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ORIGINAL ARTICLE

Isolation, molecular characterization and screening of indigenous lactobacilli for their abilities to produce bioactive conjugated linoleic acid (CLA)

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Abstract Ingestion of conjugated linoleic acid poised many health benefits; however, amount of CLA one can get through generalized diet in is inadequate in exerting the desired benefits. Therefore, presence of CLA producing lactobacilli in dairy fermented foods has a tremendous potential to increase the CLA content. Therefore, present study was focused to isolate and characterize CLA producing lactobacilli from different dairy products and human faeces. Arguably, 283 lactobacilli were isolated from various sources and tested for CLA production. Fifty-seven CLA producing (≥20 µg/ml) lactobacilli were selected from screening in de Man, Rogosa and Sharpe (MRS) broth and reconstituted with skim milk (SM), supplemented with 0.5 mg/ml of linoleic acid. Positive strains were classified into—*L. plantarum* (44%), *L. gasseri* (30%), *L. fermentum* (21%) and *L. salivarius* (5%) species. Nineteen most efficient strains (CLA ≥25 µg/ml) were further assessed in SM for CLA production. Total 08 strains produced significantly higher CLA in SM than MRS and also produced *cis* 9, *trans* 11, *trans* 10, *cis* 12 and *trans* 9, *trans* 11 isomers. Overall, *L. plantarum* HIF15 was reported as the best producer of CLA and other 08 lactobacilli may be utilized for the formulation of CLA-enriched functional foods to support these bacteria to synthesize CLA in the human gut.

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Introduction

The generic term "conjugated linoleic acids (CLA)" is defined as a set of positional and geometric (*cis* or *trans*) isomers of linoleic acid (LA, C18:2), with conjugated bonds. CLA is synthesized as an intermediate product from dietary fats in ruminants through biohydrogenation activity from two important microorganisms namely, *Butyrivibrio fibrisolvens* and *Megasphaera elsdenii* (Jenkins et al. 2008), and Δ-9 desaturase activity in ruminant' mammary glands. Of the 24 well characterized CLA isomers; *cis* 9, *trans* 11, *trans* 10, *cis* 12 and *trans* 9, *trans* 11 are of huge significance due to their reported health benefits in humans (Kim et al. 2016).

These isomers were reported to have anti-inflammatory (*c9*, *t*11), anti-obesity (*t*10, *c*12) and anti-cancerous (*t*9, *t*11) activities when supplemented to laboratory animals. Regardless of all the recognized clinical functions of CLA the fundamental mechanism is still obscured.

The daily recommended dosage (1–3 g/day) of CLA was established for humans attain the health benefits (MacDonald 2000). However, a normal diet (36–440 mg/day) is far lower for desired beneficial effects (Nunes and Torres 2010), and human system is totally inefficient to synthesize them de novo (Chung et al. 2008).

It has been reported that ruminants derived products especially milk and meat are considered as the richest source of CLA in the human diet (Herman-Lara et al. 2012). However, the concentration of CLA present in foodstuffs is lower and depends upon feedstock and animal breed (Sosa-Castañeda et al. 2015). Besides, in a majority

of these products *c*9, *t*11 isomer accounts for >70% of total CLA (Lock and Bauman 2004). Thus, looking for the safe alternative approaches to enhance the CLA content in food are desirable and is of great interest to mankind for better health.

Commercial production of CLA by alkaline isomerisation is quite expensive and yields in the production of undesirable isomers with undisclosed functions (Zheng et al. 2003). In contrary, lactic acid bacteria (LAB) fermentation produces specific CLA isomers, and some of these LABs poised other probiotic advantages due to their generally recognized as safe (GRAS) status. Previous studies have shown that strains of LAB have the abilities to synthesize CLA under in vitro and in vivo conditions (Andrade et al. 2012). Other independent studies by Taboada et al. (2015) and Özer et al. (2016) found that some strains have the ability to produce CLA in cheese products. Therefore, microbial CLA uptake in humans is visualized as the most purposeful strategy. CLA producers have the ability to crosslink with adipocytes cell lines in vitro and gut epithelial cells in human and animal model (Sosa-Castañeda et al. 2015). Moreover, in an in vitro study Dahiya and Puniya (2015) reported that CLA producing strains had good probiotic-to-functional attributes. Thus, it is significant to examine the fermented food formulations at the gut level.

