CONJUGATED FATTY ACIDS (CFAS) PRODUCTION VIA VARIOUS BACTERIAL STRAINS AND THEIR APPLICATIONS. A REVIEW

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ABSTRACT

Conjugated fatty acids (CFAs) mainly consists of Conjugated linoleic acids (CLAs) and conjugated linolenic acids (CLNAs). CFAs received significant importance because of their anti-carcinogenic, anti-obesity, anti-diabetic, lipid/ energy metabolism modulatory effects and some other health promoting properties. Though, their concentration in food stuff is inadequate for any therapeutic application to be implemented. For a biotechnological perspective, microbial production of these CFAs has been extensively explored as an alternative and various bacterial strains of *Propionbacterium*, *Lactobacillus* and *Bifidobacterium* have shown promising results. This review will amass and recap available data concerning CLA and CLNA production by various bacterial strains via various enzymatic reaction behind all the processes. Numerous studies on CFA biochemical pathways are important to understand and discourse the metabolic mechanisms behind this process showing all the gene products that could be involved in the production. Among these bacterial strains few have shown the modulation of lipid metabolism *in-vivo*, further research should be focused on this topic which would help us to understand the role of gut microbiota on human health and future foods sustainability.

Keywords: CFAs, Ruminal production, CLA, CLNA, LAB.

1. INTRODUCTION

Conjugated fatty acids (CFAs) is a collective term used for positional and geometric isomers of fatty acids with conjugated double bonds or in simple words we can say that CFAs represent polyunsaturated fatty acids with conjugated double bonds, usually found in a mixture of positional and geometric isomers [1].

The positional and geometric isomers of polyunsaturated fatty acids (PUFAs) with alternate single and double bonds are called conjugated fatty acids (CFAs) [1] and they have gained significant consideration because of their impending beneficial role in amelioration of numerous health conditions. Over the years, the biological significance of CFAs has been revealed. CFAs including conjugated linoleic acids (CLAs), conjugated linolenic acids (CLNAs), conjugated eicosapentaenoic acids (CEPAs) and conjugated docosahexaenoic acids (CDHAs) are effective for tumors and obesity, cardiovascular diseases and immune functions have been well demonstrated and intensely studied in both *in vitro* and *in vivo* [2-6]. Different aspects of life comprising diet, affect the development of various non-transmissible enduring diseases such as arthrosclerosis, cancer, diabetes, inflammation, cardiovascular problems, obesity and many others [7].

Human diet encompasses of saturated, monosaturated, omega-3 and omega-6 polyunsaturated fatty acids [8]. Moreover, two forms of structurally related conjugated octadecanoic acids explicitly, conjugated linoleic acid (CLA) and conjugated linolenic acid (CLNA) also exist in the human diet in insignificant amount [8]. CLA is polyunsaturated fatty acid which is found naturally in ruminant animal food products [9]. Polyunsaturated fatty acids (PUFAs) such as linoleic acid (LA, 18:2, ω-6), α- linolenic acid (LNA, 18:3, ω-3) and arachidonic acid (20:4, ω-6) are valuable for mammals in maintaining the bio-functional properties. It has also been reported that other Polyunsaturated fatty acids of omega series such as eicosapentaenoic acid (EPA, 20:5, ω-3) and docosahexanoic acid (DHA, 22:6, ω-3) is associated with a reduced risk of cancer and cardiovascular disease in clinical and animal studies [10].

In the past few decades, CFAs specifically CLA and CLNA showed auspicious bioactive compounds that might be used for the promotion of human health and well-being. Because of the health promoting properties like antioxidant, antitumor, immunomodulatory, anti-obesity and serum lipid lowering capacity, CFAs is getting more attention day by day [11]. CFAs have also been testified to exhibit numerous pharmacological activities related to the prevention and treatment of arthrosclerosis, obesity, cancer and hypertension [12]. The reality is that they can be only attained via microbial synthesis as it unlocks fascinating opportunities for the amplification of functional food products and future foods. However, the molecular mechanism for the production of CFAs is still not clear and conflicting data have been stated about the probable enzymes responsible for the production of CFAs as well as the regulatory mechanisms.

The aim of writing this review is to amass and recap available data concerning CLA and CLNA production by various bacterial strains via various enzymatic reaction behind all the processes. Categorically this will help in the researchers about the progress of more efficient strategies for screening and optimizing different bacterial screen for production of CFAs.

