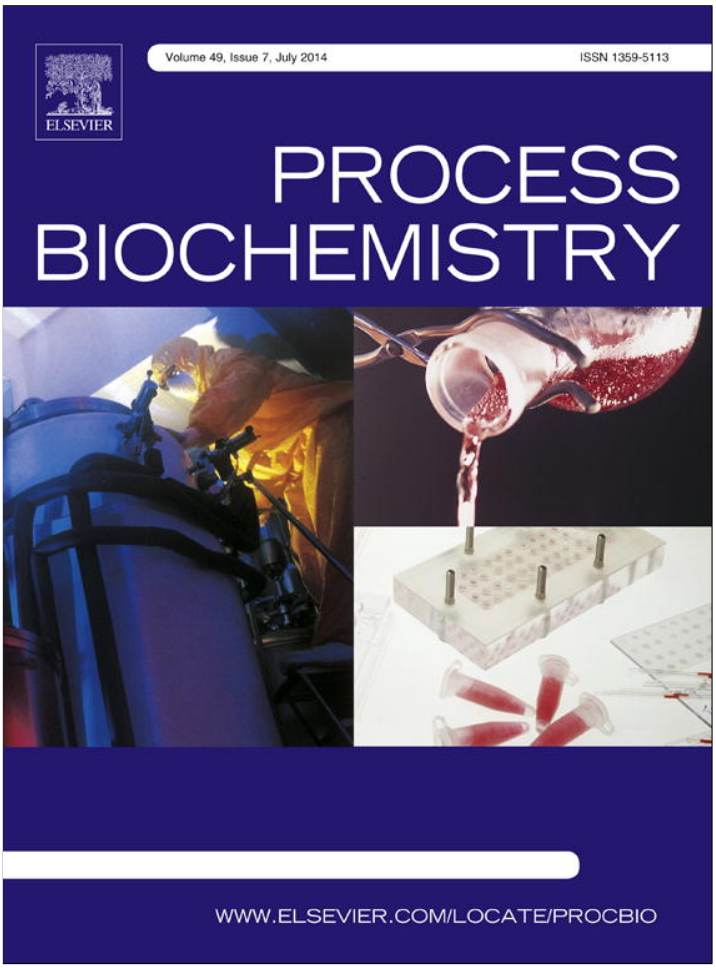

Process Biochemistry 49 (2014) 1071–1077

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Short communication

Lactic acid bacteria isolated from fish gut produce conjugated linoleic acid without the addition of exogenous substrate

María Soledad Vela Gurovic[a,*], Alejandro Raul Gentili[b], Nelda Lila Olivera[c], María Susana Rodríguez[a]

[a] INQUISUR-CONICET, Av. Alem 1253, Universidad Nacional del Sur, B8000CPB Bahía Blanca, Argentina
[b] Departamento de Biología, Bioquímica y Farmacia, San Juan 670, Universidad Nacional del Sur, B8000CPB Bahía Blanca, Argentina
[c] Centro Nacional Patagónico (CONICET), Blvd. Brown 2915, U9120ACF Puerto Madryn, Chubut, Argentina

ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form 4 April 2014
Accepted 5 April 2014
Available online 16 April 2014

Keywords:
Conjugated linoleic acid
Lactic acid bacteria
Fish
Linoleic acid

ABSTRACT

The production of conjugated linoleic acid (CLA) by four strains of lactic acid bacteria isolated from fish, i.e., *Leuconostoc mesenteroides* H20, *Leuconostoc mesenteroides* H22, *Leuconostoc lactis* H24 and *Lactobacillus pentosus* H16, was evaluated in MRS broth and on MRS agar. The bioconversion and production of CLA by resting cells were also assessed. Linoleic acid was detected in cultures grown on agar at percentages of up to 18.3% (w/w) of total fatty acid, and conjugated isomers were found in the fatty acid profiles of *Lactobacillus pentosus* H16. The percentage of CLA relative to total fatty acid increased from $5.68 \pm 1.65\%$ to $23.69 \pm 0.79\%$ when resting cells were removed from agar plates and incubated without the addition of exogenous linoleic acid as a substrate. When *Lactobacillus pentosus* H16 cells were incubated with linoleic acid, cyclization and changes in monounsaturated fatty acid percentages were observed instead of conjugation. These results show that growth on a solid support is required for CLA production. More significantly, an increase in the CLA content could be achieved by incubating resting cells without exogenous substrate.

1. Introduction

Conjugated linoleic acid (CLA) is a mixture of isomers of linoleic acid (cis, cis-9,12-octadecadienoic acid; *c*9,*c*12 18:2, Fig. 1-I) with well-known health benefits, such as anticarcinogenic, antiobesity, antidiabetic, antihypertensive, antiatherogenic, immunomodulatory and osteosynthetic properties [1,2]. While the double bonds of linoleic acid (LA) are separated by a non-conjugated methylene group ($-CH_2-$), the double bonds of the conjugated isomers are contiguous, thus allowing overlapping of p-orbitals (Fig. 1-II, III and IV). Approximately 28 different isomers, which differ in double bond geometry and position, have been identified in nature [2]. The most abundant isomer in food is *c*9,*t*11 (rumenic acid, Fig. 1-II), and the isomer *t*10,*c*12 is found in minor proportions (Fig. 1-III). CLA is naturally present in dairy products, meat and vegetable oils at low percentages. Due to its beneficial properties for human health, many efforts have been made to increase dietary CLA content. One approach consists of the addition of CLA as a functional food additive [3]. Commercially available CLA is chemically synthesized from vegetable oils rich in linoleic acid and consist mainly of a mixture of *c*9,*t*11 and *t*10,*c*12 isomers [4]. Another approach for increasing CLA intake in humans focuses on the use of probiotics capable of producing CLA from linoleic acid. The ability to produce CLA is considered a desirable property of starter cultures and probiotic strains. These studies are based on the bioconversion of linoleic acid and other exogenous substrates. Furthermore, some evidence of bacterial CLA production without the addition of linoleic acid has been found in the literature [5–8].