There is very few reports and scanty information available on indigenous lactobacilli of Indian origin; therefore, it is utmost important to hunt for novel CLA producers. Besides those extensive studies on the production of *t*10, *c*12 and *t*9, *t*11 CLA isomers from lactobacilli are also scanty.

Current study aimed to search for CLA producing lactobacilli, from dairy products and healthy human feces. The study focused on lactobacilli for CLA production as lactobacilli are the predominant allochthonous microflora of human gut and easier to handle than other known CLA producers.

Materials and methods

Sample collection

Conventional prepared fermented dairy products (Dahi and Lassi) were collected from the local market of Karnal, Haryana, India. The human breast-milk and faecal samples were collected from civil hospital, paediatric hospitals and private nursing homes of Karnal, Panipat, Sonipat and Delhi regions. In all, the study evaluated 183 faecal samples (57 adults and 126 from infants, 0–6 months of age). All faecal samples were obtained from healthier volunteers and babies with consent from their parents. In addition, 39 dairy products, 31 breast milk, 11 National Collection of Dairy Cultures (NCDC, ICAR-NDRI, Karnal, India) and two Kimchi samples were also used for isolation of bacterial strains. Samples were homogenized before used in study.

Isolation of lactobacilli

One gram or millilitre of each sample was suspended in a modified deMan, Rogosa and Sharpe (MRS) broth, containing 0.5 mg/ml of LA substrate and incubated at 37 °C for 24 h to enrich the suspension with LA tolerable lactobacilli. From the enriched broth, 1 ml of sample was added to 9 ml of BCP–MRS broth, serially diluted in 0.1% peptone water, and subsequently plated on BCP–MRS agar, and incubated at 37 °C for 48–72 h. Characteristic yellow coloured colonies were picked up from higher dilution MRS agar plates and transferred to MRS broth. Next, the isolates were streaked on MRS agar plates for further purification. The purity of cultures was examined microscopically after performing Gram staining. Apparent lactobacilli were confirmed through PCR and pure cultures were preserved at −80 °C in glycerol stocks. For routine experiments the cultures were maintained in chalk litmus milk at 4 °C and sub-cultured twice prior to use.

Lactobacilli identification

Genomic DNA extraction

A single colony from MRS plate was suspended in 2 ml of MRS broth and incubated at 37 °C for 16–18 h to attain turbidity. Thereafter, DNA extraction method of Pospiech and Neumann (1995) was followed with minor modifications. Briefly, the bacterial suspension was transferred to a 2 ml microcentrifuge tube and centrifuged at 14,000×*g* for 10 min. The pellets were washed twice with Milli Q water. Cell disruption was carried out in a mini bead beater (BioSpec; impulses 5, time 30 s, 2 min incubation on ice between intermittent cycles) with the aid of glass beads (212–300 µm) in SET buffer. Subsequently, DNA was eluted, precipitated and dissolved in TE buffer (pH 8.0). The purity and concentration of DNA was assessed in a Nano drop plate reader (Tecan-Infinite Pro 200, Switzerland) and thereafter, stored at −20 °C until use.

Molecular characterization

For identification of lactobacilli species the primers and PCR conditions of Dubernet et al. (2002) and Song et al. (2000) were used (Table 1). A 25 µl PCR reaction was prepared by adding 12.5 µl of 2× master mix green (Fermentas, Lithuania), 0.3 µl of each primer, 10.9 µl of

Table 1 Primer used for identification of lactobacilli genus and different species

Primer pair	Primer sequences (5′–3′)	Annealing temperature (°C)	Amplicon length
LbLMA-1	CTCAAAACTAAACAAAGTTTC	55	250
R-161	CTTGTACACACCGCCCGTCA		
Lpla-3	ATTCATAGTCTAGTT GGAGGT	60	248
Lpla-2	CCTGAACTGAGAGAATTTGA		
Lfer-3	ACTAACTTGACTGATCTACGA	60	192
Lfer-4	TTCACTGCTCAAGTAATCATC		
Lsal-1	AATCGCTAAACTCATAACCT	60	411
Lsal-2	CACTCTCTTTGGCTAATCTT		
Lgas-3	AGCGACCGAGAAGAGAGAGA	55	360
Lgas-2	TGCTATCGCTTCAAGTGCTT		

nucleases-free water and 1 µl of genomic DNA. Thermocycles were performed in Veriti thermocycler (Invitrogen Inc.) with initial hold at 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, annealing (as mentioned in Table 1) for 30 s and a 72 °C extension for 30 s. The final extension was performed at 72 °C for 7 min. The amplified PCR products were confirmed in agarose gel (1.8%, w/v, 1× TBE buffer) electrophoresis.