2. PRODUCTION OF CFAS

2.1 Ruminal production of CFAs

Mostly the fatty acids are present in esterfied form in concentrate feeds and forages, typically present as phospholipids and glycolipids. In forages fatty acids are present as in forages and triglycerides in plant seeds, normally used in concentrates. The most widespread and abundant fatty acids from animal diets are linoleic acid and linolenic acid. Both are taken through diet and upon reaching to the rumen, they are widely modified by the action of microbial enzymes such as lipases which further produce LA and LNA as free form for further reaction such as isomerization and hydrogenation.

Kemp and Lander in 1930 [13] divided bacteria into two groups on the basis of reactions and end products of biohydrogeantion. In group A he included those bacteria which were able to hydrogenate linoleic acid and α-linolenic acid producing *t*11- C18:1 as an end product. While group B consisted of those bacteria which were able to use *t*11- C18:1 as one of the key substrates to produce stearic acid as end product. A brief list of bacterial species of both groups has been mentioned in a review article by Harfoot and Hazlewood [14].

The isomers of LA and LNA on the basis of existence of two conjugated double bonds in different geometric (i.e. cis or trans) and positional configuration are CLA (C18:2 *c*9, *c*12) and CLNA (C18:3 *c*9, *c*12, *c*15). Both of these isomers can be produced by microorganisms. CLA has been extensively studied and so far two pathways have been defined for its production. The first pathway is biohydrogenation of LA in the rumen [15] and the second pathway involves Δ9-desaturation trans-vaccenic acid (TVA) (C18:1 *t*11) in adipose, but mostly in mammary gland tissues [17-19]. This endogenous synthesis of CLA is counted for utmost of CLA level found in milk fat according to the finding around a 64% to <80% [18, 20].

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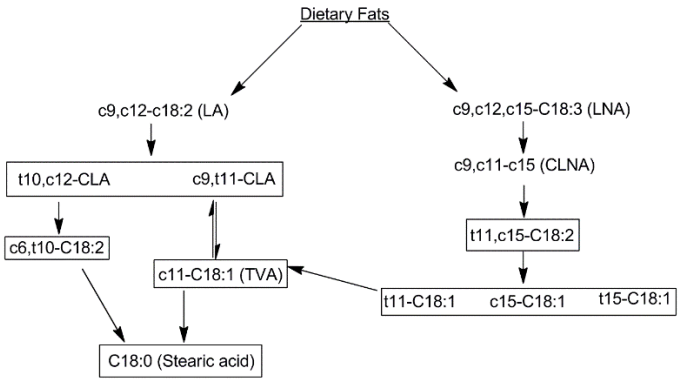

Figure 1. Conjugated fatty acids (CFAs) biohydrogenation process in rumen [14] and endogenous synthesis of CLA in mammary gland (reversible reaction arrow).

The biohydrogeantion of both CFAs occurs in the same way but differs from each other in the transitional products, as shown in Figure 1. The first step in biohydrogeantion process is lipolysis of dietary fats to release free fatty acids (FAs) [13-14]. In next step polyunsaturated fatty acids (PUFAs) are isomerized and hydrogenated into saturated fatty acids (SFAs) as end products namely, stearic acid (C18:0). In all this mechanism LA is mainly isomerized to C18:2 *c*9, *t*11 CLA which is also called Rumenic Acid (RA) [16]. Several isomers are also formed in this process and further hydrogenated to many trans C18:1 fatty acid, mainly TVA. Eventually via the biohydrogeantion process, the C18:1 isomer are converted to C18:0 in the rumen, the details are shown in figure 2.

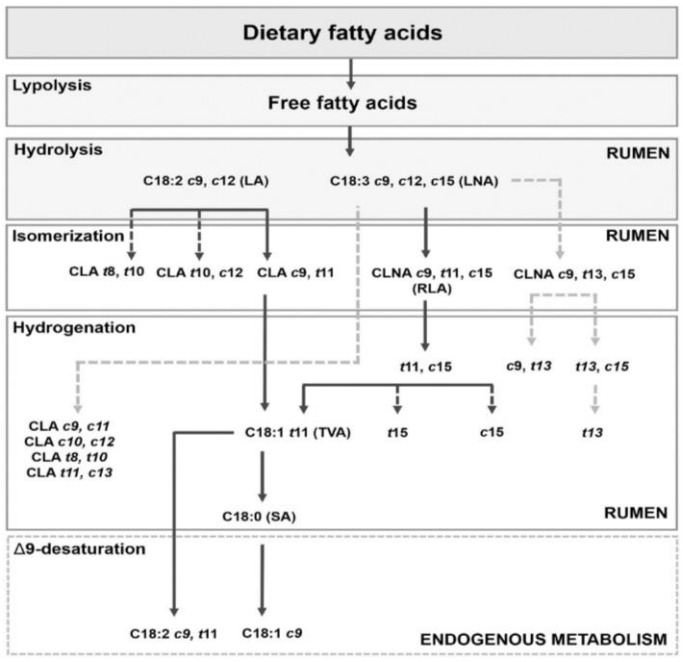

Figure 2. Mechanism of LA and LNA biohydrogenation. Bold arrows shows the main stream pathway and broken lines represents the alternative pathways.

