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Running title: CLA and CLNA production by bacteria

ABSTRACT

Conjugated linoleic acid (CLA) and conjugated linolenic acid (CLNA) isomers are present in foods derived from ruminants as a result of the respective linoleic acid (LA) and α-linolenic acid (LNA) metabolism by ruminal microorganisms and in animals' tissues. CLA and CLNA have isomer-specific, health-promoting properties, including anti-carcinogenic, anti-atherogenic, anti-inflammatory, and anti-diabetic activity, as well as the ability to reduce body fat. Besides ruminal microorganisms, such as *Butyrivibrio fibrisolvens*, many food-grade bacteria, such as bifidobacteria, lactic acid bacteria, and propionibacteria, are able to convert LA and LNA to CLA and CLNA, respectively. Linoleate isomerase activity, responsible for this conversion, is strain-dependent and probably related to the ability of the producer strain to tolerate the toxic effects of LA and LNA. Since natural concentrations of CLA and CLNA in ruminal food products are relatively low to exert their health benefits, food-grade bacteria with linoleate isomerase activity could be used as starter or adjunct cultures to develop functional fermented dairy and meat products with increased levels of CLA and CLNA or included in fermented products as probiotic cultures. However, results obtained so far are below expectations due to technological bottlenecks. More research is needed to assess if bacterial production kinetics can be increased and can match food processing requirements.

Keywords: conjugated linoleic acid, conjugated linolenic acid, food-grade bacteria, fermented foods, ruminal biohydrogenation

BACKGROUND AND RATIONALE

Conjugated linoleic acid (CLA) and conjugated linolenic acid (CLNA) refer to a mixture of positional and geometrical isomers of linoleic acid (LA; *c9c12*-C18:2) and α-linolenic acid (LNA; *c9c12c15*-C18:3), respectively, with conjugated bonds (Figure 1). They are of great interest because of their potential isomer-specific, health-promoting properties, ranging from anti-carcinogenic and anti-atherogenic properties to reducing body fat, enhancing immune functions, and improving bone mass (Pariza et al., 2001; Martin and Valeille, 2002; Wahle et al., 2004; Tricon et al., 2005; Bhattacharya et al., 2006; Benjamin and Spener, 2009; Park, 2009; de Carvalho et al., 2010; Hennessy et al., 2011). An important element concerning the biological activities of CLA is the specific and multiple effects exerted by different isomers. For instance, the *t9t11*-CLA isomer, in contrast to the *c9t11*-CLA isomer, specifically induces expression of genes involved in the lipid metabolism in human macrophages (Ecker et al., 2009). In addition, this *t9t11*-CLA isomer displays anti-carcinogenic properties that are more effective than those induced by *c9t11*-CLA (Beppu et al., 2006; Coakley et al., 2006). The *t10c12*-CLA isomer is the main responsible isomer for the reduction in body fat, but could also have detrimental effects leading to increased levels of LDL and insulin resistance (Pariza et al., 2001; Risérus et al., 2002; Tricon et al., 2004; Bhattacharya et al., 2006; Raff et al., 2008; Tholstrup et al., 2008; Park, 2009). A concern related to the potential health activities of CLA and CLNA isomers demonstrated so far is that most studies have been performed on animals. Results obtained from animal studies cannot always be extrapolated directly to humans as such because of differences

in experimental setup, e.g. CLA and CLNA doses, and diet, age, and gender of the test subjects (Plourde et al., 2008).

CLA and CLNA isomers are formed during ruminal biohydrogenation. Polyunsaturated fatty acids (PUFA), mainly LA and LNA, present in the diet of ruminants are metabolised in the rumen by several species of microorganisms (Chilliard et al., 2007; Jenkins et al., 2008). At first, dietary fat undergoes lipolysis to release free fatty acids (Jenkins et al., 2008; Lourenço et al., 2010). Then, these free PUFA are converted through isomerisation and hydrogenation to a saturated fatty acid as end-product, namely stearic acid (C18:0). In this biohydrogenation process, LA is mainly isomerised to *c9t11*-CLA and LNA to *c9t11c15*-CLNA. However, other CLA and CLNA isomers are formed as well (Figure 2) (Chilliard et al., 2007; Jouany et al., 2007; Dannenberger et al., 2009). These isomers are further hydrogenated to several *trans*-C18:1 isomers, mainly vaccenic acid (*t11*-C18:1). A second hydrogenation step converts the C18:1 isomers to C18:0. All intermediates of this biohydrogenation process are absorbed in the gut and transported through the blood stream to different body tissues. In certain tissues, and especially in the mammary gland, the *c9t11*-CLA isomer can be produced endogenously from *t11*-C18:1 by Δ9-desaturase activity, which inserts a *cis*-double bond on carbon atom 9 of different fatty acids (Griinari et al., 2000).

Since CLA and CLNA isomers mainly originate from ruminal biohydrogenation and endogenous synthesis via Δ9-desaturase, the largest source of these isomers can be found in meat and dairy products derived from ruminants. A large variability exists in the CLA and CLNA content of meat and dairy products (Table 1). This is due to species and breed variability, feeding system, as well as diverse processing parameters (Raes et al., 2004; Dhiman et al., 2005; Schmid

et al., 2006). The major CLA isomer in these food products is *c9t11*-CLA, accounting for 80-85% of the total CLA in food (Dhiman et al., 2005; Schmid et al., 2006; Decker and Park, 2010). The average CLA intake by humans differs among countries, ranging between 70 and 430 mg per day (Fritsche and Steinhart, 1998; Dhiman et al., 2005; Schmid et al., 2006; Martins et al., 2007). When including the conversion of *t11*-C18:1, also present in ruminant foods, by Δ9-desaturase in humans, a median CLA dose of 650 mg per day is reached (van Wijlen and Colombani, 2010). Optimal CLA doses to obtain beneficial effects related to cancer reduction are not well established due to the lack of human trials, but a CLA dose of 1% in the diet has been often used for animals (Bhattacharya et al., 2006). For beneficial effects on body weight or atherosclerosis in humans, daily doses of 3 to 4 g CLA are thought to be effective (Tricon et al., 2005; Bhattacharya et al., 2006). The latter required doses are thus far above the average daily CLA intake by humans, represented mainly by the *c9t11*-CLA isomer. Considering that the *t10c12*-CLA isomer seems responsible for the reduction in body fat (Gavino et al., 2000; Hur et al., 2009), effects on body weight may not be achieved.

In contrast to CLA, not much information is available on the presence and amounts of CLNA isomers in food products. The CLNA content in milk (0.3 mg/g fat) is much lower than the CLA content. However, when feeding cows a diet with elevated levels of LNA, for instance based on linseed, a concentration of 1.8 to 2.1 mg CLNA/g fat in milk can be obtained (Akraim et al., 2007; Gómez-Cortés et al., 2009). Since concentrations of CLNA in milk and meat products are lower than concentrations of CLA, average daily CLNA intake is expected to be lower than CLA intake. The major CLNA isomer, *c9t11c15*-CLNA, is present in milk (Akraim et al., 2007; Gómez-Cortés et al., 2009). Other CLNA isomers, such as *c9t13c15*-CLNA, have

been found in bovine milk and muscle (Plourde et al., 2007), while *c9t11c13*-CLNA can be found in seeds and vegetable oils (Kohno et al., 2004; Yasui et al., 2005).

To improve dietary intake of CLA and CLNA, so that health-promoting doses can be achieved, specific approaches have to be followed. The animal diet is the most important factor to enhance CLA and CLNA contents in meat and dairy products, mostly by increasing the levels by LA and LNA precursors. The latter can be obtained by feeding forage (Akraim et al., 2007; Chilliard et al., 2007; Lourenço et al., 2010) or adding certain oilseeds, vegetable or fish oils to the feed (Raes et al., 2004; Dannenberger et al., 2005; Dhiman et al., 2005; Coakley et al., 2007). While enhancing amounts of conjugated fatty acids through dietary manipulation of ruminants creates opportunities, difficulties with reduced fermentation and corresponding biohydrogenation by microorganisms remain due to toxicity of the added PUFA (Henderson, 1973; Maia et al., 2010). In monogastric animals, enhancing the CLA content in tissues of these animals can be achieved by feeding *trans* fatty acids as substrates for endogenous CLA biosynthesis or by feeding CLA and CLNA directly (Gläser et al., 2002; Sirri et al., 2003).

Alternatively, synthetic CLA or CLNA supplements can be used in the human diet to reach the required concentration for health-promoting effects. However, these synthetic supplements have a different isomer distribution than natural foodstuffs and may contain isomers of which the physiological effect is yet unknown. Synthetic CLA supplements are usually produced by chemical isomerisation of oils rich in LA. The major CLA isomers formed in this process are *c9t11*- and *t10c12*-CLA, usually in a *t10c12/c9t11*-CLA ratio of 1.03 to 1.05. Traces of *c,c*- and *t,t*-CLA isomers are found as well (Yu et al., 2003).

A more natural, but yet poorly explored solution might be to use specific CLA- and CLNA-producing bacteria in fermented foods, alone or in combination with specific starter cultures. In this review, a more in-depth overview of the literature will be given, summarizing the possibilities of these bacteria to apply in fermentation processes, aiming to increase CLA or CLNA content of fermented foods.

BACTERIAL PRODUCTION OF CLA AND CLNA ISOMERS

Microbial linoleate isomerases

The ability to produce CLA and CLNA from LA and LNA, respectively, depends on linoleate isomerase (LAI) activity, catalysing this conversion (Kepler and Tove, 1967). This enzyme has been detected in several bacterial species including butyrivibrios, lactobacilli, propionibacteria, bifidobacteria, and clostridia. Species expressing this LAI activity can be divided in two groups, namely bacteria that produce mainly *c9t11*-CLA and *c9t11c15*-CLNA from LA and LNA, respectively, and bacteria able to produce mainly *t10c12*-CLA from LA.

To date, several LAI protein sequences are available in GenBank. These include sequences of LAI of strains of *Bifidobacterium dentium*, *Bifidobacterium breve*, *Lactococcus lactis* subsp. *lactis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Propionibacterium acnes*, and *Rhodococcus erythropolis* (Farmani et al., 2010; Rosberg-Cody et al., 2011). From these strains, *P. acnes* is the species mostly recognised for its production of *t10c12*-CLA (Rosson et al., 2001). Based on the identity percentage of amino acids among these

sequences (ranging from 28% to 82% identity), four groups of LAI were established (Farmani et al., 2010). The first group contains only the LAI of *P. acnes* and shows no sequence identity to the other LAIs. The second group is comprised of the LAIs of *Lc. lactis* subsp. *lactis*, *Lb. acidophilus*, and *Lb. reuteri*. The LAIs of *Lb. plantarum* and *R. erythropolis* form the third group. The fourth group contains the LAIs of *B. dentium* and *B. breve*. All LAIs, except the one of *P. acnes*, share similarity to the myosin cross-reactive antigen protein family. Additionally, all LAIs show N-terminal similarity to the Rossmann-fold NAD(P)(+)-binding domain and similarity to a partial domain of the phytoene dehydrogenase/desaturase protein family. The phytoene dehydrogenase/desaturase is a NAD-, NADP-, or FAD-dependent enzyme. Similarity to these two domains could indicate the need of a cofactor [FAD/NAD(P)] for the conversion reaction of LA to CLA by LAI (Farmani et al., 2010; Rosberg-Cody et al., 2011). In conclusion, two different types of LAI enzymes seem to be responsible for the production of either *c9t11*-CLA or *t10c12*-CLA from LA. Recently, the LAI genes, responsible for the production of *c9t11*-CLA and *c9t11c15*-CLNA from LA and LNA, respectively, of other LAB, including strains of *Lactobacillus curvatus*, *Lactobacillus sakei*, and *Lb. plantarum*, have been identified (Gorissen et al., 2011).