UV-based spectrophotometric screening for CLA production

PCR confirmed lactobacilli were further characterized for CLA biosynthesis in MRS broth using the UV-based spectrophotometric method. Stock solution of LA (30 mg/ml, 99% purity; Sigma, St. Louis, MO, USA) was prepared in sterile distilled water with 2% (w/v) Tween-80 (Himedia, Mumbai, India) and sterilized through a 0.20 µm syringe filter. For screeningthe cultures (OD_{595} adjusted to ~3.0 nm) were inoculated @ 1% (v/v) to 10 ml MRS broth supplemented with 0.05% L-cys-Hcl supplemented with 0.5 mg/ml of LA as a substrate in 50 ml glass serum bottles. The samples were incubated at 37 °C for 48 h. Subsequently the lactobacilli were tested for the production of CLA in accordance to Barrett et al. (2007). Briefly, the samples were centrifuged at 13,000×*g*/4 °C for 5 min, the supernatant (1 ml) was vigorously mixed with 2 ml of isopropanol and left undisturbed for 3 min. To this, 1.5 ml of hexane was added for extraction of fatty acids and remained undisturbed for 3 min. An aliquot 230 µl was taken for absorbance at 233 nm in a microplate reader (Tecan-Infinite Pro 200, Switzerland). A standard curve (20–160 µg/ml) was prepared from reference *t*10, *c*12 CLA isomer to quantify total CLA. Hexane layers containing only LA were used as control. The initial selection of lactobacilli was based on CLA production and only positive strains (>20 µg/ml CLA production) were kept for further experiments. As dairy products are suitable and economical for probiotics delivery, therefore selected lactobacilli from previous experiment were tested in reconstituted skim milk (hereafter, SM) (12% w/v, containing lactose ~51%, fat ~1.0%, protein ~35%, ash ~8.20% approximately) supplemented with readily available growth promoters; 10 mg/ml yeast extracts (Hennessy et al. 2009) and 0.3% glucose (Kim and Liu 2002). Before inoculation of fresh cultures @1% to SM the medium was autoclaved at 110 °C for 10 min.

Fatty acid methyl ester (FAME) synthesis and gas chromatography (GC) analysis

Quantification of CLA isomers in LA–MRS and LA–SM were performed using gas chromatography. FAME was prepared from the fermentation medium, by following direct synthesis method of O'Fallon et al. (2007) with fewer modifications. The hexane layers containing fatty acids were dried under a stream of nitrogen and redissolved in 1 ml of hexane. The samples were stored in glass vials at −20 °C until analysed in GC.

Two microlitre sample (FAME) was injected into a fully automated GC-2010 GC machine (Shimadzu Corp, Japan) equipped with SP-2560 capillary column (100 m × 0.25 mm I.D., 0.20 µm film thickness, Supelco, USA), an automated injector (Aoc-20i) and a flame ionization detector in (1:10) split mode using hydrogen as a carrier gas. The temperatures of injector and detector were set at 270 and 280 °C, respectively. The temperature of column oven was programmed from 140 to 240 °C with step increase of 4 °C/min. The qualitative analysis of CLA isomers were performed by comparison of retention times (RTs) with methylated CLA standards (*c*9, *t*11, *t*10, *c*12 and *t*9, *t*11). For quantification, standard curves were plotted against concentrations (0–1000 µg/ml) and expressed as µg/ml.

Scanning electron microscopy (SEM)

Notably, specifically highest CLA producer was examined by SEM to analyses spore and capsule formation. The

culture was first fixed with a solution of 2.5% gluteraldehyde solution, washed with phosphate buffer saline, and again re-fixed with 1.0% osmium tetraoxide. Next, the sample was serially dehydrated in ethanol series for fixed durations. Finally, the sample was placed on a stubber, gold-coated and examined under scanning electron microscope (Zeiss, UK).

Statistical analysis

All samples were analyzed in triplicates and data are presented as mean ± SD. GraphPad prism software (ver.5.0, CA, USA) was used to perform one-way ANOVA with Turkey's test to evaluate a significance level of $P < 0.05$.