Although CLA bioconversion has been intensively studied in the last years, further efforts are needed for a better understanding of the microbial processes involved in CLA biosynthesis to find or improve biological systems that could increase the CLA food supply for humans [2]. The search for new systems that may be able to produce a blend similar to that found in natural sources has been extended from bacteria to yeast and fungi. Among them, lactic acid bacteria commonly found in dairy products, rumen and human intestine, play a major role in the metabolic processes that supply conjugated linoleic acid isomers for human intake. Fig. 1 shows the mechanisms of the isomerization process by which lactic acid bacteria (LAB) and other microorganisms conjugate linoleic acid

* Corresponding author. Tel.: +54 291 4595100; fax: +54 291 4595160.
E-mail addresses: svela@uns.edu.ar, msvelagurovic@gmail.com (M.S. Vela Gurovic), agentili@uns.edu.ar (A.R. Gentili), olivera@cenpat.edu.ar (N.L. Olivera), mrodri@uns.edu.ar (M.S. Rodríguez).

http://dx.doi.org/10.1016/j.procbio.2014.04.004
1359-5113/

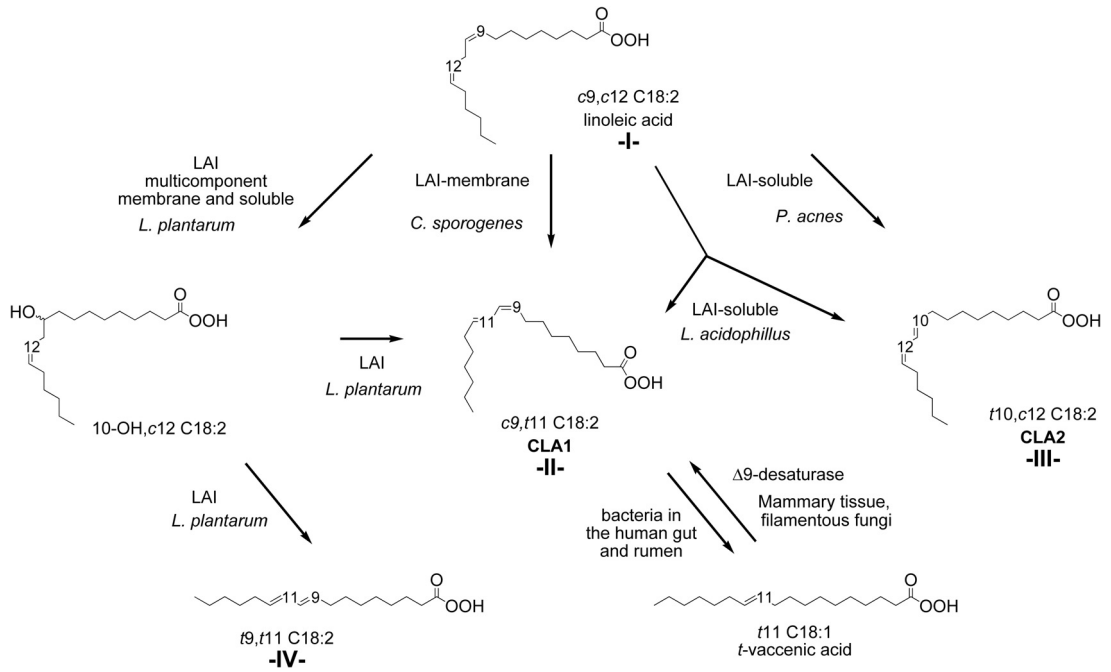

Fig. 1. Microbial conjugation of linoleic acid. *L. plantarum* = *Lactobacillus plantarum*; *L. acidophillus* = *Lactobacillus acidophillus*; *P. acnes* = *Propionibacterium acnes*; *C. sporogenes* = *Clostridium sporogenes*. LAI refers to the enzyme linoleic acid isomerase.

(LA). Conjugation of LA by the linoleic acid isomerase (LAI) from *Lactobacillus plantarum* involves the synthesis of a hydroxylated intermediate [9]. This multicomponent enzyme also catalyzes the conversion of this intermediate into *c*9,*t*11 (CLA 1) and *t*9,*t*11 isomers. *Propionibacterium acnes* converts LA into the *t*10,*c*12 isomer (CLA 2) by the action of a soluble isomerase [10]. The isomerase from *Clostridium sporogenes* is a membrane-associated enzyme that converts LA into the CLA 1 isomer [11]. The latter is also produced from *t*-vaccenic acid by Δ9 desaturase, which is present in the mammary glands of humans and cattle and in filamentous fungi [12]. The linoleate isomerase of *Lactobacillus acidophilus* is a soluble enzyme that converts linoleic acid into CLA 1 and CLA 2 and other isomers [13]. Lastly, bacteria in the human gut [14] and anaerobic bacteria in the rumen [15] hydrogenate linoleic acid to *t*-vaccenic acid after conjugation of its double bonds.

Lactic acid bacteria are also found in the fish gut. Contrasting with the predominance of anaerobic bacteria in the lower intestine of humans, the fish gut microbiota is mainly composed by aerobic or facultative anaerobic bacteria [16]. Although CLA has not been reported as a typical component of fish meat or oil [17], linoleic acid was found in the muscle tissue of some fish species at percentages that varied from 4 to 30% of the total fatty acids depending on the fatty acid composition of the diet [18]. To the best of our knowledge, the potential of LAB isolated from the fish gut has not been investigated.

Other authors reported high yields of CLA by the bioconversion of LA [2,15,19]. In these previous studies, the production of CLA by LAB has been achieved by the addition of linoleic and other fatty acids as substrates. However, this imposes a limit to bacterial CLA production due to poor LA solubility, potential LA toxicity and high production costs [2]. Some studies suggest that LAB already contain LA in their cell membranes [20,21]; therefore, the use of strains that are able to produce the endogenous substrate needed for further bioconversion into CLA would present advantages over LA- or substrate-dependent strains.