The presence of LAI activity may represent a way of detoxification, since LA and LNA inhibit growth of many microorganisms (Jiang et al., 1998; Kim et al., 2000; Coakley et al., 2003; Maia et al., 2010). The reason why PUFA such as LA and LNA are toxic could be explained by the presence of double bonds, which alter the shape of the molecule. Incorporation of these kinked unsaturated fatty acids in the cell membrane can disrupt the lipid bilayer structure. Another possibility is that diffusion of fatty acids across the membrane causes

chemiosmotic difficulties, disturbs the membrane potential, or disconnects intramembrane pathways (Maia et al., 2010).

Production of conjugated fatty acids by ruminal microorganisms

One of the principal rumen bacteria, *Butyrivibrio fibrisolvens*, member of *Clostridium* cluster XIVa, has been of great importance in understanding ruminal biohydrogenation and thus the production of CLA and CLNA. The bacterium converts LA to *c9t11*-CLA, which is then hydrogenated to *t11*-C18:1 (Kepler et al., 1966). The formation of *c9t11*-CLA is extremely rapid compared to the hydrogenation reaction (Kepler et al., 1966; Kim et al., 2000). The primary intermediate formed from LNA by *Bu. fibrisolvens* is *c9t11c15*-CLNA (Kepler and Tove, 1967). This CLNA isomer is further hydrogenated to a non-conjugated C18:2 fatty acid containing at least one *trans* double bond.

The available concentration of LA may affect microbial growth and CLA production, depending on the growth status of the cells. Inoculated cells of *Bu. fibrisolvens* A38 are inhibited by a concentration of LA as low as 35 μM, whereas actively growing cells tolerate concentrations up to 1000 μM (Kim et al., 2000). Also, concentrations of LA higher than 350 μM inhibit the hydrogenation reaction by *Bu. fibrisolvens* A38, so that CLA is not further converted to C18:1. The latter effect is also observed under aerobic conditions, leading to CLA concentrations of ± 30 μM obtained from 350 μM LA by washed cells of *Bu. fibrisolvens* A38, whereas anaerobic conditions lead to only 13 μM CLA maximally. Prolonged incubation or higher LA concentrations do not result in higher CLA formation by *Bu. fibrisolvens* A38,

indicating that the LAI does not recycle as a common enzyme to catalyse more substrate. Strain variability seems to occur, since *Bu. fibrisolvens* TH1 displays higher isomerisation ability than *Bu. fibrisolvens* A38 (Fukuda et al., 2005). As for *Bu. fibrisolvens* A38, no vaccenic acid production is obtained with *Bu. fibrisolvens* TH1 under aerobic conditions (Kim et al., 2000).

The LAI enzyme from *Bu. fibrisolvens* has been purified and characterised (Kepler and Tove, 1967). From the purification results, it has become clear that the LAI is localised in the cell envelope. Addition of cofactors, such as ATP, ADP, AMP, Mg^{2+}, NAD^{+}, and CoA, do not increase the activity of the enzyme. Remarkably, anaerobic conditions are not required for isomerisation, although ruminal biohydrogenation occurs in an overall anaerobic environment. The pH optimum of the enzyme is between pH 7.0 and 7.2 and the K_m values for LA and LNA are 1.2 10^{-5} M and 2.3 10^{-5} M, respectively. An absolute requirement for isomerisation is the *c9c12*-diene system and a free C1-carboxyl group in C18 fatty acids (Kepler et al., 1970). Higher enzyme specificity is obtained for substrates having a straight carbon atom chain bearing an ω chain length of six carbon atoms. Thus, an 18-carbon straight chain acid is the preferred substrate (Kepler et al., 1971). Three features of the substrate are involved in binding to the enzyme: the π system of the substrate double bond, hydrophobic interaction, and hydrogen bonding of the substrate carboxyl group. The presence of a double bond in the substrate is important, since several unsaturated but no saturated fatty acids can inhibit LAI. The significance of hydrophobic interaction is reflected in the fact that only unsaturated fatty acids longer than 16 carbon atoms inhibit the enzyme. The importance of hydrogen bonding of the substrate carboxyl group results in enzyme inhibition of substrate carboxyl derivatives, such as linoleyl amide and linoleyl alcohol (Kepler et al., 1970). As a result, a general mechanism for isomerisation has been

proposed (Kepler et al., 1971). A hydration-dehydration mechanism was quickly eliminated, leading research towards a direct isomerisation mechanism. After eliminating a carbocation and a carbanion intermediate in this mechanism, it was concluded that isomerisation involves a combined reaction of both or an enzyme-bound carbanion in which protonation occurred prior to dissociation from the enzyme surface. In addition, it has been assumed that the substrate is bound to the enzyme in the form of a loop, since the two binding groups at the active centres of the enzyme would be in close proximity of each other.

Production of CLA from LA by another ruminal bacterium, namely *Megasphaera elsdenii* YJ-4, a Gram-negative, anaerobic bacteria, has also been reported (Kim et al., 2002). In contrast to *Bu. fibrisolvens*, this bacterium is able to produce *t10c12*-CLA instead of *c9t11*-CLA. It has not been reported if further hydrogenation of this CLA isomer occurs. The *t10c12*-CLA isomer is produced very rapidly but only a small fraction of LA is converted to *t10c12*-CLA.

Production of conjugated fatty acids by bifidobacteria

Bifidobacteria are important residents of the human digestive tract, mainly the colon. Some bifidobacteria are used as probiotics in dairy products, such as fermented milks (Collins and Gibson, 1999; Gomes and Malcata, 1999; Acharya and Shah, 2002; Senok et al., 2005). It has been suggested that the production of CLA or CLNA isomers can contribute to the probiotic properties of these intestinal bacteria (Ross et al., 2010; Russell et al., 2011; O'Shea et al., 2012).

Several bifidobacteria produce CLA and CLNA isomers from LA and LNA, respectively, which has been demonstrated mostly in de Man-Rogosa-Sharpe (MRS) medium. Out of a total of

fifteen tested bifidobacteria, five *B. breve* strains, a *B. dentium* strain, and a *Bifidobacterium lactis* strain showed good conversion of LA (0.55 mg/l) to CLA in MRS medium (Coakley et al., 2003). Conversion percentages ranged from 23% (for *B. dentium* NCFB 2243) to 66% (for *B. breve* NCFB 2258) after 48 h. The isomers produced were mainly *c9t11*-CLA and, in a smaller amount, *t9t11*-CLA. Furthermore, the screening has revealed some interspecies and intraspecies variation. This strain-dependent character of CLA and CLNA production has been confirmed in a large screening of 36 different bifidobacteria, of which four *B. breve*, one *Bifidobacterium bifidum*, and one *Bifidobacterium pseudolongum* subsp. *pseudolongum* strains were able to produce *c9t11*-CLA and *t9t11*-CLA from LA (0.50 mg/ml) in MRS medium, with conversion percentages ranging from 20% to 54% (Gorissen et al., 2010). According to Oh et al. (2003), a *B. breve* strain as well as *Bifidobacterium pseudocatenulatum* strain, both isolated from infant faecal samples, were capable of producing 0.16 and 0.14 mg/ml of *c9t11*-CLA, respectively, from 0.50 mg/ml LA in MRS medium. This corresponded with conversion percentages of 32% and 27% after 48 h, respectively. In another study, the most efficient CLA-producing bifidobacteria, isolated from faecal samples of neonates, were *B. breve* strains, resulting in 8% to 29% conversion of LA to *c9t11*-CLA after 72 h in MRS medium supplemented with 0.50 mg/ml LA (Rosberg-Cody et al., 2004). In addition, a *Bifidobacterium longum* and a *B. bifidum* strain have been identified as *c9t11*-CLA producers with conversion percentages of 3% and 18%, respectively.

In addition to MRS medium, bifidobacteria are also able to produce CLA in milk-based media. Yet, when MRS and milk-based media are compared, differences in CLA production may be observed. Several isolates from infant stool (all *B. breve* strains) were capable of producing

CLA (mainly *c9t11*-CLA) in skim milk supplemented with 0.50 mg/ml LA, of which *B. breve* LMC 520 produced the highest CLA concentration (0.11 mg/ml) with a corresponding conversion percentage of 23% after 48 h (Choi et al., 2008). This conversion percentage could nevertheless be increased up to 90% in MRS medium containing 280 mg/ml LA at pH 5.5 under anaerobic conditions for 24 h (Park et al., 2009). Likewise, formation of CLA by several bifidobacteria in a milk-based medium, supplemented with LA, was reduced compared to CLA production in MRS medium (Hennessy et al., 2009). In contrast, the conversion percentage of LA to CLA obtained with a *B. bifidum* strain was higher in buffalo milk supplemented with 0.20 mg/ml LA was higher (39%) compared to that obtained in MRS medium (25%) after 24 h (Van Nieuwenhove et al., 2007b). Based on the fact that results seem to be medium-dependent, medium modifications could be used to increase CLA levels. Formation of CLA from different substrates by *B. breve* LMC 520 has been tested, indicating that skim milk supplemented with 0.05% monolinolein (a monoglyceride form of LA) yields the highest concentration of CLA (0.41 mg/ml) (Choi et al., 2008). Addition of sodium salts, short chain fatty acids (such as sodium acetate), cys-HCl, or yeast extract to milk-based media can significantly increase the production of *c9t11*-CLA. For *B. breve* NCIMB 702258, the highest increase in CLA was obtained with a combination supplement of 200 mmol/l sodium acetate and 10 mg/ml yeast extract. This corresponded to a conversion percentage of 48% for *c9t11*-CLA, compared to 16% for *c9t11*-CLA in milk medium without supplementation. In addition, supplementation of 20 mg/ml inulin and optimisation of the inoculum (up to 7 log CFU/ml) increased the conversion percentage of *c9t11*-CLA to 55%, a value comparable to that obtained in MRS medium.

Production of CLA isomers by bifidobacteria is not only related to the composition of the growth medium but also to the growth phase of the bacteria. Formation of the *c9t11*-CLA isomer mainly occurs during the exponential growth phase and ceases when cells enter the stationary phase (Coakley et al., 2003; Oh et al., 2003; Park et al., 2009). This is followed by a gradual decrease in the concentration of *c9t11*-CLA and a gradual increase in the concentration of *t9t11*-CLA in the stationary phase, as demonstrated for *B. breve* NCIMB 702258 (Coakley et al. 2003; Hennessy et al., 2009), as well as other *B. breve* strains (LMG 11040, LMG 11084, LMG 11613, and LMG 13194), and for *B. bifidum* LMG 10645 and *B. pseudolongum* subsp. *pseudolongum* LMG 11595 (Gorissen et al., 2012a). These results suggest formation of *t9t11*-CLA as a product of the metabolism of *c9t11*-CLA. Furthermore, a high specific formation of CLA isomers by bifidobacteria seems not to be related to a fast growth. For instance, *B. breve* LMG 13194 and *B. pseudolongum* subsp. *pseudolongum* LMG 11595 show a higher conversion of LA to CLA isomers in MRS medium compared to *B. breve* LMG 11084, *B. breve* LMG 11040, and *B. breve* LMG 11613, despite slower growth and lower biomass yields (Gorissen et al., 2012a).