Regarding CLNA production pathway, LNA biohydrogenation comprises reaction parallel to those defined above for LA, yielding both CLA and CLNA in changed proportions. After lipolysis, isomerization the released LNA occurs at the *cis*-12 position producing C18:3 *c9, t*11, *c*15 (rumenlenic acid "RLA") which was for the first time identified by Destaillats [21], in milk fat. Furthermore, RLA is reduced C18:2 *t*11, *c*15 and finally converted to TVA, C18:1 *c*15 and C18:1 *t*15. Substitute pathways have been suggested i.e. the transformation of isorumenlenic acid (iRLA) (C18:3 *c*9, *t*13, *c*15) to C18:2 *c9*, *t*13, *c*15 and eventually to C18:1 *t*13 [21].

Some other researchers have demonstrated that some of the resulting compounds have not been identified yet. During quantitative and qualitative determination 13C transfer to CFA, mixed microbial population from dairy rumen the cow was examined in batch cultures in the presence of LNA (13C) [22]. Throughout the incubation time (48 h), both conjugated 18:2 and non-conjugated 18:3 isomers were enriched with 13C. Remarkably, for C18:2, six unidentified isomers were reported, whereas for C18:3, there were 10 plus 2 conjugated forms listed. Formation of RA and isomers C18:2, *t*10, *c*12 plus 9, *t*11; *t*9, c11; *c*9, *c*11; *t*11, *t*13; *t*8, *t*10; and *c*10,12 were also found.

Hence, it was concluded that ruminal microbes have the capability of transforming LNA into both CLA and 18:3 compounds. Lastly, these intermediates are hydrogenated into stearic acid (C18:0) [23-24] whereas fatty acids are absorbed in the gut and then transported to various body tissues by the bloodstream [19, 22].

The biohydrogenation pathways mentioned earlier have been proposed to have a detoxifying mechanism for bacteria and due to this process, growth inhibiting free PUFAs are converted to less toxic SFAs [25-26]. To know the effects of these compounds on the bacterial cell and their various mechanisms, many scientists tried their best to disclose this mystery. In this regard, it was noticed that Fab I, and enoyl-acyl carrier protein (ACP) reductase which catalyzes the final and rate limiting step of chain elongation in bacterial fatty acid synthesis was inhibited by LNA [26]. To support this hypothesis an investigation was done by [28], with other organism for example, the cis-12 linoleate isomerase (LAI) gene of Fusarium graminearum (FgLAI12) which is responsible for the conversion of LA into RA, was characterized. Reduction of mycelial growth was observed due to gene deletion mutants test. Hence the wheat plant produces LA as a response to Fusarium graminearum infection.

It has been confirmed that linolenic, arachidonic and eicosapentaenoic acids modified membrane bounded proteins, ATPase and the histocompatibility complex as well as brought about the control of FA binding proteins [28-29]. On the basis of interpretations, it was observed that bacterial growth inhibitory effect of FA specifically LA is associated to high bacterial membrane permeability because of its surfactant action [30].

Fascinatingly, in the presence of LA and LNA the growth of *Butyrivibro fibrisolvens* was induced only when these PUFAs were converted to TVA [31]. Available data showed that the toxicity was induced by an energy metabolism deficiency as a substantial decrease in the Acyl-CoA pools in LA containing cultures were reported.

2.2 Propionibacteria

Generally *Pripionibacteria* are found in milk and dairy products and some species among them play a vigorous role in the manufacturing of cheeses for example emmental cheese. Another groups of bacteria in which the capability of LA isomerization in vitro was confirmed is represented by *Pripionibacteria*¸ being more precise since it could be incorporated in fermented products as cheeses. Several studies demonstrated that *P. freudenerhichii* is capable for the production of CLA mainly as *c*9, *t*11 form [32-36], however another study displayed eight different isomers of CLA produced by enzyme extract in this bacteria [36]. The first scientist who reported CLA production in *propionibacteria was* Verhuslt [37], in his study he reported that *p.freudenreichii subsp, freudenreicbii, P. freudenreichii subsp. Shermanii, P.acidi-propionici* and *P. tecbnicum* can produce *c*9, *t*11- CLA.