The aim of the present study was to explore the properties of LAB isolated from the fish gut for the production of CLA. For that purpose, we evaluated whether the cells contained preexisting LA and CLA isomers in their membranes or whether the addition of exogenous linoleic acid was needed for their bioconversion into CLA.

2. Materials and methods

2.1. Chemicals

Linoleic acid and fatty acid methyl ester standards were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Synthetic De Man, Rogosa and Sharpe broth (MRS) and bacteriological agar were purchased from Laboratorios Britania S.R.L. (Buenos Aires, Argentina). Agar was added at 1.5% (w/v) for the preparation of solid media.

2.2. Bacterial strains, cultivation and preparation of resting cells

The strains *Leuconostoc mesenteroides* H20, *Leuconostoc mesenteroides* H22 and *Leuconostoc lactis* H24 were isolated from the intestinal tract of rainbow trout (*Oncorhynchus mykiss*), as explained in a previous work [22]. *Lactobacillus pentosus* H16 from the CENPAT microbial collection was previously isolated from the gut of hake (*Merluccius hubbsi*). After incubation, individual colonies were selected from plates containing less than 300 colonies based on differences in form, size, colour, elevation, border and Gram reaction [23]. The selected colonies were purified by repeated streaking on MRS agar and stored at −80 °C in MRS broth supplemented with 20% (v/v) glycerol (Anedra, 99.0%, Buenos Aires, Argentina). *Lactococcus lactis* CNRZ481 isolated from dairy products [24] was kindly provided by Dr. J.-C. Piard (Institut Micalis, INRA, France) and used as a control due to its ability to conjugate linoleic acid [2]. Strains were activated in MRS broth at 25 °C for 48 h and further incubated in broth for 24 h without agitation. All experiments were run in triplicate.

Bacterial cells grown on MRS broth were pelleted at 4300 rpm for 20 min at room temperature (Luguimac LC-20, maximum centrifugal force 2500 × *g*, Buenos Aires, Argentina) and washed twice with 0.85% NaCl (w/v) solution. To construct the growth curve of

Lactobacillus pentosus H16 that is shown in Fig. 4, aliquots were taken at different time points and subjected to plate viable counting. The optical density of each aliquot was measured at 600 nm using a UV–visible spectrophotometer (UV-1603, Shimadzu).

To analyze the bacterial cells grown on MRS agar, the plates were inoculated according to the quadrant streak technique described in the Microbial Identification System protocol (MIS Operating Manual 3.1, 1999) produced by Microbial ID (MIDI, Newark, DE 19713, USA). Colonies from confluent zones (quadrants 1 and 2) were removed from the Petri dishes (20–40 mg) for fatty acid analyses.

For free cell analysis, colonies were picked from the same zones after incubation for 48 h at 25 °C. A solution containing 20 mg/mL Tween 80 (used as a solubilizing agent, Biopack, polyoxyethylene-20-sorbitan monooleate, 99–100%, Buenos Aires, Argentina) and 4.4 mg/mL linoleic acid in potassium phosphate buffer 0.1 M, pH 6.5 was sterilized with a 0.22-μm filter and added to the biomass at a final LA concentration of 1.8 mg LA/mL. This concentration was selected according to a previous report [25]. Anhydrous monobasic potassium phosphate and dibasic potassium phosphate were from Anedra (98.0%, Buenos Aires, Argentina). Cells were incubated at 25 °C without agitation. Aliquots of the cell suspension were taken at 0, 12 and 24 h for fatty acid analyses. Control cells were suspended in potassium phosphate buffer 0.1 M, pH 6.5 without LA. The cell density was kept at 200 mg wet biomass per mL (20% wet cell, w/v) for all treatments.

2.3. 16S rRNA sequencing

The strains *Leuconostoc mesenteroides* H20, *Leuconostoc mesenteroides* H22 and *Leuconostoc lactis* H24 were identified by 16S rRNA sequencing. Sequences were PCR amplified as described previously [22] using the DNA thermal cycler Multigene Gradient (Labnet International Inc., Woodbridge, NJ). Sequencing of both strands of the amplified fragments was performed using the dideoxy chain termination method by the commercial services of CENPAT Molecular Biology Laboratory (Argentina). The 16S rRNA gene sequences were compared to the EzTaxon-extended database [26]. The sequences generated in this study were deposited into the GenBank database under the accession numbers KC937069, KC937070 and KC937071 for strains H20, H22 and H24, respectively, and KC921993 for strain H16.

2.4. Fatty acid analyses

Lipid extraction and reaction conditions were previously described [19]. Briefly, fatty acids were extracted with 1.5 mL of chloroform/methanol (1:2, v/v). The organic phase was collected, washed once with 1 mL of 0.85% NaCl (w/v) and dried over anhydrous sodium sulfate. The clear organic phase was poured into a clean glass tube, and 1 mL of 10% methanolic HCl (v/v) was added [19]. This solution was prepared with 37% HCl (w/v) (for analysis, Merck, Buenos Aires, Argentina) and anhydrous methanol (Biopack, ≥99.8%, Buenos Aires, Argentina). The reaction was held at 50 °C for 20 min. These mild conditions were selected to avoid the disruption of fragile fatty acids, as previously reported [5]. After cooling the samples to room temperature, fatty acid methyl esters were extracted with 1 mL of n-hexane (chromatographic grade, U.V.E. Dorwil, Buenos Aires, Argentina). Samples were immediately dried before chromatographic analysis or stored under a nitrogen atmosphere.