In contrast to the conversion of LA into CLA, the production of CLNA isomers (mostly *c9t11c15*-CLNA and, in much lower concentration, *t9t11c15*-CLNA) from LNA is less well documented. In MRS medium supplemented with 0.24 mg/ml LNA, five *B. breve* strains have been shown to produce CLNA after 42 h of incubation (Coakley et al., 2009). Conversion percentages ranged from 68% to 81%, once more indicating intraspecies variation. Next to *B. breve* strains, strains of other *Bifidobacterium* species are able to produce CLNA from LNA. A *B. bifidum* and a *B. pseudolongum* subsp. *pseudolongum* strain, together with four *B. breve* strains, produced *c9t11c15*-CLNA and *t9t11c15*-CLNA from LNA (0.50 mg/ml) supplemented

to MRS medium (Gorissen et al., 2010). Furthermore, conversion percentages, ranging from 56% to 78%, were higher compared to conversion percentages of LA to CLA isomers (from 20% to 54%) with these strains, possibly resulting from the more toxic effect of LNA compared to LA on the strains. Also, bifidobacterial strains seem to produce higher amounts of CLNA-isomers when their growth is more inhibited and further conversion of *c9t11c15*-CLNA to *t9t11c15*-CLNA may take place (Gorissen et al., 2012a).

The health-promoting effects of CLA and CLNA produced by bifidobacteria have been investigated in several studies. Ewaschuk et al. (2006) have tested the CLA-producing ability of probiotic bifidobacterial strains (VSL3). Feeding *Bifidobacterium infantis*, a *B. breve*, and a *B. longum* strain, capable of producing both *c9t11*-CLA and *t10c12*-CLA, to adult 129/SvEv mice for three days, led to a 100-fold increase in CLA when faecal pellets were incubated overnight at 37°C in MRS with 0.50 mg/ml LA. In addition, it has been shown that the viability and proliferation of colonic carcinoma cells (HT-29) is reduced by filtrated culture medium (containing CLA) of *B. infantis*, *B. breve*, or *B. longum* strains (Ewaschuk et al., 2006). Seemingly, *t9t11*-CLA has a more potent anti-proliferative effect on colonic carcinoma cell lines than *c9t11*-CLA. For instance, Coakley et al. (2006) have investigated the anti-proliferative effect of pure *c9t11*- and *t9t11*-CLA on SW480 and HT-29 colon cancer cells, since these two isomers are produced by the tested bifidobacteria. Growth of SW480 cancer cells was inhibited up to 45% by *t9t11*-CLA compared to 37% by *c9t11*-CLA at a concentration of 0.01 mg/ml CLA after 4 days. For HT-29 cells, growth inhibition values amounted to 94% and 57%, respectively, at a concentration of 0.02 mg/ml CLA. Concerning CLNA isomers, a 85% reduction in cell numbers of SW480 colon cancer cells can be achieved after incubation of 5 days with 0.05

mg/ml CLNA produced by *B. breve* (a mixture of *c9t11c15*- and *t9t11c15*-CLNA) compared to a 50% reduction by LNA in the same conditions (Coakley et al., 2009).

Production of conjugated fatty acids by lactic acid bacteria

Different species of lactic acid bacteria (LAB) are able to produce CLA (Lin et al., 1999; Ogawa et al., 2001; Alonso et al., 2003; Lee et al., 2003b, 2006; Puniya et al., 2008). Instead of assessing CLA production by LAB through addition of LA directly to the growth medium, their CLA-producing potential has been mainly demonstrated by adding LA to washed cells of LAB (Ogawa et al., 2001, 2005; Lee et al., 2003b) or their enzyme extracts (Lin et al., 2002; Lin, 2006), due to the inhibitory effect of LA on the growth of the bacteria (Lin et al., 2002; Lee et al., 2006).

When using enzyme extracts, formation of many different CLA isomers from LA has been reported. However, incomplete cell lysis is a major obstacle for efficient isolation of LAI (Irmak et al., 2006). Also, results depend on both the bacterial strain used and the experimental conditions. When using an enzyme preparation (20 mg) of a *Lb. acidophilus,* eight CLA isomers (*t8t10-*, *t9t11-*, *t10t12-*, *t11t13-*, *t8c10-*, *c9t11-*, *t10c12-*, and *c11t13*-CLA) were formed from 50 mg LA, independent of pH (5 to 8) (Lin et al., 2002). The highest values were obtained for *t10c12*- (436 μg), *c9t11*- (379 μg), and *c11t13*-CLA (261 μg), and the maximum total CLA amount (1700 μg) was reached at pH 5. When 25 mg LA was treated with 50 mg enzyme preparation of a *Lactobacillus delbrueckii* subsp. *bulgaricus* strain, the total amount of CLA (8.5 μg) was lower compared to *Lb. acidophilus* (Lin, 2006). Six different CLA isomers (*t8t10-*,

t9t11-, *t8c10*-, *c9t11*-, *t10c12*-, and *c11t13*-CLA) were detected, of which *c9t11*-CLA was the major isomer (3.1 μg). The LAI of *Lb. reuteri* ATCC 55739, of which CLA production has been patented, produces mainly *c9t11*, with an optimum at 4°C and pH 8.5 (Pariza and Yang, 1999).

Similarly, the use of washed cells indicates variability in CLA production due to differences in producer strain and production conditions. For instance, washed cells of the probiotic strain *Lb. acidophilus* La-5 were able to produce 4.21 mg/l of CLA from 320 mg/l LA (Macouzet et al., 2009). Washed cells of *Lactobacillus rhamnosus* PL60 could generate *c9t11*-CLA (2.8 mg/mg protein) and *t10c12*-CLA (1.6 mg/mg protein) from 20 μg LA (Lee et al., 2006). Immobilisation of cells of *Lb. reuteri* ATCC 55739 on silica gel increased the amount of *c9t11*-CLA produced from 500 mg/l LA, compared to the use of washed cells (from 32 mg/l to 175 mg/l CLA) (Lee et al., 2003a, 2003b). Washed cells of *Lb. acidophilus* AKU 1137 were able to form *c9t11*- and *t9t11*-CLA from LA under microaerobic conditions, reaching higher levels when the culture was pre-incubated with LA (Ogawa et al., 2001). In addition to LA conversion, conjugated fatty acids can also be formed from LNA and γ-linolenic acid (*c6c9c12*-C18:3) by washed cells. This has been shown for washed cells of *Lb. plantarum* AKU 1009a that were able to produce *c9t11c15*-, *t9t11c15*-CLNA, and *t10c15*-C18:2 from LNA and *c6c9t11*-C18:3, *c6t9t11*-C18:3, *c6t10*-C18:2, and *t10*-C18:1 from γ-linolenic acid (Kishino et al., 2009, 2010).

Two possible intermediates in the production of *c9t11*- and *t9t11*-CLA from LA by washed cells of *Lb. acidophilus* AKU 1137 have been detected and identified as 10-hydroxy-*cis*-12-C18:1 and 10-hydroxy-*trans*-12-C18:1 (Ogawa et al., 2001). Other LAB strains belonging to *Lb. plantarum*, *Lactobacillus paracasei*, *Lb. rhamnosus*, *Lactobacillus pentosus*, and *Lactobacillus brevis* strains, have also been identified as CLA producers with hydroxy fatty acids (HFA) as

intermediates (Kishino et al., 2002b; Ogawa et al., 2005). The mechanism of CLA production has been proposed to be one of hydration of LA to 10-hydroxy-18:1 and subsequent dehydration of this HFA to CLA. This mechanism has been demonstrated to be a multi-component enzyme system. In enzyme fractions of *Lb. plantarum* AKU 1009a, the hydrating activity was found in the membrane fraction whereas the dehydrating activity was present in both the soluble and the membrane fraction (Kishino et al., 2011a, 2011b). Washed cells of *Lb. plantarum* AKU 1009a were able to form CLA from ricinoleic acid (12-hydroxy-*cis*-9-C18:1), structurally similar to 10-hydroxy-12-C18:1 (Kishino et al., 2002a). Pre-incubation of *Lb. plantarum* AKU 1009a cells in a medium containing LA resulted in a better HFA yield (Demir and Talpur, 2010). Lipase activity on castor oil results in the release of ricinoleic acid acting as a substrate for CLA production, as shown for *Lb. plantarum* JCM 1551 (Ando et al., 2004).

In comparison to experiments with enzyme extracts or washed cells, CLA production during LAB cultivation in media enriched with LA is less easily obtained due to growth inhibition by LA and CLA (Jiang et al., 1998; Coakley et al., 2003). Indeed, LA and CLA inhibit different LAB (*Lactobacillus zeae*, *Lactobacillus helveticus*, *Lb. acidophilus*, and *Lb. reuteri*) in a dose-dependent manner (Jenkins and Courtney, 2003). In the latter study, a *Lb. reuteri* strain, capable of producing CLA from LA, was the most inhibited strain by LA while a CLA mixture had no inhibitory effect on growth. This suggests that fatty acid isomerisation has a detoxifying effect for LAB. Nevertheless, several studies have demonstrated CLA production by LAB cultures, even when applied in MRS medium. For instance, probiotic strains of *Lb. acidophilus*, *Lb. plantarum*, *Lactobacillus casei*, *Lb. delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* strain were able to produce CLA in MRS medium inoculated with LA (0.50

mg/ml) (Ewaschuk et al., 2006). Also, *Lb. plantarum* strains were the highest producer of CLA from LA in MRS medium among other LAB, isolated from natural sauerkraut fermentations or fermented Chinese pickles (Zeng et al., 2009; Liu et al., 2011). Conversion percentages of CLA ranging from 17% to 36% were found among several *Lb. casei*, *Lb. rhamnosus*, and *S. thermophilus* strains grown in MRS medium supplemented with LA (0.20 mg/ml) (Van Nieuwenhove et al., 2007b). Strains of *Lb. paracasei*, *Lb. brevis*, *Lb. rhamnosus*, *Lb. acidophilus*, and *Lb. casei* were found to produce CLA in MRS medium supplemented with LA (1.0 mg/ml) after cultures reached steady-state phase and were incubated for 48 h at 37°C (Xu et al., 2008). From the latter strains, the *Lb. acidophilus* strain showed the highest CLA production (0.05 mg/ml). For *Lb. curvatus* and *Lb. sakei* strains, a poor conversion percentage of LA into CLA (2% to 5%) has been found in MRS medium in contrast to a rather high conversion of LNA into CLNA (1% to 60%) (Gorissen et al., 2011).