Another study performed by Jiang [32], in which he checked dairy cultures for the competency to produce CLA from free LA in MRS broth. Three *propionibacteria* strains namely *P. freudenreichii subsp, freudenreichii propionic-6 Wiseby* showed high efficiency to produce CLA with about 35.3% conversion rate. *P. shermanii* AKU1254 can produce 0.11g/l CLA in reaction mixture with 4 g/l free LA and the CLA produced was the mixture of *c*9, *t*11-CLA and *t*9, *t*11-CLA [38-39].

Production of CLA in fat milk model supplemented with hydrolyzed soy oil for 24-48 h was checked in two *Pripionibacteria* strains namely *P. freudenreichii ssp shermanii* and *P. freudenreichii ssp freudenreichii* by Xu [33]. In addition to that higher CLA levels were found in skim milk rather than MRS broth. The capability of *P. acnes* isolated from sheep was assessed to produce CLA as *t*10, *c*12 form by Wallace [34]. From all these studies and results its manifested that *propionibacteria* strains shows a great unpredictability regarding CLA production, depending upon numerous factors such specie, origin, substrate and culture conditions.

As far as CLNA production by *propionibacteria* is concerned it has been recently reported by Henessy [40], in his work he cultured bacteria in the manifestation of various fatty acid which were used as substrate to evaluate its supplementary conversion into the conjugated form. Therefore, LA, α and γ LNA, steridonic (c6, c9, c15-18:4) and some other polyunsaturated fatty acids were individually assimilated to culture medium. Strains of *P. freudenreiichii ssp shermanii* and *P. freudenreichii ssp freudenreichii* were capable to conjugate several PUFA, presenting diverse percentage conversion of each specific fatty acid.

Hence, for conversion of LA, α-LNA and stearidonic acid by *P. freudenreiichi ssp shermanii* grasped a conversion rate of 50.5; 53.5 and 3.09 whereas *P. freudenreihichii ssp freudenreichii* propioni-6 achieved a conversion rate of 44.65; 8.94 and 3.58 for the same fatty acids. The γ-LNA isomerization process was not reported by these bacteria. A decrease on the percentage of bioconversion was reported by increasing the substrate concentration which is shown in table 1.

Table 1. CLNA isomers production by bacterial strains cultured in the presence of α-LNA

Strain	*c*9,*t*11,*c*15	*t*9,*t*11,*c*15	CLA(%)	CLNA(%)	Author	Year
***B. breve* NCBIMB 8807**	+	+	66%	68%	Hennessy	2012
***B. breve* DPC6330**	+	+	67%	83%	Hennessy	2012
***B. longum* DPC6315**	-	-	12%	0.0%	Hennessy	2012
***P.freudenreihichii ssp freudenreichii* propioni-6**	+	+	44.6%	8.6%	Hennessy	2012
P.freudenreiichii ssp shermanii 9093 **	+	+	50.5%	53.5%	Hennessy	2012
***L.curvatus* LMG1353**	+	+	1.6%	22.4%	Gorissen	2011
***L. plantarum* ATCC8014**	+	+	4.6%	26.8%	Gorissen	2011
***L. sakei* LMG 13558, CG1**	+	+	4.2 ND	60.1 28.4	Gorissen	2011
B. bifidum LMG 10645	+	+	40.7%	78.4%	Gorissen	2010
***B. breve* LMG 11040**	+	+	44%	65.5%	Gorissen	2010
***B. breve* LMG 11084**	+	+	53.5%	72.0%	Gorissen	2010
***B. breve* LMG 11613**	+	+	19.5%	55.6%	Gorissen	2010
***B. breve* LMG 13194**	+	+	24.2%	63.3%	Gorissen	2010
***B. pseudolongum ssp pseudongum* LMG 11595**	+	+	42.2%	62.7%	Gorissen	2010

* production of conjugated isomers of γ-LNA and stearidonic acid were also reported.
** production of conjugated stearidonic acid was reported. ND: Not detected.

2.3 Lactic Acid Bacteria

Many studies have been accomplished for the production of CFA by lactic acid bacteria (LAB). The mechanism of CFA (CLA/CLNA) production, isomer and optimum conditions makes LAB the most inconstant and complex group of bacteria. Even though Lactobacilli have attained more attention as compared to other CLA producing strain because of its beneficial health properties. Several species of LAB have been described to own the capability of CLA production. The first specie of LAB to exhibit high CLA production ability was *L. reutri* [41]. Some other strains such as *L. plantarum* AKU1009 was reported to have the competency of CLA production in two steps reaction. First step demonstrated the hydration of linoleic acid to 10-hydroxy-18:1 and in the second step dehydration of the resulting hydroxyl acid changes to CLA. In the said bacterial strain CLA was molded as *c*9, *t*11 (CLA1) and *t*10, *c*12 (CLA2) isomers [42].