Result and discussion

Sample collection and lactobacilli identification

The selective media, BCP–MRS broth helped in isolation of LA tolerable lactobacilli. Consequently, 390 distinct colonies were picked from different samples. No colony was obtained from breast milk samples and that might be primarily due to the antimicrobial effect of colostrums and infusion of antibiotics during the maternity period. Microscopically, only 311 isolate were found Gram positive and of these, 283 were confirmed as lactobacilli through PCR analysis.

UV-based spectrophotometric screening for CLA production

There is a concomitant interest in the industrial demand for multifaceted lactobacilli that in addition to increasing the CLA content of foods, could further improve health (Andrade et al. 2012; Gorissen et al. 2013; Lee et al. 2006). Now with the availability of rapid UV screening methods, it becomes easier to screen high CLA producer and their further CLA isomers characterization by GC analysis. Therefore, large array of 283 lactobacilli strain can be screened for CLA biosynthesis. Only 57 of the 283 lactobacilli were able to produce CLA ≥20 μg/ml. CLA production among these lactobacilli ranged from 19.5 to 71.5 μg/ml (Fig. 1). Here, strain HIF15 reported as the highest CLA producer (71.5 μg/ml) in LA-MRS broth. Our findings are in consistent with previous reports of CLA production from different LAB strains (Andrade et al. 2012; Gorissen et al. 2013). Although, the exact mechanism for CLA production is still not clear and needs a thorough investigation on mechanistic aspects. But it has been suggested that a LA detoxifying mechanism works behind it. Incorporation of LA into bacterial cell membrane changes the lipid bilayer chemistry, membrane potential and even intramembrane pathways (Sosa-Castañeda et al. 2015); therefore for survival bacteria could have to detoxify the LA.

Our major concern was to identify the high *t*10, *c*12 and *t*9, *t*11 CLA producer in addition to *c*9, *t*11 isomer. As earlier stated CLA isomer-*c*9, *t*11 accounts >70% of the total CLA produced, and these other isomers were produced in little amounts. The LA–MRS screening revealed that only 19 lactobacilli produced CLA ≥25 μg/ml (Fig. 2) and therefore, assessed in LA-SM medium. Strikingly, culturing in SM significantly ($P < 0.005$) enhanced CLA production abilities of 08 lactobacilli strains viz. HIF15, HIF27, HIF64, HIF70, HIF77, HIF221, HAF13 and HAF28 in comparison to LA-MRS broth with similar substrate (0.5 mg/ml) concentration (Fig. 2).