The production of CLA by LAB during cultivation may be affected by different factors, such as the concentration of added LA and the pH and temperature of the incubations, as well as by the cultivation medium. For instance, production of CLA (0.11 mg/ml) by *Lb. reuteri* ATCC 55739 was maximal in cultures supplemented with 20 mg/ml LA at 10°C for 30 h at pH 6.5, compared to other concentrations of LA (5, 10, and 30 mg/ml), temperatures (4, 16, 22, and 30°C), and pH values (5.5) tested (Hernandez-Mendoza et al., 2009). No production of CLA isomers from LA and a decreased production of CLNA isomers from LNA in MRS medium at 30°C and pH 5.5 compared to 6.2 has been observed for *Lb. sakei* LMG 13558 as well (Gorissen et al., 2011). For the same strain, an increase in temperature to 37°C compared to 30°C, at pH 6.2, resulted in a lower production of CLA and CLNA from LA and LNA, respectively (Gorissen

et al., 2011). In a similar study, maximum production of CLA (0.08 to 0.13 mg/ml) by strains of *Lb. acidophilus* and *Lb. casei* was obtained in MRS supplemented with 0.2 mg/ml LA during stationary phase at 37°C, compared to other LA concentrations tested (0, 0.05, 0.1, and 0.5 mg/ml) (Alonso et al., 2003). The amounts of CLA produced by the latter strains were lower (ranging from 0.045 to 0.100 mg/ml) in skim milk supplemented with 0.2 mg/ml LA compared to MRS medium supplemented with the same amount of LA (Alonso et al., 2003). The effect of LA concentration on CLA production has been shown for several LAB (sub)species (*Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, and *S. thermophilus*) in skim milk (Lin et al., 1999). In the latter study, the largest increase in CLA was obtained with *Lb. acidophilus* incubated with 1 mg/ml LA instead of 5 mg/ml LA, resulting in 0.11 mg/ml CLA compared to 0.09 mg/ml CLA, respectively.

Studies with addition of substrates other than free LA supplemented to the cultivation medium have been conducted, with considerable effects. When adding sunflower oil to skim milk, two *Lb. brevis* rumen isolates showed higher amounts of CLA produced after 12 h at 37°C (10.5 and 8.3 mg/g fat) when a small concentration of sunflower oil was used (0.25%) (Puniya et al., 2008). At higher levels of sunflower oil (1%), CLA production by *Lb. brevis* was inhibited and rumen isolates belonging to *Lb. lactis* and *Lb. viridescens* were the best CLA producers, with 9.2 and 5.7 mg/g fat of CLA, respectively. When hydrolysed sesame oil was added to skim milk (0.6% - 0.8%) to evaluate its effect on the production of CLA by a whole range of potentially probiotic LAB, a *Lc. lactis* subsp. *lactis* biovar *diacetylactis* and *Leuconostoc mesenteroides*

subsp. *mesenteroides* strain were the highest CLA producers, reaching levels of about 0.20 mg/ml CLA (Abd El-Salam et al., 2010).

When feeding mice and humans with CLA-producing LAB, health-promoting effects may be expected (Lee et al., 2006, 2007; Lee and Lee, 2009). *Lactobacillus plantarum* PL62, a strain isolated from infant faeces and capable of producing *c9t11*-CLA (0.027 mg/ml) and *t10c12*-CLA (0.006 mg/ml) in skim milk supplemented with 0.1% LA, could be recovered from the faeces of mice from the first day after administration through feeding, and led to a weight loss of 16% after eight weeks of feeding compared to control mice (Lee et al., 2007). Another CLA-producing infant faeces isolate, *Lb. rhamnosus* PL60, could be recovered from faeces of mice receiving a high-fat diet supplemented with this strain, leading also to elevated levels of *t10c12*-CLA in sera and a decreased weight gain (Lee et al., 2006). In humans, consumption of *Lb. rhamnosus* PL60 increased concentrations of *c9t11* and *t10c12*-CLA in the serum (Lee and Lee, 2009).

Production of conjugated fatty acids by propionibacteria

Four *Propionibacterium* (sub)species have been found to produce *c9t11*-CLA from LA, namely *Propionibacterium freudenreichii* subsp. *freudenreichii*, *P. freudenreichii* subsp. *shermanii*, *Propionibacterium acidipropionici*, and *Propionibacterium technicum* (Verhulst et al., 1987). They can isomerise the *c12*-double bond of LNA and γ-linolenic acid as well. On the contrary, *P. acnes* is able to convert LA to *t10c12*-CLA and to isomerise the *c9*-double bond of γ-linolenic acid into a *t10*-double bond.

According to Jiang et al. (1998), two strains of *P. freudenreichii* subsp. *freudenreichii* (ATCC 6207 and Propioni-6) and one strain of *P. freudenreichii* subsp. *shermanii* (9093) are able to produce mainly *c9t11*-CLA, and in smaller amounts *t10c12*- and *t9t11/t10t12*-CLA, when incubated in MRS supplemented with LA (0.03 mg/ml). Different concentrations of LA can affect the CLA production by propionibacteria, since growth may be affected by too high LA levels in a strain-dependent manner. For instance, *Propionibacterium freudenreichii* subsp. *freudenreichii* ATCC 6207 showed a high production of *c9t11*-CLA (0.17 mg/ml) at a LA level of 0.10 mg/ml in MRS medium. Production of CLA decreased when 0.20 mg/ml LA or more was administered, since growth of this strain was largely inhibited. On the contrary, *P. freudenreichii* subsp. *freudenreichii* Propioni-6 had the highest production of *c9t11*-CLA (0.25 mg/ml) at a concentration of 0.75 mg/ml LA in MRS and no growth inhibition was observed. Additionally, different results have been obtained depending on the cultivation medium. For instance, when comparing sodium lactate medium (SLM) with skim milk, a 50% conversion of LA (0.10 mg/ml) to CLA by *P. freudenreichii* subsp. *freudenreichii* Propioni-6 was found in SLM compared to 60% to 90% conversion in skim milk.

The positive correlation between CLA production and the ability to tolerate growth inhibition caused by LA, has directed Jiang et al. (1998) to suggest LA conversion to CLA as a detoxification mechanism for propionibacteria. The use of detergents can help to eliminate the growth inhibitory effect of LA on propionibacteria. For instance, when using polyoxyethylene sorbitan monooleate (SO) at a LA:SO ratio of 1:15, a culture of *P. freudenreichii* subsp. *shermanii* JS can tolerate a concentration of 1 mg/ml LA added to whey permeate medium, resulting in a CLA yield of 57% (Rainio et al., 2001). Furthermore, when using LA (0.51 mg/ml)

in a micellar solution with SO (ratio 1:15) a higher yield (90%) of CLA was obtained with resting cells of *P. freudenreichii* subsp. *shermanii* JS (Rainio et al., 2002).

Propionibacteria can also produce CLA from sunflower oil instead of free LA, the success being strain-dependent. For instance, *P. freudenreichii* subsp. *shermanii* CGMCC 1.2227 had a higher CLA production from sunflower oil in SLM and MRS media compared to *P. freudenreichii* subsp. *freudenreichii* CGMCC 1.2236, whereas in skim milk the situation was reversed (Wang et al., 2007). As observed by Jiang et al. (1998), a positive relationship exists between CLA production and the ability to tolerate sunflower oil. Maximum production of CLA (0.079 mg/ml) was observed after 36 h by *P. freudenreichii* subsp. *shermanii* CGMCC 1.2227 in MRS medium supplemented with 12 mg/ml sunflower oil.

In contrast to the LAI of *Bu. fibrisolvens*, the LAI from *P. acnes*, which catalyses the isomerisation of LA to *t10c12*-CLA, is not bound to the cell membrane. It is a soluble protein, facilitating its identification (Deng et al., 2007; Liavonchanka and Feussner, 2008). This LAI converts LA to *t10c12*-CLA and has a pH optimum of 7.3 and a K_m of 17.2 μM at a pH of 7.5 and a temperature of 20°C. A precise alignment of the substrate is needed to complete isomerisation and is achieved by hydrogen bonding between the carboxyl group of the fatty acid and two residues (Arg 88 and Phe 193) of the LAI. This lock ensures selectivity for free fatty acids over esterified fatty acids (Liavonchanka et al., 2009). This LAI gene has been successfully introduced in other microorganisms, such as *Saccharomyces cerevisiae* (Hornung et al., 2005), as well as in *Escherichia coli* and *Lc. lactis* (Rosson et al., 2001; Rosberg-Cody et al., 2007), resulting in production of *t10c12*-CLA. Cloning this LAI gene in tobacco seeds (Hornung et al.,

2005) and rice (Kohno-Murase et al., 2006), leads to detection of *t10c12*-CLA in the transformed plants.

Production of conjugated fatty acids by other bacterial species

The ability to produce CLA is also present in other intestinal bacteria than bifidobacteria and LAB, namely *Roseburia* species (members of *Clostridium* cluster XIVa), which are important inhabitants of the human colon (Devillard et al., 2007). However, these bacteria possess a different route of CLA biosynthesis than bifidobacteria and LAB. Strains of *Roseburia inulinivorans* and *Roseburia hominis* produce vaccenic acid (*t11*-C18:1) as end-product in incubations with LA, similar to *Bu. fibrisolvens*. Strains of *Roseburia intestinalis* and *Roseburia faecis* have 10-hydoxy-*cis*-12-C18:1 as end-product. Production of HFA as intermediates from LA to CLA has been observed in certain *Lactobacillus* species as well (Ogawa et al., 2001). Mixing culture supernatant fluid from *R. intestinalis* L1-952 (rich in HFA) with human faeces, containing *Roseburia* spp., results in the production of mainly *c9t11*-CLA, *t11*-C18:1, and C18:0. Because synthesis of these three fatty acids corresponds with a decrease in HFA, it has been suggested that HFA is a precursor of *c9t11*-CLA (Devillard et al., 2007). Different mechanisms of CLA production for bacteria of the rumen and for those of the human intestine have been proposed (McIntosh et al., 2009). Certain human intestinal bacteria, such as *R. intestinalis*, metabolise LA to CLA with HFA as transient intermediates, whereas ruminal bacteria, such as *Bu. fibrisolvens*, produce CLA through a direct isomerisation mechanism

without HFA intermediates, since these vaccenic acid-producing species do not metabolise HFA to CLA or vaccenic acid.

Clostridia can also produce CLA. Incubations of *Clostridium sporogenes* with LA results in production of *c9t11*-, *t9t11*-, and *t10c12*-CLA (Peng et al., 2007). When incubated aerobically, formation of *c9t11*-CLA is rapid, within 30 min, followed by a decrease in *c9t11*-CLA and an increase in *t9t11*- and *t10c12*-CLA. However, under anaerobic conditions, no production of *t9t11*- and *t10c12*-CLA takes place and conversion of LA to *c9t11*-CLA is much slower. The LAI of *C. sporogenes* is a membrane-associated enzyme (Peng et al., 2007). Based on the molecular mass of the LAI on a native polyacrylamide gel, the active enzyme has been suggested to be a homotetramer. The purified enzyme is able to convert LA into *c9t11*-CLA. Its optimum pH is about pH 7.5, which is slightly higher than the value observed for the LAI of *Bu. fibrisolvens* (Kepler and Tove, 1967). The enzyme displays a preference towards substrates with *c9,c12* double bonds in C18 fatty acids with a free carboxyl group and is not affected by the cofactors ATP, ADP, NAD, NADH, NADPH, or CoA.