An experiment with LAB and *propionibacteria* strains cultured in a fat milk model supplemented with hydrolyzed soy oil from 24 to 48h performed by Xu [33] in which he noticed the production of CLA as *c*9, *t*11, *c*2 isomer of CLA at different ration [33]. *L. plantarum, L. rhamnosus*, *L. acidophilus, L. casei*, , *E. faceium, predicoccus (Ped.) acidilactici* and yogurt cultures (mixture of *L. delbruekii ssp. bulgaricus* and *Str. Salivarius ssp. thermophiles*, 1:1 ratio) among these strains were testified as increase CLA content, except in *Ped. acidilactici* and *L. rhamnosus* strains. The key isomer found was *c*9, *t*11 followed by *t*10, *c*12 after 24h of incubation except in *E. faecium* which were *t*10, *c*12 they were not reported. Another study done by Lee [44], reported that 5.5 times more CLA can be obtained by *L. reuteri* cells as compared to conversion by free washed cells. Bioconversion ability of CLA in *L. reuteri* at various condition were studied by Hernandez Mendoza [45] and he noticed that at 10 °C for 30h high concentration of CLA was achieved in broth containing 20mg/L free LA aerobically. Among LAB strains the most widely studied for CLA production is *L. plantarum*. Kishino [43] performed a study with various bacterial strains for CLA production in which he noticed that *L. plantarum* AKU1009a exhibited a highest conversion rate up to 85% from LA to *c*9, *t*11-CLA. *L. plantarum* JCM1551 has the capability to accumulate CLA up to a level of 2700 mg/l with ricinoleic acid (12-hydroxycias-9-octadecenoic acid) as a substrate [39].

Yang [46] reported that *L. plantarum* ZS2058 growing culture and washed cells has the efficiency to convert LA into CLA deliberately at the rate of 54.3% and 46.75% respectively. Furthermore, Khosravi [48] noticed that washed cells and substrate concentration, influence of the content of yeast extract and glucose in MRS broth increased the CLA yield ominously in *L. plantarum.* Some studies proposed an optimistic relationship between CLA production and tolerance to LA [50-51] by using various substrate concentration. Though, in some LAB and *bifidobacterium* the efficiency of CLA production is reduced at higher levels of free LA in the medium [49].

In contrast to CLNA production, the only strain which was able to transform ricinoleic acid to CLA (CLA1 and CLA2 was *L. plantarum* AKU1009a [43]. More studies showed that lactobacilli strains have the competency of using α- and γ- linoleic acid as substrate to produce the corresponding conjugated trienoic acids [43] named CALAA and CGLA respectively.

The authors described the production rate of CALA up to 40% under two isomeric forms namely c9, t11, c15-C18:3 (CALA 1, 67% of total CALA) and t9, t11, c15- C18:3 (CALA 2, 33% of total CALA).. In this study a higher rate of CGLA production was noted as a micture isomer, c6, c9, t11 –C18:3 (CGLA 1, 40% of total CGLA) and c6, t9, t11- C18:3 (CGLA 2, 60% of total CGLA). Production of CLNA by other LAB Strains were recently determined [50]. Among these strains *L. sakei* and *L. curvatus* showed a high production rate of 22.4% and 60.1% respectively. The authors demonstrated that isomerization process of LA to CLA and LNA to CLNA is different according to several LAB strains, so as isomer resulting after culturing. Few microorganisms were able to form both conjugated fatty acids both conjugated fatty acids, but mostly convert LNA to CLNA, while the rest were not able to form CLA but efficiently converted LNA to CLNA. The results have been shown in the following table 2.

Table 2. CLA production by LAB strains cultured in the presence of free LA.