2.5. Gas chromatography–mass spectrometry analysis

Fatty acid methyl esters were analyzed by GC–MS with a HP6890 chromatograph equipped with a mass spectrometer HP5972A. The ionization energy was 70 eV. Samples (1 μL) were injected into a HP-5 capillary column (30 m × 0.25 mm i.d. × 0.25-μm film thickness). The temperature was programmed from 85 °C to 250 °C at a rate of 4 °C/min and held at the final temperature for 15 min. The temperature of the injector and detector was 280 °C; and the carrier gas was helium at a flow rate of 1 mL/min and a split ratio of 20:1. The fatty acid compositions were expressed as relative percentages (w/w).

Fatty acids were identified by comparing their retention times and mass fragmentation patterns with those of the standards. CLA1 refers to *c*9,*t*11-octadecadienoic acid; CLA2 refers to *t*10,*c*12; and CLA3 is a mixture of other isomers, including *t*9,*t*11. Cyclic fatty acid methyl esters were identified by comparison of their mass spectra with data from the literature [27] and from the database of the chromatograph.

2.6. Statistical analysis

The relative percentages are expressed as the mean and standard error of the mean. Significant differences were tested using a two-tailed, paired *t*-test. *p* values less than 0.05 were considered significant. The data of Table 1 was analyzed using two-way ANOVA, and a post test was performed using the Bonferroni method with $p < 0.001$ for multiple comparisons between treatments [28].

3. Results and discussion

3.1. 16S rRNA sequencing

The closest match of the 16S rRNA sequences (~1400 bp) of isolates H20 and H22 was to that of *Leuconostoc mesenteroides* subsp. *dextranicum* NRIC 1539^T (99.77 and 99.85% homology, respectively), while 100% homology was obtained when comparing the 16S rRNA sequences of isolate H24 and *Leuconostoc lactis* KCTC 3528^T.

3.2. Fatty acid profile of LAB isolated from the fish gut in MRS broth vs. MRS agar

The fatty acid composition of cells grown in culture broth comprised primarily saturated fatty acids except 20–25%, which was oleic acid (Fig. 2). Cells of these strains removed from agar cultures displayed a more miscellaneous pattern. *Lactococcus lactis* CNRZ481 was previously isolated from dairy products. The fatty acid profile of this strain when grown in broth contained saturated and oleic acids and vaccenic and cyclic fatty acids (Fig. 2). The results for this strain agree with those reported for *Lactococcus* strains [29].

The membranes of the strains *Leuconostoc mesenteroides* H22, *Leuconostoc lactis* H24 and *Lactobacillus pentosus* H16 contained between 15 and 18% linoleic acid when grown on MRS agar. There is evidence that linoleic acid is present in the membranes of lactic acid bacteria under certain conditions [8,30,31] and in MRS broth in small amounts: 0.1 μg LA/mL MRS broth, representing 0.1% of the total fatty acid [7]. In the present study, linoleic acid was not detected as a component of the utilized medium, supporting the idea that these strains could have produced linoleic acid. The cell membranes of *Lactococcus lactis*, a strain isolated from sources other than fish, did not contain this fatty acid when grown under the same conditions (Fig. 2).

Linoleic acid was found at percentages of up to 20% in membranes of a *Lactobacillus acidophilus* strain at 25 °C in MRS broth, and it was suggested that the production of this fatty acid, which decreased at higher temperatures, would be essential for adaptation to freeze-thaw stress [20]. In contrast, a thermotolerant *Lactobacillus helveticus* strain increased its linoleic acid to 10% of its cellular lipids at superoptimal temperatures combined with low pH values [30]. Other authors found that LAB produced linoleic acid as

Table 1
Fatty acid profile of *Lactobacillus pentosus* H16 free cell suspension incubated with linoleic acid vs. untreated cells.

	Free cells + LA			Free cells
Incubation time (h)	0	12	24	24
19Cyc 9/11 (CYC)	n.d.[a]	4.44 ± 0.51[a]	11.42 ± 0.92[b]	n.d.[a]
C16:1 Δ9 (PA)	3.53 ± 0.39[a]	1.37 ± 0.59[a]	0.83 ± 0.12[a]	n.d.[a]
C18:1 Δ9 (OLE)	67.83 ± 1.16[b]	11.01 ± 1.89[a]	8.14 ± 1.94[a]	12.31 ± 2.11[a]
C18:1 Δ11 (VA)	8.14 ± 0.09[a]	18.04 ± 2.17[b]	24.34 ± 0.98[c]	n.d.[d]
C18:2 *c*9,*c*12 (LA)	11.79 ± 0.19[b]	1.57 ± 0.49[a]	n.d.[a]	n.d.[a]
C18:2 *c*9,*t*11/*t*10,*c*12 (CLA)	n.d.[a]	1.36 ± 0.68[a]	n.d.[a]	23.69 ± 0.79[b]
C14	0.84 ± 0.79[a]	6.39 ± 0.58[a]	2.19 ± 0.37[a]	3.47 ± 1.52[a]
C16	3.93 ± 0.29[a]	32.63 ± 0.56[b]	32.22 ± 1.72[b]	29.52 ± 1.70[b]
C18	1.53 ± 0.04[a]	23.52 ± 1.91[b]	20.28 ± 0.55[b]	26.13 ± 3.16[b]

Values are mean ± SEM. n.d.: not detected. Different superscript letters within a row means significant differences ($p < 0.001$).

a response to desiccation [21]. In all of the aforementioned cases, an increase in linoleic acid content seemed to be linked to stress conditions. In addition, some processes in LAB, such as bacteriocin production, occurred in a constitutive manner on solid, but not in liquid media [32,33].

The production of linoleic acid on agar plates that is shown in the present study could be the consequence of several factors that affect lipid unsaturation and develop differently in liquid media with respect to solid supports, such as the growth rate [34,35]. These previous studies revealed that *Lactococcus lactis* cells growing in liquid media show different growth rates than those growing on solid supports. Additionally, the low growth rates are associated with changes in the lipid unsaturation of the cell membranes.

