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Short communication

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

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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

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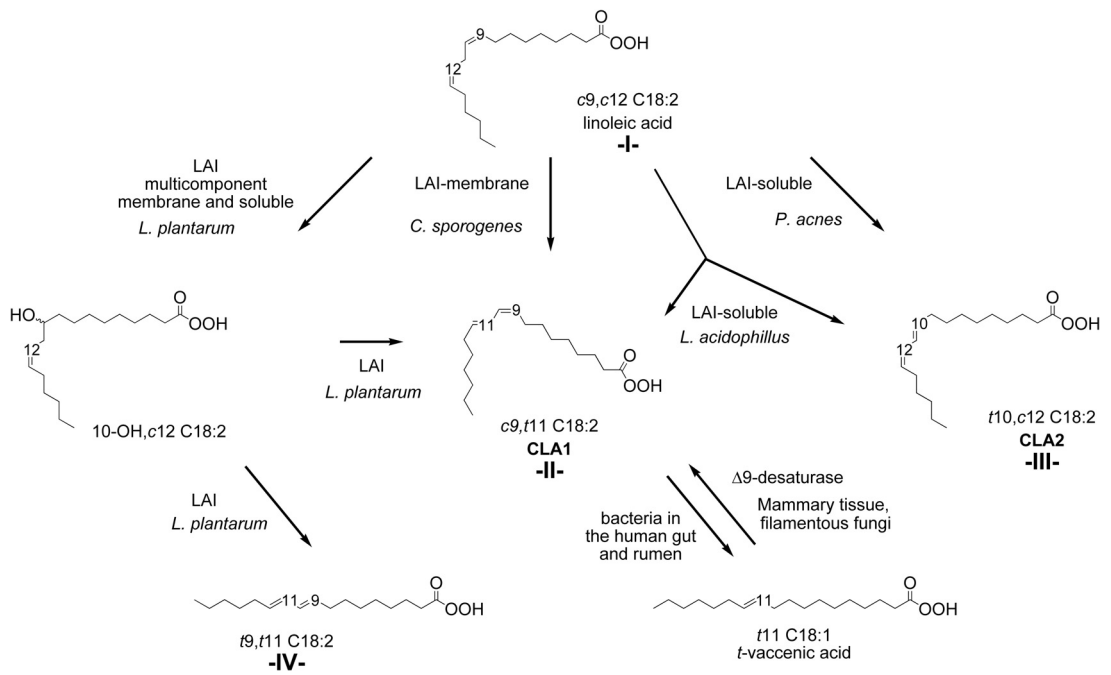

Fig. 1. Microbial conjugation of linoleic acid. *L. plantarum* = *Lactobacillus plantarum*; *L. acidophillus* = *Lactobacillus acidophillus*; *P. acnes* = *Propionibacterium acnes*; *C. sporogenes* = *Clostridium sporogenes*. LAI refers to the enzyme linoleic acid isomerase.

(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

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