Strain	*c*9,*t*11	*t*10,*c*12	Other isomer	LA conversion (%)	Author	Year
L. curvatus	+	+	-	1.6	Gorissen *et al*	2011
L. plantarum	+	+	-	4.6	Gorissen *et al*	2011
					Kishino *et al*	
	+	-	+	N.D	Ogawa *et al*	2002
	+	+	-	N.D	Rodriguez-alcha *et al*	2005
	+	+	-	N.D		2011
					Xu *et al*	2004
	+	+	-	N.D		
L. sakei	+	+	-	4.2	Gorissen *et al*	2011
L. reuteri	+	+		26	Lee *et al*	2003
L. rhmanosus	+	+	-		Lee *et al*	2006
	+	+	-		Ogawa *et al*	2002
	+	-	-	34	Van Nieuwenhove *et al*	2007
L. paracasei	+	-	-	N.D	Lee *et al*	2006
L. pentosus	+	+	-	N.D	Lee *et al*	2006
	+	+	-		Ogawa *et al*	2002
Strep. thermophilus	+	-	-	33	Van Nieuwenhove *et al*	2007
L. brevis	+	+	-	N.D	Ogawa *et al*	2002
L. curvatus	+	+	-	1.6	Gorissen	2011
L. acidophilus	+	-	-	20	Van Nieuwenhove *et al*	2007
			-	N.D	Ogawa *et al*	
	+	+	-	N.D	Xu *et al*	2002
	+	+				2004
L. reutri	N.I	N.I	N.I	26	Lee *et al*	2003
Lact. lactis	+	+	-	N.D	Rodriguez-alcha *et al*	2011

+ positive production, - no production, N.D. not determined, N.I. not informed.

2.4. *Bifidobacterium*

Bifidobacterium are amid the first colonizers of the sterile gastrointestinal tracks (GIT) of newborns [52] and they are also found as inhabitants of human gut. Because of the health promoting properties *bifidobacterium* strains are used as probiotic and their activities are unquestionable [51]. After years of frequent research across different countries in the world various functional foods have been developed by adding *bifidobacterium* to the food matrix [52-54] and that's the reason that it's not astonishing that numerous studies on the capability of these bacterial strains to produce CLA / CLNA have been made for a long time.

For the first time Coakley [57], reported that *Bifidobacteria* species are capable of producing CLA and he also demonstrated a considerable interspecies variation. In his study he screened 15 different strains among them 9 showed very efficient CLA production. The most efficient producers of CLA among different range of evaluated *Bifidobacterium* strains were *Bifidobacterium breve* and *B. dentium*. *B. breve* showed the highest percentage of 65% (c9, t11 – CLA) of LA conversion. .. In this study strains speckled considerably with respect to their tolerance to the linoleic acid concentration in the medium. Some authors demonstrated that strains of *Bifidobacterium breve* and *B. pseudocatenulatum* isolated from human feces, were able to transform CLA in a conversion rate of 69% and 78% correspondingly [58].

Furthermore, Xu [49], in another study reported the production of CLA in *B. bifidum* cultured in skim milk using as substrate hydrolyzed soy oil where they noticed CLA production after 24-48 h only as c9, t11 isomer and traces of t10, c12 form. Another study made by Rodríguez [64] reported the capability of two strains to produce CLA by *B. animalis*, they noticed CLA production from free LA and safflower oil added to MRS broth and skim milk. Strains were able to transform LA to CLA after 24-48 h of incubation. The most imperative isomer produced by this strain was c9, t11 followed by t10, c12. Chung [59], isolated one hundred and fifty strains of *bifidobacteria* strains from human intestines and four isolates among them presented 80% conversion of LA to CLA in MRS broth. *B. breve* LMC017 among these strain was capable of converting 90% of linoleic acid or 78.8% of monolinolein into CLA among [59]. Thirty-six *bifidobacteria* strains were screened to scrutinize them for the capability of producing CLA or CLNA as a substrate of free LA and α-LNA by Gorissen [60] and subsequently four B. breve strains were noticed to transform LA into CLA with a conversion rate ranging from 19.5% to 53.5% > 70% of CLA isomers produced by B. breve were c9, t11 CLA, in addition to that 38% of CLA isomers t9, t11 were produced by B. breve LMG13194. Another high CLA producing strain among various *Bifidobacterium* species was B. longum. The rapid screening of CLA producing *bifidobacterium* was discovered by Barret [61], in this method four *B. longum* strains isolated from feaces were found capable of converting <20% of free LA to CLA. *B. longum* DPC6320 showed 43.89% c9, t11 CLA conversion while *B. longum* DPC6315 was able to convert only 11.02% of free LA into c9, t11 CLA [40]. In another study performed by Gursoy [63], demonstrated that *b. longum* could increase the content of CLA in the cheese by 20.44%. One of the most widely used probiotics *B animalis* Bb12, could transfer 27% of free LA into c9, t11 CLA in MRS broth [59]. The best CLA production with free LA substrate was shown by *B. animalis* while the highest conversion rate of CLA with ricninoleic acid as substrate was shown by *B. animalis* B12-1 [65]. Moreover, *B. dentium* NCFB 2243 was capable of converting 29% of LA into 9,11 CLA [66]. Van Nieuwenhove [49], reported the CLA conversion rate of B. bifidum CRL 1399 up to 24.8% in MRS broth. Gorrisen [63], demonstrated that *B. bifidum* LMG 10645 can produce CLA from LA upto 40.7%. Yang and Rosberg Cody, demonstrated that *B. animalis* sub sp. *Lactis* Bb-12 [67], B. breve NCIMB 702258 [68] could produce 10-HOE during CLA production respectively.