CLA isomers were only detected in *Lactobacillus pentosus* H16 cells growing on MRS agar at low percentages of 5.68 ± 1.35 (Fig. 3). Further studies on liquid cultures of this strain showed that the cellular fatty acid composition remained relatively constant (the means of each fatty acid at different time points were not significantly different, $p > 0.05$), while neither linoleic acid nor CLA were detected at any growth stage (Fig. 4). These results confirmed that linoleic acid and CLA isomers were only observed in *Lactobacillus pentosus* H16 when grown on MRS agar.

3.3. The addition of exogenous LA to resting cells of Lactobacillus pentosus H16

The presence of LA and CLA isomers in the membranes of *Lactobacillus pentosus* H16 cells grown on MRS agar led us to test the bioconversion of exogenous LA by this strain. Resting cells that were removed from agar plates were incubated with linoleic acid, and the fatty acid profiles were obtained at different incubation times (Table 1). The fatty acid profile of untreated cells was also analyzed.

The percentage of oleic acid was significantly higher at the beginning of incubation due to oleic acid being supplemented by Tween 80, which is the solubilizing agent of linoleic acid. Noteworthy, the percentage of oleic acid decreased after 24 h of incubation, showing that the resting cells consumed oleic acid. Saturated fatty acids did not display significant differences, with the exception of values observed at the beginning of the incubation. The high percentage of oleic acid from the solubilizing agent accounts for the low percentages of saturated fatty acids that were observed at time 0.

The following cyclic fatty acids were detected after 12 and 24 h of incubation with LA (Fig. 5A): dihydrosterculic (9,10-methyleneoctadecanoic acid, 19cyc9) and lactobacillic acid (11,12-methyleneoctadecanoic acid, 19cyc11). Neither controls (untreated cells) nor cells at an incubation time of 0 h showed cyclic fatty acids, but percentages shown at 24 h of incubation with LA were significantly higher ($p < 0.001$). No significant differences were found for palmitoleic acid between treatments. The percentage of vaccenic acid was significantly different between treatments, increasing after incubation with LA.

Linoleic acid decreased during the incubation period and was not detectable at 24 h. This fatty acid was also not detected in control cells (Fig. 5B). The desirable conjugated product was not detected or was detected at low percentages after treatment with

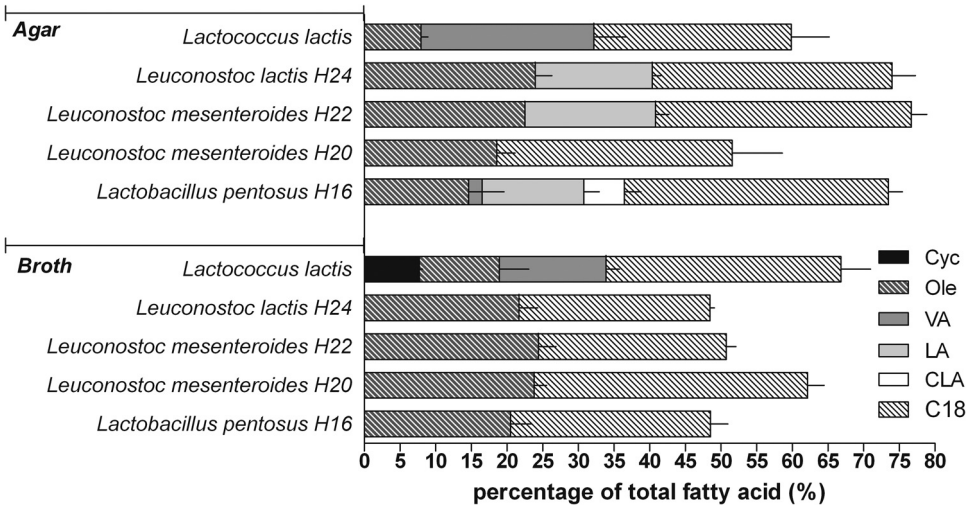

Fig. 2. Relative percentages of cellular fatty acids of LAB grown on MRS agar vs. those obtained from liquid culture in MRS broth. Fatty acid percentages relative to the total fatty acid of lactic acid bacteria isolated from fish cultured on MRS agar (above) vs. the fatty acid profile displayed by MRS liquid cultures (below). Cyc = cyclic fatty acids; OLE = oleic acid; VA = vaccenic acid; LA = linoleic acid; CLA = conjugated linoleic acid. C18 = stearic acid. The strains were incubated for 24 h at 25 °C.