BACTERIAL FORMATION OF CONJUGATED FATTY ACIDS IN FERMENTED PRODUCTS

Fermented dairy products

Since several CLA- and CLNA-producing bacteria (LAB, bifidobacteria, and propionibacteria) are associated with dairy technology, their application during milk

fermentation seems promising. Naturally, the CLA content of milk and derived products, such as yoghurt, varies between 3.4 to 8.8 mg/g fat (Jiang et al., 1997). Processing of the milk does not influence the CLA content (Dhiman et al., 2005). Nevertheless, the amounts of CLA and CLNA isomers in dairy products, such as cheese, butter, and yoghurt, can be slightly higher than the amounts of these isomers found in milk (Sieber et al., 2004). The use of starter cultures, such as LAB and propionibacteria, may contribute to the CLA content. However, the resulting increase in CLA due to fermentation remains very moderate (Table 1). Using other food-grade bacteria with high conversion capacity of LA and LNA to CLA and CLNA isomers, respectively, during milk processing may result in further augmentation of the CLA and CLNA contents of fermented dairy products. Several studies have looked into the potential of food-grade bacteria to produce CLA and CLNA isomers in milk-based media, such as skim milk. Since skim milk contains no or extremely low levels of fat, additional sources of LA or LNA need to be added to investigate CLA or CLNA production by bacteria cultivated in this medium. Also, variability in fatty acid composition of milk needs to be considered when evaluating the obtained results. When using strains of *P. freudenreichii* subsp. *shermanii*, able to produce CLA from free LA in MRS medium, in combination with lipolytic yeasts (*Geotrichum candidum* and *Yarrowia lipolytica*), an increase in free CLA from 0.01 to 0.5 mg CLA/g could be obtained in cheese (Das et al., 2005). However, this increase should be ascribed to the lipolytic activity of the yeasts, rather than to conversion of free LA to CLA by the propionibacteria. Furthermore, when adding safflower oil (rich in LA) to the cheese curd, no increase in CLA concentration could be observed, confirming that there was no conversion of LA to CLA by the *Propionibacterium* strains. The reason for the failure of CLA production is thought to be the lower pH and lower

water activity of the cheese compared to laboratory conditions. On the contrary, slightly elevated levels of *c9t11*-CLA and *t10c12*-CLA could be obtained in fermented milk products with hydrolysed soy oil as a source of LA, when inoculated with either *P. freudenreichii* subsp. *freudenreichii* 23, *P. freudenreichii* subsp. *shermanii* 56, or *P. freudenreichii* subsp. *shermanii* 51 (Xu et al., 2005). Furthermore, inoculation with these propionibacteria strains in combination with a traditional yoghurt culture (*Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*; YC-180) gave higher CLA contents compared to the yoghurt culture alone, indicating that these *Propionibacterium* strains were able to convert LA to CLA in fermented milk. A maximum *c9t11*-CLA content of 0.65 mg/g fat has been obtained with *P. freudenreichii* subsp. *freudenreichii* 23 and YC-180 and a maximum *t10c12*-CLA content of 0.50 mg/g fat has been obtained with *P. freudenreichii* subsp. *shermanii* 56 and YC-180.

The ability of different bifidobacteria (*Bifidobacterium animalis* subsp. *animalis* strains) in co-culture with *S. thermophilus* to increase CLA content in conventional milk and organic milk has been assessed in the absence of an external source of LA (Florence et al., 2009). No increase in CLA content in the conventional milk during fermentation could be obtained. However, in the fermented organic milks, amounts of CLA were slightly higher compared to the unfermented organic milk. Similarly, no production of CLA and CLNA isomers could be detected in fermented milk products with bifidobacteria, despite the fact that the chosen strains had the capacity to do so *in vitro* (Gorissen et al., 2012b). Although, LA and LNA were present in sufficient amounts in milk, their availability as free fatty acids was likely too low.

Similar studies have been performed with CLA-producing LAB. In whole milk, CLA production (8 mg/g fat) improved after fermentation with *Lc. lactis* I-01 when sunflower oil was

added (0.1 mg/ml) (Kim and Liu, 2002). In buffalo cheeses, an increase in CLA content (from 4.4 to 5.9 mg/g fat) was obtained after one day with *S. thermophilus* (Van Nieuwenhove et al., 2007a). Other strains (a *Lb. casei* and a *Lb. rhamnosus* strain) could only increase CLA contents in buffalo cheeses after addition of sunflower oil, corresponding to 0.20 mg/ml LA, to the pasteurised milk. The highest CLA level (6.96 mg/g fat) was obtained for *Lb. rhamnosus*. In fermented milk products, a *Lb. rhamnosus* strain was more effective than propionibacteria in formation of CLA (Xu et al., 2005). The highest levels of *c9t11*-CLA (0.97 mg/g fat) and *t10c12*-CLA (0.71 mg/g fat) were reached in a co-culture of *Lb. rhamnosus* and a commercial yoghurt culture (YC-180) after 14 days (Xu et al., 2006).

Special interest goes to CLA-producing abilities of probiotic strains in fermented milk products, for the generation of added value. In dahi, an Indian-type yoghurt, *Lb. acidophilus* NCDC 14 together with *Lb. casei* NCDC 19 increased the CLA content from 6 to 10.5 mg/ml during fermentation (Yadav et al., 2007). In yoghurt, an increase in *c9t11*-CLA could be obtained with the probiotic strains *Lb. acidophilus* La-5 or *B. bifidum* Bb-12 in combination with a commercial starter culture (Akalin et al., 2007). An 1.58 fold increase in CLA concentration (up to 3.18 mg/g fat) was obtained with *Lb. acidophilus* La-5 and an 1.75 fold increase (up to 3.53 mg/g fat) with *B. bifidum* Bb-12. The same two strains also effectively increased CLA content in fermented cream samples, in the absence of a commercial starter culture (0.70 and 0.73 mg/g fat with *Lb. acidophilus* La-5 or *B. bifidum* Bb-12, respectively), compared to the raw cream (0.64 mg/g fat) (Ekinci et al., 2008). In Ras cheese, supplemented with sesame oil, the combination of two probiotic strains, *Lb. acidophilus* and *Lb. casei*, increased the CLA content to maximal 8.5 mg/g fat after 60 days of storage (Abd El-Salam et al., 2011).

Prebiotics or other carbohydrate chains may affect the fatty acid profile in non-fat fermented milk, as shown for oligofructose, maltodextrin, and polydextrose (Oliveira et al., 2009). Addition of such compounds slightly increases the *c9t11*-CLA content in fermented milk products. This has been shown for a commercial *S. thermophilus* starter strain in combination with *Lb. delbrueckii* subsp. *bulgaricus* LB340, *Lb. acidophilus* LAC4, *Lb. rhamnosus* LBA, or *B. animalis* subsp. *lactis* BL04, probably due to a better growth of the strains. The strongest effect has been observed with maltodextrin and *Lb. rhamnosus* LBA, resulting in an increase of 38% *c9t11*-CLA compared to the control. In probiotic yoghurts, containing *Lb. acidophilus* La-5 or *B. bifidum* Bb-12 in combination with a commercial starter culture, addition of 2% fructooligosaccharide resulted in an increase of the CLA content (to 5.51 and 5.82 mg/g fat, respectively), exceeding the amounts in yoghurts produced without addition of fructooligosaccharide (Akalin et al., 2007).

Fermented meat products

In meat products, such as salami and cooked ham, the CLA content is comparable to that of the raw material and does not seem to be influenced by the processing method (Chin et al., 1992; Fritsche and Steinhart, 1998). Variability in CLA content in these meat products has been explained by variability in the raw meat. The CLA and CLNA contents of meat varies not only due to animal species variability, but also among different tissues of an animal, partially as an effect of the feed composition (Raes et al., 2004; Dannenberger et al., 2005; Lourenço et al., 2008). The influence of specific CLA- and CLNA-producing bacteria on the amount of these

conjugated fatty acids in fermented meat products has hardly been investigated. However, since *Lb. plantarum, Lb. pentosus,* and *Lb. sakei* strains are sometimes associated with fermented meats, their use as CLA-producing functional starter cultures for meat fermentation has been suggested (Leroy et al., 2006). Several strains of the meat-associated species *Lb. sakei* are able to produce CLA and CLNA in MRS medium supplemented with LA and LNA, respectively (Gorissen et al., 2011). However, no increase in CLA and CLNA isomers could be found in fermented meat products with the CLA and CLNA producer *Lactobacillus sakei* LMG 13558, probably due to the low pH of the meat during acidification (Gorissen et al., 2012b). It still needs to be analysed further if it is possible to match CLA- and CLNA-production kinetics with meat fermentation technology.

CONCLUSION

The major source of CLA and CLNA in the human diet originates from dairy and meat products from ruminants. Since these conjugated fatty acids have important biological properties, increasing the CLA and CLNA intake could be beneficial for human health. Altering the animal diet is a practical way of changing the CLA and CLNA contents in meat and dairy products. Another approach is the use of CLA- and CLNA-producing food-grade bacteria in fermented food products. Different food-grade microorganisms, such as bifidobacteria, LAB, and propionibacteria, are able to produce CLA and CLNA isomers from LA and LNA, respectively. However, production of CLA and CLNA isomers is strain-dependent and probably dependent on the ability of the strain to tolerate the toxic effects of LA and LNA. It has been suggested that increased levels of CLA in fermented dairy products can be achieved by addition of bacteria with high CLA-producing ability during food processing. However, the increase in CLA content obtained in these studies was moderate or absent. To achieve increased amounts of CLA and CLNA in fermented dairy or meat products, the potential of using carefully selected bacteria with high CLA- and CLNA-producing ability or the introduction of modifications in food-processing parameters will need to be investigated. Also, possible probiotic benefits related to *in vivo* CLA and CLNA production by bifidobacteria or other human gut-associated bacteria, to be included in foods or food supplements, need further exploration.

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REFERENCES

Abd El-Salam, M.H., El-Shafei, K., Sharaf, O.M., Effat, B.A., Asem, F.M., and El-Aasar, M. (2010). Screening of some potentially probiotic lactic acid bacteria for their ability to synthesis conjugated linoleic acid. *Int. J. Dairy Technol.* **63**: 62-69.

Abd El-Salam, M.H., Hippen, A.R., Assem, F.M., El-Shafei, K., Tawfik, N.F., and El-Aassar, M. (2011). Preparation and properties of probiotic cheese high in conjugated linoleic acid content. *Int. J. Dairy Technol.* **64**: 64-74.

Acharya, M.R. and Shah, R.K. (2002). Selection of human isolates of bifidobacteria for their use as probiotics. *Appl. Biochem. Biotechnol.* **102**: 81-98.

Akalin, A.S., Tokuşoğlu, Ö., Gönç, S., and Aycan, Ş. (2007). Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. *Int. Dairy J.* **17**: 1089-1095.

Akraim, F., Nicot, M.C., Juaneda, R., and Enjalbert, F. (2007). Conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA) and other biohydrogenation intermediates in plasma and milk fat of cows fed raw or extruded linseed. *Animal* **1**: 835-843.

Alonso, L., Cuesta, E.P., and Gilliland, S.E. (2003). Production of free conjugated linoleic acid by *Lactobacillus acidophilus* and *Lactobacillus casei* of human intestinal origin. *J. Dairy Sci.* **86**: 1941-1946.

Ando, A., Ogawa, J., Kishino, S., and Shimizu, S. (2004). Conjugated linoleic acid production from castor oil by *Lactobacillus plantarum* JCM 1551. *Enzyme Microbiol. Technol.* **35**: 40-45.

Benjamin, S. and Spener, F. (2009). Conjugated linoleic acids as functional food: an insight into their health benefits. *Nutr. Metab.* **6**: 36.