The isomerization process of various fatty acids by *bifidobacterium* strains were reported by Hennessy and his colleagues [39]. Moreover, various PUFA such as stearidonic, araquidonic and docosapentanoic and docosahexanoic acid were supplemented to the culture. An inclusive patron of isomerization was reported on *B. breve* and *B. longun* strains being able to transform LA, α and γ-LNA and steraridonic acid to its conjugated form. It was noticed in *prpoionibacteria* before, the percentage of conversion mottled among strains displaying around 12 to 67% of LA conversion chiefly into c9, t11 and t10, c12 isomer. A-LNA was converted from 0 to 83% among various strains and lower

conversion rate was determined for γ-LNA was 0.5 to 37%. The conjugation of stearidonic acid varied from 3.8 to 27%. *B. breve* DPC6330 was the most efficient conjugated fatty acid producer indicating a bioconversion rate of 70% for LA, 90% of α-LNA, 17% for γ-LNA and 28% for stearidonic acid. In addition to that the capability to isomerize fatty acid was reported in LAB and *propionibacteria*, *bifidobacteria*, they also exhibit a wide range of bioconversion rate.

There are many factors which affect the mechanism of the fatty acid isomerization, such as culture condition and substrate concentration. The production of various isomers ration was assessed for all evaluated strains. To the best of our knowledge, this is the only work reporting the conjugation of stearidonic acid by bacteria. The results are shown in Table. 1.

2.5. *Clostridium*

Several strains of *Clostridium bifermentas*, *C. sporogenes* and *C. sordelli* demonstrated to hydrogenate LA into trans-vaccenic acid *in-vitro* with c9, t11 CLA as intermediate [67]. Peng [68], confirmed that c9, t11 CLA accumulated in *C. sporogenes* ATCC22762 within 30 min and then t9, t11 CLA and t10, t12 CLA increased at the disbursement of c9, t11 CLA until these reached the same level.

2.6. Other C9, t11 CLA producers

Different other strains showed the capability to produce CLA. Lin [69], reported that *Lactococcus lactis subsp. Cermoris* CCRC12586, *L. lactis subsp. Lactis* CCRC 10791 and *S. thermphophilus* CCRC 12257 are able to convert free LA in skim milk plus 12 % free LA. Furthermore, some other *Lactococcus* [70], *Streptococcus* [38] , *Leuconostoc* and *Pediococcus* [38, 71] have shown the ability to produce CLA with different substrates.

3. CFAS AS PROBIOTICS AND FUNCTIONAL FOOD:

Recently CFAs have attracted significant consideration because of its potentially advantageous health and biological promoting properties/ effects on both humans and animal models including anti-tumor, anti-obesity, anti-atherogenic and anti-diabetic properties. The development of healthier food is observing for taking into account to their benefits for humans. Among these, dairy products represent a good alternative to manufacture functional and probiotic foods. Functional food includes processed food or foods fortified with health promoting additives. On the contrary, probiotics are live microorganism where when administered in suitable amounts deliberate a health benefit to host. Many bacteria are informed as probiotic strains during years, while several positive effects on health have been supported [72]. Microbiota present in the intestine plays an important physiological role to the host, modulating some metabolic functions, conferring resistance to microorganism infection and enhancing immune response among other functions.

The bioconversion of LA to CLA and LNA to CLNA by bacteria at intestinal level, marks a novel and interesting topic to be developed with the objective to obtain probiotic food with microorganism able to produce it or functional foods with high levels of CLA or CLNA. The used of CLA or CLNA producing bacteria as probiotics have obtained great attention for nutrition, since many studies showed their benefits for the promotion of human health.

It has been noticed by Cho [73], that the isomer of CLA has different function and according to research t10, c12 more potent than c9, t11 CLA to prevent cancer cell proliferation. This isomer is linked to decrease the body fat in animals [74-76] and humans [76-77]. Previous studies showed informed that CLA content in cheeses varied according to strain used as starter or adjunct culture [69]. Consequently, the inclusion of bacteria able to form it during the fermentation process has got great attention and concern by researchers.

Currently, different functional foods such as yogurt, cheese and fermented milk are manufactured with CLA producing bacteria, gaining a final product with a high CLA content. Cheese prepared with CLA producing bacteria were developed using sunflower oil as exogenous source of LA by Van Niuenhowe [78], reported a modification of fatty acids profile in mice tissues after its administration. Mice fed functional cheese demonstrated a protective effect on feasibility of intestinal cells after treatment of 1,2-dimethylhydrazine drug used as oxidant compound.