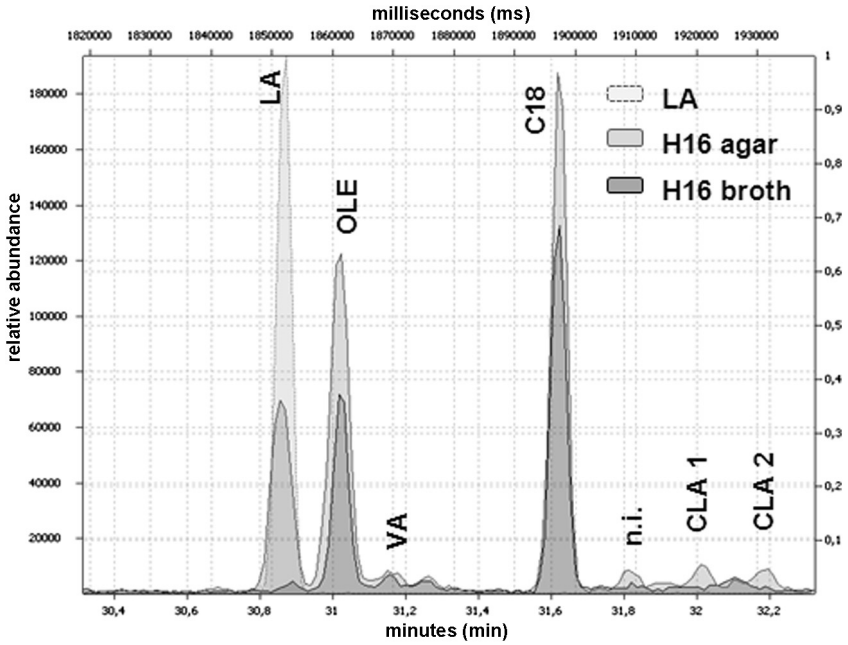

Fig. 3. Fatty acid profiles of *Lactobacillus pentosus* H16 grown on MRS agar vs. those of cells grown in broth. A section of the overlapped chromatograms showing the cellular fatty acid profile of strain H16 grown in MRS broth (highlighted in dark grey, solid line) and that obtained after cultivation on agar (light grey, solid line). The peak of the LA standard appears in light grey (dashed line). All results were obtained after a 24 h incubation at 25 °C. CLA 1 = *c*9,*t*11 C18:2 methyl ester (ME); CLA 2 = *t*10,*c*12 C18:2 ME; LA = linoleic acid ME; VA = Δ11 octadecenoic acid ME (vaccenic acid); OLE = oleic acid ME; C18 = stearic acid ME; n.i. = not identified.

linoleic acid. In contrast, the controls displayed the highest percentage of the conjugated isomers. The CLA percentages found for resting cells of *Lactobacillus pentosus* H16 without the addition of exogenous LA comprised 10.46 ± 0.51% of *c*9,*t*11 CLA, 13.24 ± 0.27% of *t*10,*c*12 CLA and less than 1% of a mixture of *t*,*t* and other isomers (Fig. 5B).

The results shown in the present study suggest that LAB produce linoleic acid when grown on solid medium (Fig. 2), which may serve as the substrate for further conversion into its conjugated isomers (Fig. 5B). These results are in agreement with previous reports that showed CLA production by lactobacilli at small percentages ranging from 2.7 to 3.4% [8]. Other authors found up to 5% CLA in lyophilized LAB [5]. However, a deeper insight into the literature revealed that MRS broth also contains small quantities of CLA. Percentages of CLA of 1.2 μg/mL MRS broth (1.8%) and 17 μg/mL MRS broth (6.4%) have been found, [7,8]. These values are even higher than those detected in cultures. This fact raised the question of whether these small relative percentages of CLA are truly due to bacterial activity. In the present study, the percentage of CLA that was detected in control cells released from medium (Table 1) was significantly different ($p < 0.001$) from that found in the MRS broth used in this study (1.27 ± 0.35%), which supports the hypothesis that *Lactobacillus pentosus* H16 produces CLA from internal precursors rather than from the medium.

Growth on solid medium was shown to be a critical factor for increasing the linoleic acid content in *Leuconostoc* strains (H22 and H24) and *Lactobacillus pentosus* H16 (Fig. 2), which are native gut bacteria from wild rainbow trout and hake, respectively.

The addition of exogenous LA to *Lactobacillus pentosus* H16 free cells picked from agar plates led to an increase in the percentage of cyclic fatty acids (19Cyc 9/11) and changes in the ratio of vaccenic to oleic acid from 0.1 to 3.0 after 24 h of incubation (Table 1). Neither linoleic acid nor its conjugated isomers were detected after 24 h (Fig. 5A). When the cells were released from media and further incubated, linoleic acid was nearly absent, while the percentages of CLA increased (Table 1, Fig. 5B), which supports the idea that conjugated isomers result from the conjugation of preexisting LA in cells. The main outcome of this study is the evidence showing that the CLA content in the cell membranes can be significantly different from that found in MRS and that this bioconversion does not consume exogenous linoleic acid as a substrate. These findings are of great value for dairy science and aquaculture as well as for

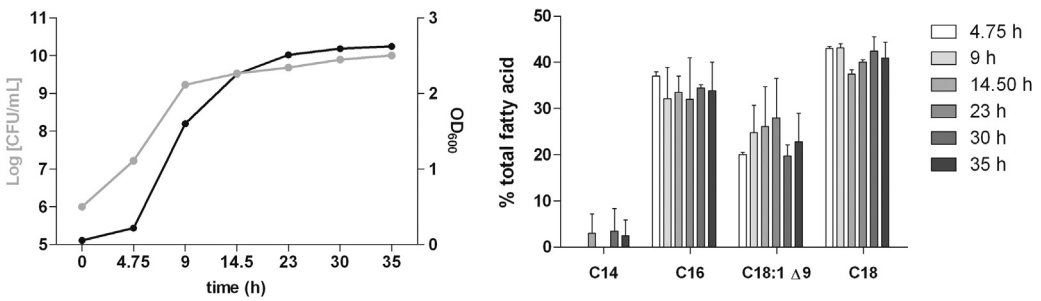

Fig. 4. Cellular fatty acid profiles of *Lactobacillus pentosus* H16 liquid cultures. The growth and fatty acid profiles of *L. pentosus* H16 in MRS broth at 25 °C without agitation were monitored at several time points. Left: Growth was measured as the logarithm of the number of colony forming units per mL (CFU/mL) on the left *Y*-axis (light line). Optical density at 600 nm was registered on the right *Y*-axis (dark line). Right: Fatty acid percentages comprised of saturated C14 (myristic acid); C16 (palmitic acid); C18 (stearic acid); and unsaturated C18:1 Δ9 (oleic acid).