Beppu, F., Hosokawa, M., Tanaka, L., Kohno, H., Tanaka, T., and Miyashita, K. (2006). Potent inhibitory effect of *trans*9, *trans*11 isomer of conjugated linoleic acid on the growth of human colon cancer cells. *J. Nutr. Biochem.* **17**: 830-836.

Bhattacharya, A., Banu, J., Rahman, M., Causey, J., and Fernandes, G. (2006). Biological effects of conjugated linoleic acids in health and disease. *J. Nutr. Biochem.* **17**: 789-810.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. (2007). Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *Eur. J. Lipid Sci. Technol.* **109**: 828-855.

Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L., and Pariza, M.W. (1992). Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Compos. Anal.* **5**: 185-197.

Choi, N.J., Park, H.G., Kim, Y.J., Kim, I.H., Kang, H.S., Yoon, C.S., Yoon, H.G., Park, S.I., Lee, J.W., and Chung, S.H. (2008). Utilization of monolinolein as a substrate for conjugated linoleic acid production by *Bifidobacterium breve* LMC 520 of human neonatal origin. *J. Agric. Food Chem.* **56**: 10908-10912.

Coakley, M., Banni, S., Johnson, M.C., Mills, S., Devery, R., Fitzgerald, G., Ross, R.P., and Stanton, C. (2009). Inhibitory effect of conjugated α-linolenic acid from bifidobacteria of intestinal origin on SW480 cancer cells. *Lipids* **44**: 249-256.

Coakley, M., Barrett, E., Murphy, J.J., Ross, R.P., Devery, R., and Stanton, C. (2007). Cheese manufacture with milk with elevated conjugated linoleic acid levels caused by dietary manipulation. *J. Dairy Sci.* **90**: 2919-2927.

Coakley, M., Johnson, M.C., McGrath, E., Rahman, S., Ross, R.P., Fitzgerald, G.F., Devery, R., and Stanton, C. (2006). Intestinal bifidobacteria that produce *trans*-9, *trans*-11 conjugated linoleic acid: a fatty acid with antiproliferative activity against human colon SW480 and HT-29 cancer cells. *Nutr. Cancer* **56**: 95-102.

Coakley, M., Ross, R.P., Nordgren, M., Fitzgerald, G., Devery, R., and Stanton, C. (2003). Conjugated linoleic acid biosynthesis by human-derived *Bifidobacterium* species. *J. Appl. Microbiol.* **94**: 138-145.

Collins, M.D. and Gibson, G.R. (1999). Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am. J. Clin. Nutr.* **69**: 1052S-1057S.

Dannenberger, D., Nuernberg, K., and Nuernberg, G. (2009). Diet-dependent occurrence of CLA isomers in rumen and duodenal digesta of slaughtered bulls. *Eur. J. Lipid Sci. Technol.* **111**: 553-562.

Dannenberger, D., Nuernberg, K., Nuernberg, G., Scollan, N., Steinhart, H., and Ender, K. (2005). Effect of pasture vs. concentrate diet on CLA isomer distribution in different tissue lipids of beef cattle. *Lipids* **40**: 589-598.

Das, S., Holland, R., Crow, V.L., Bennett, R.J., and Manderson, G.J. (2005). Effect of yeast and bacterial adjuncts on the CLA content and flavour of a washed-curd, dry-salted cheese. *Int. Dairy J.* **15**: 807-815.

de Carvalho, E.B.T., de Melo, I.L.P., and Mancini-Filho, J. (2010). Chemical and physiological aspects of isomers of conjugated fatty acids. *Ciência e Tecnologia de Alimentos* **30**: 295-307.

Decker, E.A. and Park, Y. (2010). Healthier meat products as functional foods. *Meat Sci.* **86**: 49-55.

Demir, A.S. and Talpur, F.N. (2010). Chemoenzymatic conversion of linoleic acid into conjugated linoleic acid. *J. Agric. Food Chem.* **58**: 1646-1652.

Deng, M.D., Grund, A.D., Schneider, K.J., Langley, K.M., Wassink, S.L., Peng, S.S., and Rosson, R.A. (2007). Linoleic acid isomerase from *Propionibacterium acnes*: purification, characterization, molecular cloning, and heterologous expression. *Appl. Biochem. Biotechnol.* **143**: 199-211.

Devillard, E., McIntosh, F.M., Duncan, S.H., and Wallace, R.J. (2007). Metabolism of linoleic acid by human gut bacteria: different routes for biosynthesis of conjugated linoleic acid. *J. Bacteriol.* **189**: 2566-2570.

Dhiman, T.R., Nam, S.H., and Ure, A.L. (2005). Factors affecting conjugated linoleic acid content in milk and meat. *Crit. Rev. Food Sci. Nutr.* **45**: 463-482.

Ecker, J., Liebisch, G., Patsch, W., and Schmitz, G. (2009). The conjugated linoleic acid isomer *trans*-9,*trans*-11 is a dietary occurring agonist of liver X receptor α. *Biochem. Biophys. Res. Commun.* **388**: 660-666.

Ekinci, F.Y., Okur, O.D., Ertekin, B., and Guzel-Seydim, Z. (2008). Effects of probiotic bacteria and oils on fatty acid profiles of cultured cream. *Eur. J. Lipid Sci. Technol.* **110**: 216-224.

Ewaschuk, J.B., Walker, J.W., Diaz, H., and Madsen, K.L. (2006). Bioproduction of conjugated linoleic acid by probiotic bacteria occurs *in vitro* and *in vivo* in mice. *J. Nutr.* **136**: 1483-1487.

Farmani, J., Safari, M., Roohvand, F., Razavi, S.H., Aghasadeghi, M.R., and Noorbazargan, H. (2010). Conjugated linoleic acid-producing enzymes: a bioinformatics study. *Eur. J. Lipid Sci. Technol.* **112**: 1088-1100.

Florence, A.C.R., da Silva, R.C., Santo, A.P.D., Gioielli, L.A., Tamime, A.Y., and de Oliveira, M.N. (2009). Increased CLA content in organic milk fermented by bifidobacteria or yoghurt cultures. *Dairy Sci. Technol.* **89**: 541-553.

Fritsche, J. and Steinhart, H. (1998). Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. *Z. Lebensm.-Unters. -Forsch. A* **206**: 77-82.

Fukuda, S., Furuya, H., Suzuki, Y., Asanuma, N., and Hino, T. (2005). A new strain of *Butyrivibrio fibrisolvens* that has high ability to isomerize linoleic acid to conjugated linoleic acid. *J. Gen. Appl. Microbiol.* **51**: 105-113.

Gavino, V.C., Gavino, G., Leblanc, M.J., and Tuchweber, B. (2000). An isomeric mixture of conjugated linoleic acids but not pure *cis*-9,*trans*-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. *J. Nutr.* **130**: 27-29.

Gläser, K.R., Wenk, C., and Scheeder, M.R.L. (2002). Effects of feeding pigs increasing levels of C 18:1 trans fatty acids on fatty acid composition of backfat and intramuscular fat as well as backfat firmness. *Arch. Anim. Nutr.-Arch. Tierernahr.* **56**: 117-130.

Gomes, A.M.P. and Malcata, F.X. (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends Food Sci. Technol.* **10**: 139-157.

Gómez-Cortés, P., Tyburczy, C., Brenna, J.T., Juárez, M., and de la Fuente, M.A. (2009). Characterization of *cis*-9 *trans*-11 *trans*-15 C18:3 in milk fat by GC and covalent adduct chemical ionization tandem MS. *J. Lipid Res.* **50**: 2412-2420.

Gorissen, L., De Vuyst, L., Raes, K., De Smet, S., and Leroy, F. (2012a). Conjugated linoleic and linolenic acid production kinetics by bifidobacteria differ among strains. *Int. J. Food Mircobiol.* **155**: 234-240.

Gorissen, L., Raes, K., De Smet, S., De Vuyst, L., and Leroy, F. (2012b). Microbial production of conjugated linoleic and linolenic acids in fermented foods: Technological bottlenecks. *Eur. J. Lipid Sci. Technol.* **114**: 486-491.

Gorissen, L., Raes, K., Weckx, S., Dannenberger, D., Leroy, F., De Vuyst, L., and De Smet, S. (2010). Production of conjugated linoleic acid and conjugated linolenic acid isomers by *Bifidobacterium* species. *Appl. Microbiol. Biotechnol.* **87**: 2257-2266.

Gorissen, L., Weckx, S., Vlaeminck, B., Raes, K., De Vuyst, L., De Smet, S., and Leroy, F. (2011). Linoleate isomerase activity occurs in lactic acid bacteria strains and is affected by pH and temperature. *J. Appl. Microbiol.* **111**: 593-606.

Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V., and Bauman, D.E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. *J. Nutr.* **130**: 2285-2291.

Henderson, C. (1973). The effects of fatty acids on pure cultures of rumen bacteria. *J. Agric. Sci.* **81**: 107-112.

Hennessy, A.A., Ross, R.P., Devery, R., and Stanton, C. (2009). Optimization of a reconstituted skim milk based medium for enhanced CLA production by bifidobacteria. *J. Appl. Microbiol.* **106**: 1315-1327.

Hennessy, A.A., Ross, R.P., Devery, R., and Stanton, C. (2011). The health promoting properties of the conjugated isomers of α-linolenic acid. *Lipids* **46**: 105-119.

Hernandez-Mendoza, A., Lopez-Hernandez, A., Hill, C.G., and Garcia, H.S. (2009). Bioconversion of linoleic acid to conjugated linoleic acid by *Lactobacillus reuteri* under different growth conditions. *J. Chem. Technnol. Biotechnol.* **84**: 180-185.

Hornung, E., Krueger, C., Pernstich, C., Gipmans, M., Porzel, A., and Feussner, I. (2005). Production of (10*E*, 12*Z*)-conjugated linoleic acid in yeast and tobacco seeds. *Biochim. Biophys. Acta* **1738**: 105-114.

Hur, S.J., Whitcomb, F., Rhee, S., Park, Y., Good, D.J., and Park, Y. (2009). Effects of *trans*-10,*cis*-12 conjugated linoleic acid on body composition in genetically obese mice. *J. Med. Food* **12**: 56-63.

Irmak, S., Dunford, N.T., Gilliland, S.E., Banskalieva, V., and Eisenmenger, M. (2006). Biocatalysis of linoleic acid to conjugated linoleic acid. *Lipids* **41**: 771-776.

Jenkins, J.K. and Courtney, P.D. (2003). *Lactobacillus* growth and membrane composition in the presence of linoleic or conjugated linoleic acid. *Can. J. Microbiol.* **49**: 51-57.

Jenkins, T.C., Wallace, R.J., Moate, P.J., and Mosley, E.E. (2008). Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *J. Anim. Sci.* **86**: 397-412.

Jiang, J., Björck, L., and Fondén, R. (1997). Conjugated linoleic acid in Swedish dairy products with special reference to the manufacture of hard cheeses. *Int. Dairy J.* **7**: 863-867.

Jiang, J., Björck, L., and Fondén, R. (1998). Production of conjugated linoleic acid by dairy starter cultures. *J. Appl. Microbiol.* **85**: 95-102.

Jouany, J.P., Lassalas, B., Doreau, M., and Glasser, F. (2007). Dynamic features of the rumen metabolism of linoleic acid, linolenic acid and linseed oil measured in vitro. *Lipids* **42**: 351-360.