Today, CLA production by probiotic bacteria has gained special interest in research field being well understood that bacteria isolated from intestine or fecal samples can form it. However, *in vitro* production was intensely informed, while few studies have established an *in vivo* CLA production after ingestion of bacteria. Some authors revealed that according to the administered strain a high t10, c12 isomer [79-81] or c9, t11 isomer [82] content in animal tissues occurs.

Edionwe and Kies [83], reported that linoleic acid excretion in humans occurs about 340 mg/day, being this fatty acid available for further isomerization process by intestinal microbiota. Nonetheless, this local CLA production was only reported after probiotic treatment, but if CLA amount produced is adequate to exert a preventive effect require better understanding.

Another study done by Hui young [80], confirmed that strains daily administered as probiotic for short term study, formed an increase on CLA systemic content. Lee [84] indicated that consumption of *L.rhamnosus* PL60 (10^7-10^9 CFU/day) during 8 weeks increased t10, c12 isomer content in plasma and tissues of diet induced obese mice. Animal receiving PL60 showed a significant reduction of fat adipose tissue (epididymal and perineal). No liver steatosis was observed in this research, being the most adverse effect informed to t10, c12-CLA. The increasing amount of CLA in tissues after oral treatment with *L. rhamnosus* was explained as an intestinal production once bacterium has been colonized in the intestine. Lower leptin levels in PL60 group were also noticed. Obese mice selection as animal model was maintined by t10, c12 as the main isomer formed by this probiotic strain.

4. CFAS AND FUTURE FOODS

Considering all the previous research done on CFAs showed that conjugate Trienic fatty acids have stronger cytotoxic activity compared with conjugated diene fatty acid, evaluation physiological and biological activities of CFA isomers other than CLA for atherosclerosis, diabetes, allergies and high blood pressure and they would have great interest in future studies. Development of functional foods enriched on conjugated fatty acids is being widely studied by researchers, since benefits of health properties were related to humans. The physiological role of conjugated fatty acids like CLA or CLNA is well documented on the literature. The capability of some species of LAB including *propionibacteria* and *bifidobacteria* to *in-vitro* conjugate the LA or LNA has been made over the years. Manufacturing of functional food enriched in conjugated fatty acids by using it as starter or adjunct culture is a promising topic to be developed and studied more deeply.

The variation on CLA and CLNA production among bacteria be contingent on various factors such as intrinsic characteristic of each particular strain, conditions of experimental design and methodology for isomer determination. For this reason, studies must carefully be done before the inclusion of strain during food manufacturing.

CONCLUSION

Nevertheless, one of the most active method to increase CLA uptake by humans consists of increase CLA levels in milk and dairy products by modification of animal diet or the inclusion of bacteria able to form it during manufacturing process in the last years the *in vivo* CLA production appears as a substitute way to make it.

Meanwhile CLA was documented as a significant bio-lipid with health beneficial properties there was a snowballing interest in this field. Still, there is another conjugated fatty acid recently included in studies, conjugated linoleic acid (CLNA). This fatty acid is also creating great consideration because of its anti-atherogenic properties.

Some bacteria are capable to produce CLNA using as substrate linolenic acid. CLNA isomers in foods and its biological effects in animal models were slighter understandable than CLA, being the mechanism of its production by bacteria recently investigated. Thus in the literature there is not yet any recommended dose for this compound for humans. Roughly few authors have disclosed the action of bacteria intake on *in-vivo* CLA production using experimental animal models and human, but results are promising in this field. Instead of some technological developments have been performed, many points remain undiscovered, such as CLA enriched products are also high in fat, being difficult to recommend a single daily use of CLA after food intake. As we earlier stated, not all isomers are incorporated at in the same way into tissue fat.

Certainly, captivating in account the lack of information and availability of data in respect to some epidemiological and technological aspects of CFAs, further studies are needed to fully understand the utility of CLA and CLNA in disease prevention. The development of products as probiotics or functional foods to ensure the bioavailability of both compounds for humans is an appreciated approach to be deliberated.

DECLARATION OF COMPETING INTERESTS

The authors have no competing interests to declare.

ABBREVIATIONS USED

CFAs, conjugated fatty acids; PUFAs, polyunsaturated fatty acids; CLAs, conjugated linoleic acids; CLNAs, conjugated linolenic acids; CEPAs, conjugated eicosapentaenoic acids; CDHAs, conjugated docosahexaenoic acids.

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