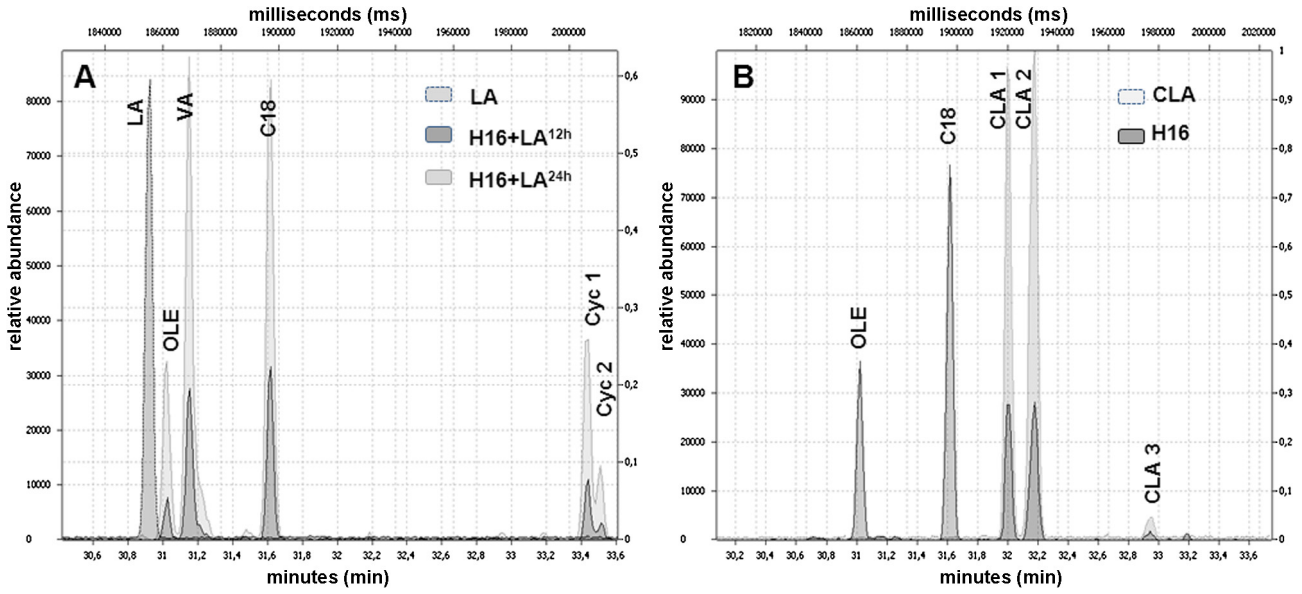

Fig. 5. Fatty acid profiles of *Lactobacillus pentosus* H16 free cells after incubation at 25 °C. (A) A section of the chromatogram obtained from free cells grown on MRS agar and further incubated with 1.8 mg LA/mL for 12 h (dark grey) and 24 h (light grey) vs. the LA methyl ester standard (CLAME, dashed line). The chromatogram in (B) shows the fatty acid profile of cells previously grown on MRS agar and further incubated in buffer without the addition of LA vs. the overlapping chromatogram of the CLAME standard. Cyc 1 = dihydrosterculic acid ME (C19cyc9); Cyc 2 = lactobacillic acid ME (C19cyc11); CLA 1 = *c*9,*t*11 C18:2 ME; CLA 2 = *t*10,*c*12 C18:2 ME; CLA 3 = mixture of *t*9,*t*11 C18:2 ME and other isomers; OLE = oleic acid ME; VA = vaccenic acid ME; C18 = stearic acid ME; LA = linoleic acid ME.

the industrial production of CLA by bio-catalysis. The use of strains that are able to produce linoleic acid and convert it into CLA could help to reduce the production costs of industrial processes.

Acknowledgments

The authors acknowledge Dr. J.-C. Piard (INRA, France) for kindly supplying *Lactococcus lactis* CNRZ481 and Dr. C. Riva-Rossi and Lic. M. Tagliaferro (CENPAT-CONICET) for providing fish samples. This work was supported by Universidad Nacional del Sur. N.L. Olivera is a research member of the National Scientific and Technical Research Council of Argentina (CONICET). M. S. Vela Gurovic is grateful to CONICET for a postdoctoral grant.