Kepler, C.R., Hirons, K.P., McNeill, J.J., and Tove, S.B. (1966). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. *J. Biol. Chem.* **241**: 1350-1354.

Kepler, C.R. and Tove, S.B. (1967). 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**: 5686-5692.

Kepler, C.R., Tucker, W.P., and Tove, S.B. (1970). Biohydrogenation of unsaturated fatty acids. IV. Substrate specificity and inhibition of linoleate Δ^{12}-*cis*, Δ^{11}-*trans*-isomerase from *Butyrivibrio fibrisolvens*. *J. Biol. Chem.* **245**: 3612-3620.

Kepler, C.R., Tucker, W.P., and Tove, S.B. (1971). Biohydrogenation of unsaturated fatty acids. V. Stereospecificity of proton addition and mechanism of action of linoleic acid Δ^{12}-*cis*, Δ^{11}-*trans*-isomerase form *Butyrivibrio fibrisolvens*. *J. Biol. Chem.* **246**: 2765-2771.

Kim, Y.J. and Liu, R.H. (2002). Increase of conjugated linoleic acid content in milk by fermentation with lactic acid bacteria. *J. Food Sci.* **67**: 1731-1737.

Kim, Y.J., Liu, R.H., Bond, D.R., and Russell, J.B. (2000). Effect of linoleic acid concentration on conjugated linoleic acid production by *Butyrivibrio fibrisolvens* A38. *Appl. Environ. Microbiol.* **66**: 5226-5230.

Kim, Y.J., Liu, R.H., Rychlik, J.L., and Russell, J.B. (2002). The enrichment of a ruminal bacterium (*Megasphaera elsdenii* YJ-4) that produces the *trans*-10, *cis*-12 isomer of conjugated linoleic acid. *J. Appl. Microbiol.* **92**: 976-982.

Kishino, S., Ogawa, J., Ando, A., Omura, Y., and Shimizu, S. (2002a). Ricinoleic acid and castor oil as substrates for conjugated linoleic acid production by washed cells of *Lactobacillus plantarum*. *Biosci., Biotechnol., Biochem.* **66**: 2283-2286.

Kishino, S., Ogawa, J., Ando, A., Yokozeki, K., and Shimizu, S. (2010). Microbial production of conjugated γ-linolenic acid from γ-linolenic acid by *Lactobacillus plantarum* AKU 1009a. *J. Appl. Microbiol.* **108**: 2012-2018.

Kishino, S., Ogawa, J., Omura, Y., Matsumura, K., and Shimizu, S. (2002b). Conjugated linoleic acid production from linoleic acid by lactic acid bacteria. *J. Am. Oil Chem. Soc.* **79**: 159-163.

Kishino, S., Ogawa, J., Yokozeki, K., and Shimizu, S. (2009). Metabolic diversity in biohydrogenation of polyunsaturated fatty acids by lactic acid bacteria involving conjugated fatty acid production. *Appl. Microbiol. Biotechnol.* **84**: 87-97.

Kishino, S., Ogawa, J., Yokozeki, K., and Shimizu, S. (2011a). Linoleic acid isomerase in *Lactobacillus plantarum* AKU1009a proved to be a multi-component enzyme system requiring oxidoreduction cofactors. *Biosci., Biotechnol., Biochem.* **75**: 318-322.

Kishino, S., Park, S.-B., Takeuchi, M., Yokozeki, K., Shimizu, S., and Ogawa, J. (2011b). Novel multi-component enzyme machinery in lactic acid bacteria catalyzing C=C double bond migration useful for conjugated fatty acid synthesis. *Biochem. Biophys. Res. Commun.* **416**: 188-193.

Kohno-Murase, J., Iwabuchi, M., Endo-Kasahara, S., Sugita, K., Ebinuma, H., and Imamura, J. (2006). Production of *trans*-10, *cis*-12 conjugated linoleic acid in rice. *Transgenic Res.* **15**: 95-100.

Kohno, H., Suzuki, R., Yasui, Y., Hosokawa, M., Miyashita, K., and Tanaka, T. (2004). Pomegranate seed oil rich in conjugated linolenic acid suppresses chemically induced colon carcinogenesis in rats. *Cancer Sci.* **95**: 481-486.

Lee, H.Y., Park, J.H., Seok, S.H., Baek, M.W., Kim, D.J., Lee, K.E., Paek, K.S., Lee, Y., and Park, J.H. (2006). Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochim. Biophys. Acta* **1761**: 736-744.

Lee, K. and Lee, Y. (2009). Production of c9,t11- and t10,c12-conjugated linoleic acids in humans by *Lactobacillus rhamnosus* PL60. *J. Microbiol. Biotechnol.* **19**: 1617-1619.

Lee, K., Paek, K., Lee, H.Y., Park, J.H., and Lee, Y. (2007). Antiobesity effect of *trans*-10,*cis*-12-conjugated linoleic acid-producing *Lactobacillus plantarum* PL62 on diet-induced obese mice. *J. Appl. Microbiol.* **103**: 1140-1146.

Lee, S.O., Hong, G.W., and Oh, D.K. (2003a). Bioconversion of linoleic acid into conjugated linoleic acid by immobilized Lactobacillus reuteri. *Biotechnol. Prog.* **19**: 1081-1084.

Lee, S.O., Kim, C.S., Cho, S.K., Choi, H.J., Ji, G.E., and Oh, D.K. (2003b). Bioconversion of linoleic acid into conjugated linoleic acid during fermentation and by washed cells of *Lactobacillus reuteri*. *Biotechnol. Lett.* **25**: 935-938.

Leroy, F., Verluyten, J., and De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. *Int. J. Food Mircobiol.* **106**: 270-285.

Liavonchanka, A. and Feussner, I. (2008). Biochemistry of PUFA double bond isomerases producing conjugated linoleic acid. *Chembiochem* **9**: 1867-1872.

Liavonchanka, A., Rudolph, M.G., Tittmann, K., Hamberg, M., and Feussner, I. (2009). On the mechanism of a polyunsaturated fatty acid double bond isomerase from *Propionibacterium acnes*. *J. Biol. Chem.* **284**: 8005-8012.

Lin, T.Y. (2006). Conjugated linoleic acid production by cells and enzyme extract of *Lactobacillus delbrueckii* ssp. *bulgaricus* with additions of different fatty acids. *Food Chem.* **94**: 437-441.

Lin, T.Y., Lin, C.W., and Lee, C.H. (1999). Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. *Food Chem.* **67**: 1-5.

Lin, T.Y., Lin, C.W., and Wang, Y.J. (2002). Linoleic acid isomerase activity in enzyme extracts from *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* ssp. *shermanii*. *J. Food Sci.* **67**: 1502-1505.

Liu, P., Shen, S.-r., Ruan, H., Zhou, Q., Ma, L.-l., and He, G.-q. (2011). Production of conjugated linoleic acids by *Lactobacillus plantarum* strains isolated from naturally fermented Chinese pickles. *J. Zhejiang Univ. Sci. B.* **12**: 923-930.

Lourenço, M., Ramos-Morales, E., and Wallace, R.J. (2010). The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. *Animal* **4**: 1008-1023.

Lourenço, M., Van Ranst, G., Vlaeminck, B., De Smet, S., and Fievez, V. (2008). Influence of different dietary forages on the fatty acid composition of rumen digesta as well as ruminant meat and milk. *Anim. Feed Sci. Technol.* **145**: 418-437.

Macouzet, M., Lee, B.H., and Robert, N. (2009). Production of conjugated linoleic acid by probiotic *Lactobacillus acidophilus* La-5. *J. Appl. Microbiol.* **106**: 1886-1891.

Maia, M.R.G., Chaudhary, L.C., Bestwick, C.S., Richardson, A.J., McKain, N., Larson, T.R., Graham, I.A., and Wallace, R.J. (2010). Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. *Bmc Microbiol.* **10**: 52.

Martin, J.C. and Valeille, K. (2002). Conjugated linoleic acids: all the same or to everyone its own function? *Reprod. Nutri. Dev.* **42**: 525-536.

Martins, S.V., Lopes, P.A., Alfaia, C.M., Ribeiro, V.S., Guerreiro, T.V., Fontes, C., Castro, M.F., Soveral, G., and Prates, J.A.M. (2007). Contents of conjugated linoleic acid isomers in ruminant-derived foods and estimation of their contribution to daily intake in Portugal. *Br. J. Nutr.* **98**: 1206-1213.

McIntosh, F.M., Shingfield, K.J., Devillard, E., Russell, W.R., and Wallace, R.J. (2009). Mechanism of conjugated linoleic acid and vaccenic acid formation in human faecal suspensions and pure cultures of intestinal bacteria. *Microbiol.* **155**: 285-294.

O'Shea, E.F., Cotter, P.D., Stanton, C., Ross, R.P., and Hill, C. (2012). Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: Bacteriocins and conjugated linoleic acid. *Int. J. Food Mircobiol.* **152**: 189-205.

Ogawa, J., Kishino, S., Ando, A., Sugimoto, S., Mihara, K., and Shimizu, S. (2005). Production of conjugated fatty acids by lactic acid bacteria. *J. Biosci. Bioeng.* **100**: 355-364.

Ogawa, J., Matsumura, K., Kishino, S., Omura, Y., and Shimizu, S. (2001). Conjugated linoleic acid accumulation via 10-hydroxy-12 octadecaenoic acid during microaerobic transformation of linoleic acid by *Lactobacillus acidophilus*. *Appl. Environ. Microbiol.* **67**: 1246-1252.

Oh, D.K., Hong, G.H., Lee, Y., Min, S.G., Sin, H.S., and Cho, S.K. (2003). Production of conjugated linoleic acid by isolated *Bifidobacterium* strains. *World J. Microbiol. Biotechnol.* **19**: 907-912.

Oliveira, R.P.S., Florence, A.C.R., Silva, R.C., Perego, P., Converti, A., Gioielli, L.A., and Oliveira, M.N. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. *Int. J. Food Mircobiol.* **128**: 467-472.

Pariza, M.W., Park, Y., and Cook, M.E. (2001). The biologically active isomers of conjugated linoleic acid. *Prog. Lipid Res.* **40**: 283-298.

Pariza, M.W. and Yang, X.Y. (1999) Method of producing conjugated fatty acids. US.

Park, H.G., Cho, S.D., Kim, J.H., Lee, H., Chung, S.H., Kim, S.B., Kim, H.S., Kim, T., Choi, N.J., and Kim, Y.J. (2009). Characterization of conjugated linoleic acid production by *Bifidobacterium breve* LMC 520. *J. Agric. Food Chem.* **57**: 7571-7575.

Park, Y. (2009). Conjugated linoleic acid (CLA): good or bad *trans* fat? *J. Food Compos. Anal.* **22**: S4-S12.

Peng, S.S., Deng, M.D., Grund, A.D., and Rosson, R.A. (2007). Purification and characterization of a membrane-bound linoleic acid isomerase from *Clostridium sporogenes*. *Enzyme Microbiol. Technol.* **40**: 831-839.

Plourde, M., Destaillats, F., Chouinard, P.Y., and Angers, P. (2007). Conjugated α-linolenic acid isomers in bovine milk and muscle. *J. Dairy Sci.* **90**: 5269-5275.