References

[1] Koba K, Yanagita T. Health benefits of conjugated linoleic acid (CLA). Obes Res Clin Pract 2013, http://dx.doi.org/10.1016/j.orcp.2013.10.001 (in press).
[2] Andrade JC, Ascençao K, Gullón P, Henriques SMS, Pinto JMS, Rocha-Santos TAP, et al. Production of conjugated linoleic acid by food-grade bacteria: a review. Int J Dairy Technol 2012;65:467–81.
[3] Kovacs EMR, Mela DJ. Metabolically active functional food ingredients for weight control. Obes Rev 2006;7:59–78.
[4] Kennedy A, Martinez K, Schmidt S, Mandrup S, LaPoint K, McIntosh M. Antiobesity mechanisms of action of conjugated linoleic acid. J Nutr Biochem 2010;21:171–9.
[5] Dionisi F, Golay P-A, Elli M, Fay LB. Stability of cyclopropane and conjugated linoleic acids during fatty acid quantification in lactic acid bacteria. Lipids 1999;34:1107–15.
[6] Lin TY. Conjugated linoleic acid production by cells and enzyme extract of *Lactobacillus delbrueckii* ssp. *bulgaricus* with additions of different fatty acids. Food Chem 2006;94:437–41.
[7] Jiang J, Bjorck L, Fonden R. Production of conjugated linoleic acid by dairy starter cultures. J Appl Microbiol 1998;85:95–102.
[8] Kankaanpää P, Yang B, Kallio H, Isolauri E, Salminen S. Effects of polyunsaturated fatty acids in growth medium on lipid composition and on physicochemical surface properties of *Lactobacilli*. Appl Environ Microbiol 2004;70:129–36.
[9] Kishino S, Ogawa J, Yokozeki K, Shimizu S. Linoleic acid isomerase in *Lactobacillus plantarum* AKU1009a proved to be a multi-component enzyme system requiring oxidoreduction cofactors. Biosci Biotechnol Biochem 2011;75:122–318.
[10] Liavonchanka A, Hornung E, Feussner I, Rudolph MG. Structure and mechanism of the *Propionibacterium acnes* polyunsaturated fatty acid isomerase. Proc Natl Acad Sci U S A 2006;103:2576–81.
[11] Peng SS, Deng M-D, Grund AD, Rosson RA. Purification and characterization of a membrane-bound linoleic acid isomerase from *Clostridium sporogenes*. Enzyme Microb Technol 2007;40:831–9.
[12] Ando A, Ogawa J, Sugimoto S, Kishino S, Sakuradani E, Yokozeki K, et al. Selective production of cis-9,trans-11 isomer of conjugated linoleic acid from trans-vaccenic acid methyl ester by *Delacroixia coronate*. J Appl Microbiol 2009;106:1697–704.
[13] Lin TY, Lin C-W, Wang Y-J. Production of conjugated linoleic acid by enzyme extract of *Lactobacillus acidophilus* CCRC 14079. Food Chem 2003;83:27–31.
[14] Devillard E, McIntosh FM, Duncan SH, Wallace RJ. Metabolism of linoleic acid by human gut bacteria: different routes for biosynthesis of conjugated linoleic acid. J Bacteriol 2007;189:2566–70.
[15] Ogawa J, Kishino S, Ando A, Sugimoto S, Mihara K, Shimizu S. Production of conjugated fatty acids by lactic acid bacteria. J Biosci Bioeng 2005;100:355–64.
[16] Rurangwa E, Laranja JL, Van Houdt R, Delaedt Y, Geraylou Z, Van de Wiele T, et al. Selected nondigestible carbohydrates and prebiotics support the growth of probiotic fish bacteria mono-cultures in vitro. J Appl Microbiol 2009;106:932–40.
[17] Rosa R, Andrade AM, Bandarra NM, Nunes ML. Physiological and biochemical effects of conjugated linoleic acid and its use in aquaculture. Rev Aquacult 2010;2:59–72.
[18] Benedito-Palos L, Bermejo-Nogales A, Karampatos AI, Ballester-Lozano GF, Navarro JC, Diez A, et al. Modeling the predictable effects of dietary lipid sources on the fillet fatty acid composition of one-year-old gilthead sea bream (*Sparus aurata* L.). Food Chem 2011;124:538–44.
[19] Kishino S, Ogawa J, Omura Y, Matsumura K, Shimizu S. Conjugated linoleic acid production from linoleic acid by lactic acid bacteria. J Am Oil Chem Soc 2002;79:159–63.
[20] Fernández Murga ML, Cabrera GM, Font de Valdez G, Disalvo A, Seldes AM. Influence of growth temperature on cryotolerance and lipid composition of *Lactobacillus acidophilus*. J Appl Microbiol 2000;88:342–8.
[21] Santivarangkna C, Kulozik U, Kienberger H, Foerst P. Changes in membrane fatty acids of *Lactobacillus helveticus* during vacuum drying with sorbitol. Lett Appl Microbiol 2009;49:516–21.
[22] Sequeiros C, Vallejo M, Marguet ER, Olivera NL. Inhibitory activity against the Fish pathogen *Lactococcus garvieae* produced by *Lactococcus lactis* TW34, a lactic acid bacterium isolated from the intestinal tract of a Patagonian fish. Arch Microbiol 2010;192:237–45.
[23] Sica MG, Olivera NL, Brugnoni LI, Marucci PL, López Cazorla AC, Cubitto MA. Isolation, identification and antimicrobial activity of lactic acid bacteria from the Bahía Blanca Estuary. Rev Biol Mar Oceanogr 2010;45:389–97.
[24] Piard J-C, Delorme F, Giraffa G, Commissaire J, Desmazeaud M. Evidence for a bacteriocin produced by *Lactococcus lactis* CNRZ 481. Neth Milk Dairy J 1990;44:143–58.
[25] Lee SO, Kim CS, Cho SK, Choi HJ, Ji GE, Oh D-K. Bioconversion of linoleic acid into conjugated linoleic acid during fermentation and by washed cells of *Lactobacillus reuteri*. Biotechnol Lett 2003;25:935–8.
[26] Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, et al. Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 2012;62:716–21.

[27] Hanus LO, Goldshlag P, Dembitskya VM. Identification of cyclopropyl fatty acids in walnut (*Juglans regia* L.) oil. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2008;152:41–5.
[28] Neter J, Wasserman W, Kutner MH. Applied Linear Statistical Models. 3rd ed. Boston: Irwin; 1990. p. 741–744, 771.
[29] Johnsson T, Nikkila P, Toivonen L, Rosenqvist H, Laakso S. Cellular fatty acid profiles of *Lactobacillus* and *Lactococcus* strains in relation to the oleic acid content of the cultivation medium. Appl Environ Microbiol 1995;61:4497–9.
[30] Guerzoni ME, Lanciotti R, Cocconcelli PS. Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*. Microbiology 2001;147:2255–64.
[31] Montanari C, Sado Kamdem SL, Serrazanetti DI, Etoa F-X, Guerzoni ME. Synthesis of cyclopropane fatty acids in *Lactobacillus helveticus* and *Lactobacillus sanfranciscensis* and their cellular fatty acids changes following short term acid and cold stresses. Food Microbiol 2010;27:493–502.
[32] Maldonado-Barragán A, Ruiz-Barba JL, Jiménez-Díaz R. Knockout of three-component regulatory systems reveals that the apparently constitutive plantaricin-production phenotype shown by *Lactobacillus plantarum* on solid medium is regulated via quorum sensing. Int J Food Microbiol 2009;130:35–42.
[33] Tagg JR, Dajani AS, Wannamaker LW. Bacteriocins of gram-positive bacteria. Bacteriol Rev 1976;40:722–56.
[34] Kabanova N, Stulova I, Vilu R. Microcalorimetric study of the growth of bacterial colonies of *Lactococcus lactis* IL1403 in agar gels. Food Microbiol 2012;29:67–79.
[35] Špitsmeister M, Adamberg K, Vilu R. UPLC/MS based method for quantitative determination of fatty acid composition in Gram-negative and Gram-positive bacteria. J Microbiol Methods 2010;82:288–95.