Plourde, M., Jew, S., Cunnane, S.C., and Jones, P.J.H. (2008). Conjugated linoleic acids: why the discrepancy between animal and human studies? *Nutr. Rev.* **66**: 415-421.

Puniya, A.K., Chaitanya, S., Tyagi, A.K., De, S., and Singh, K. (2008). Conjugated linoleic acid producing potential of lactobacilli isolated from the rumen of cattle. *J. Ind. Microbiol. Biotechnol.* **35**: 1223-1228.

Raes, K., De Smet, S., and Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. *Anim. Feed Sci. Technol.* **113**: 199-221.

Raff, M., Tholstrup, T., Basu, S., Nonboe, P., Sørensen, M.T., and Straarup, E.M. (2008). A diet rich in conjugated linoleic acid and butter increases lipid peroxidation but does not affect atherosclerotic, inflammatory, or diabetic risk markers in healthy young men. *J. Nutr.* **138**: 509-514.

Rainio, A., Vahvaselkä, M., and Laakso, S. (2002). Cell-adhered conjugated linoleic acid regulates isomerization of linoleic acid by resting cells of *Propionibacterium freudenreichii*. *Appl. Microbiol. Biotechnol.* **60**: 481-484.

Rainio, A., Vahvaselkä, M., Suomalainen, T., and Laakso, S. (2001). Reduction of linoleic acid inhibition in production of conjugated linoleic acid by *Propionibacterium freudenreichii* ssp. *shermanii*. *Can. J. Microbiol.* **47**: 735-740.

Risérus, U., Arner, P., Brismar, K., and Vessby, B. (2002). Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* **25**: 1516-1521.

Rosberg-Cody, E., Johnson, M.C., Fitzgerald, G.F., Ross, P.R., and Stanton, C. (2007). Heterologous expression of linoleic acid isomerase from *Propionibacterium acnes* and anti-proliferative activity of recombinant *trans*-10, *cis*-12 conjugated linoleic acid. *Microbiol.* **153**: 2483-2490.

Rosberg-Cody, E., Liavonchanka, A., Göbel, C., Ross, P.R., O'Sullivan, O., Fitzgerald, G., Feussner, I., and Stanton, C. (2011). Myosin-cross-reactive antigen (MCRA) protein from *Bifidobacterium breve* is a FAD-dependent fatty acid hydratase which has a function in stress protection. *BMC Biochem.* **12**: 9.

Rosberg-Cody, E., Ross, R.P., Hussey, S., Ryan, C.A., Murphy, B.P., Fitzgerald, G.F., Devery, R., and Stanton, C. (2004). Mining the microbiota of the neonatal gastrointestinal tract for conjugated linoleic acid-producing bifidobacteria. *Appl. Environ. Microbiol.* **70**: 4635-4641.

Ross, R.P., Mills, S., Hill, C., Fitzgerald, G.F., and Stanton, C. (2010). Specific metabolite production by gut microbiota as a basis for probiotic function. *Int. Dairy J.* **20**: 269-276.

Rosson, R.A., Deng, M.D., Grund, A.D., and Peng, S.S. (2001) Linoleate isomerase.

Russell, D.A., Ross, R.P., Fitzgerald, G.F., and Stanton, C. (2011). Metabolic activities and probiotic potential of bifidobacteria. *Int. J. Food Mircobiol.* **149**: 88-105.

Schmid, A., Collomb, M., Sieber, R., and Bee, G. (2006). Conjugated linoleic acid in meat and meat products: a review. *Meat Sci.* **73**: 29-41.

Senok, A.C., Ismaeel, A.Y., and Botta, G.A. (2005). Probiotics: facts and myths. *Clin. Microbiol. Infect.* **11**: 958-966.

Sieber, R., Collomb, M., Aeschlimann, A., Jelen, P., and Eyer, H. (2004). Impact of microbial cultures on conjugated linoleic acid in dairy products - a review. *Int. Dairy J.* **14**: 1-15.

Sirri, F., Tallarico, N., Meluzzi, A., and Franchini, A. (2003). Fatty acid composition and productive traits of broiler fed diets containing conjugated linoleic acid. *Poult. Sci.* **82**: 1356-1361.

Tholstrup, T., Raff, M., Straarup, E.M., Lund, P., Basu, S., and Bruun, J.M. (2008). An oil mixture with *trans*-10, *cis*-12 conjugated linoleic acid increases markers of inflammation and in vivo lipid peroxidation compared with *cis*-9, *trans*-11 conjugated linoleic acid in postmenopausal women. *J. Nutr.* **138**: 1445-1451.

Tricon, S., Burdge, G.C., Kew, S., Banerjee, T., Russell, J.J., Jones, E.L., Grimble, R.F., Williams, C.M., Yaqoob, P., and Calder, P.C. (2004). Opposing effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid on blood lipids in healthy humans. *Am. J. Clin. Nutr.* **80**: 614-620.

Tricon, S., Burdge, G.C., Williams, C.M., Calder, P.C., and Yaqoob, P. (2005). The effects of conjugated linoleic acid on human health-related outcomes. *Proc. Nutr. Soc.* **64**: 171-182.

Van Nieuwenhove, C.P., Oliszewski, R., González, S.N., and Pérea Chaia, A.B. (2007a). Influence of bacteria used as adjunct culture and sunflower oil addition on conjugated linoleic acid content in buffalo cheese. *Food Res. Int.* **40**: 559-564.

Van Nieuwenhove, C.P., Oliszewski, R., González, S.N., and Pérez Chaia, A.B. (2007b). Conjugated linoleic acid conversion by dairy bacteria cultured in MRS broth and buffalo milk. *Lett. Appl. Microbiol.* **44**: 467-474.

van Wijlen, R.P.J. and Colombani, P.C. (2010). Grass-based ruminant production methods and human bioconversion of vaccenic acid with estimations of maximal dietary intake of conjugated linoleic acids. *Int. Dairy J.* **20**: 433-448.

Verhulst, A., Janssen, G., Parmentier, G., and Eyssen, H. (1987). Isomerization of poylunsaturated long-chain fatty acids by propionibacteria. *Syst. Appl. Microbiol.* **9**: 12-15.

Wahle, K.W.J., Heys, S.D., and Rotondo, D. (2004). Conjugated linoleic acids: are they beneficial or detrimental to health? *Prog. Lipid Res.* **43**: 553-587.

Wang, L.M., Lv, J.P., Chu, Z.Q., Cui, Y.Y., and Ren, X.H. (2007). Production of conjugated linoleic acid by *Propionibacterium freudenreichii*. *Food Chem.* **103**: 313-318.

Xu, H., Lee, H.Y., Hwang, B., Nam, J.H., Kang, H.Y., and Ahn, J. (2008). Kinetics of microbial hydrogenation of free linoleic acid to conjugated linoleic acids. *J. Appl. Microbiol.* **105**: 2239-2247.

Xu, S., Boylston, T.D., and Glatz, B.A. (2005). Conjugated linoleic acid content and organoleptic attributes of fermented milk products produced with probiotic bacteria. *J. Agric. Food Chem.* **53**: 9064-9072.

Xu, S., Boylston, T.D., and Glatz, B.A. (2006). Effect of inoculation level of *Lactobacillus rhamnosus* and yogurt cultures on conjugated linoleic acid content and quality attributes of fermented milk products. *J. Food Sci.* **71**: C275-C280.

Yadav, H., Jain, S., and Sinha, P.R. (2007). Production of free fatty acids and conjugated linoleic acid in problotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* during fermentation and storage. *Int. Dairy J.* **17**: 1006-1010.

Yasui, Y., Hosokawa, M., Sahara, T., Suzuki, R., Ohgiya, S., Kohno, H., Tanaka, T., and Miyashita, K. (2005). Bitter gourd seed fatty acid rich in 9*c*,11*t*,13*t*-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPARγ in human colon cancer Caco-2 cells. *Prostraglandins Leukot. Essent. Fatty Acids* **73**: 113-119.

Yu, L., Adams, D., and Watkins, B.A. (2003). Comparison of commercial supplements containing conjugated linoleic acids. *J. Food Compos. Anal.* **16**: 419-428.

Zeng, Z., Lin, J., and Gong, D. (2009). Identification of lactic acid bacterial strains with high conjugated linoleic acid-producing ability from natural sauerkraut fermentations. *J. Food Sci.* **74**: M154-M158.

Table 1 Mean CLA content in various meat and dairy products (adapted from Schmid et al., 2006; Jiang et al., 1997; and Dhiman et al., 2005)

Dairy products	CLA (mg/g fat)	Meat products	CLA (mg/g fat)
Fluid milk products			
whole milk	3.4 -6.8	Lamb	4.3-5.6
evaporated milk	4.9	Beef	1.2-10.0
UHT milk	8.0	Veal	2.7
homogenised milk	5.5	Pork	0.6-0.7
condensed milk	6.3-7.0	Chicken	0.9
cultured buttermilk	5.4-6.7	Turkey	2.5
Cheeses		Horse	0.6
cheddar	4.0-5.3		
feta	4.9		
cottage	4.5-5.9		
mozzarella	3.4-5.0		
processed parmesan	5.3		
Fermented products			
plain yoghurt	3.8-8.8		
low fat yoghurt	4.4		
butter	4.7-9.4		
sour cream	4.6-7.5		
ice cream	3.6-5.0		

Figure 1 Structures of LA and CLA isomers (left) and LNA and CLNA isomers (right)

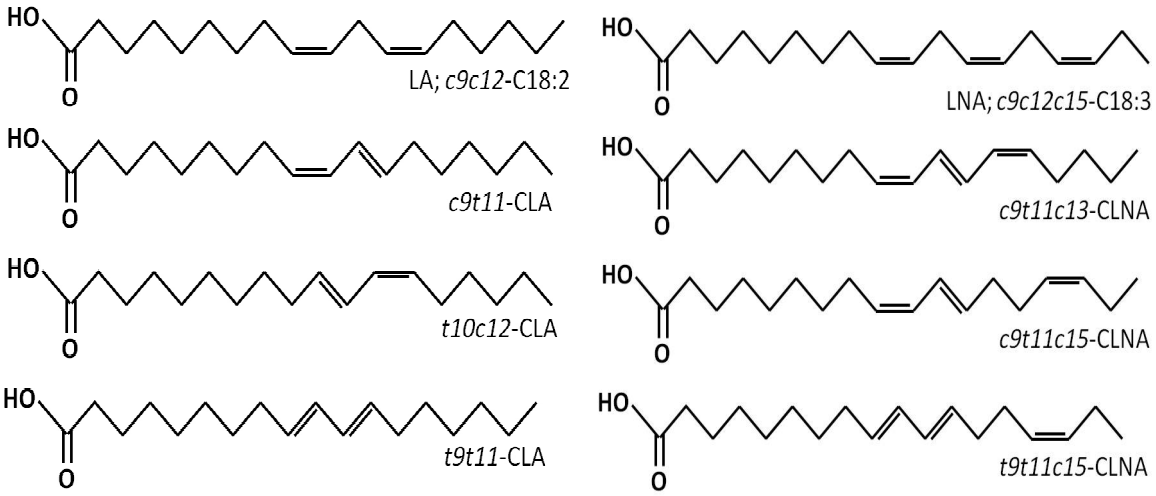

Figure 2 Ruminal biohydrogenation pathways of LA (*c9c12*-C18:2) and LNA (*c9c12c15*-C18:3) (adapted from Chilliard et al., 2007)