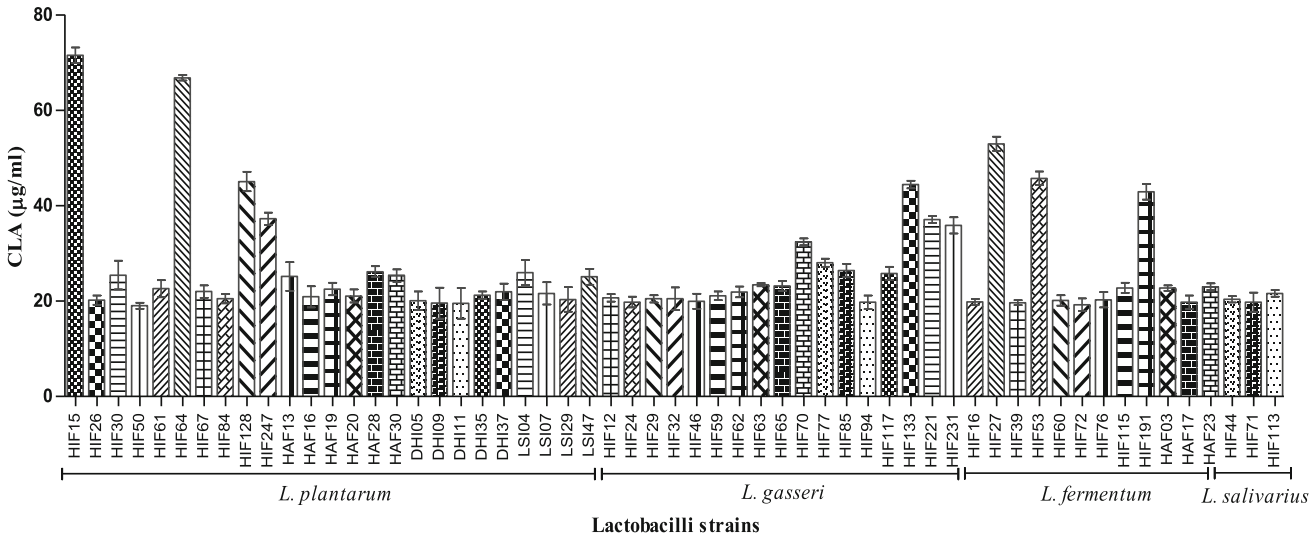

Fig. 1 CLA productions from different lactobacilli isolates on the basis of UV-based spectrophotometeric method

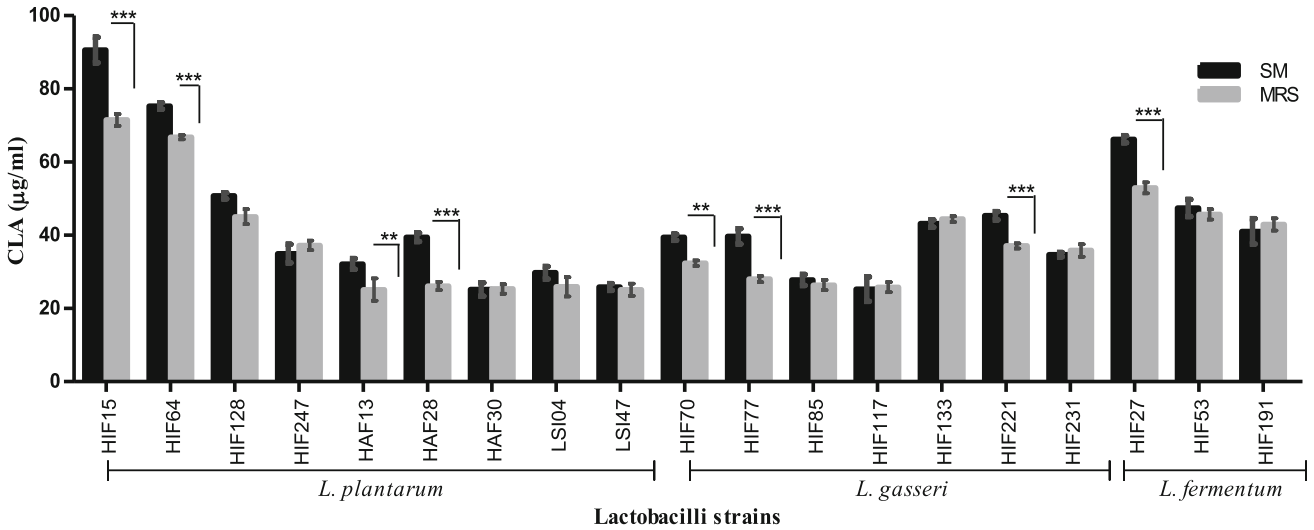

Fig. 2 Comparison of CLA production in MRS and SM medium by different lactobacilli. *HIF* human infant feces, *HAF* human adult feces, *LSI* Lassi, *DHI* Dahi

Kim and Liu (2002) and Van Nieuwenhove et al. (2007) reported similar observations for addition of non-fat dry milk powder into the fermentation medium. The proposed mechanism states that SM prevents CLA oxidation in addition to support better growth (Shantha and Decker 1993) and some of the milk proteins (α- and β-lactoglobulin) shielded the bacteria from LA toxicity effect.

The frequency distribution chart (Fig. 3) showed that more numbers of CLA producers were obtained from infant feces than adults, followed by dairy products. This variation in CLA production among LAB is well documented in literature. Barrett et al. (2007) tested 18 human feces bifidobacterial strains for CLA biosynthesis with variation (2.60–76.65%) in total CLA production. Chung et al. (2008) characterized 04 bifidobacterial strains with high (>80%) LA conversion potential from a pool of 150. Li et al. (2012) targeted 06 *L. plantarum* isolates with minor variations (3.85–4.90%) from traditional dairy origin. The results strongly support the facts that different strains of lactobacilli have the varying ability to produce CLA. In our case, higher production was observed for the faecal originated lactobacilli. However, it is not yet clear, how the bacteria origin determines different LA metabolism.

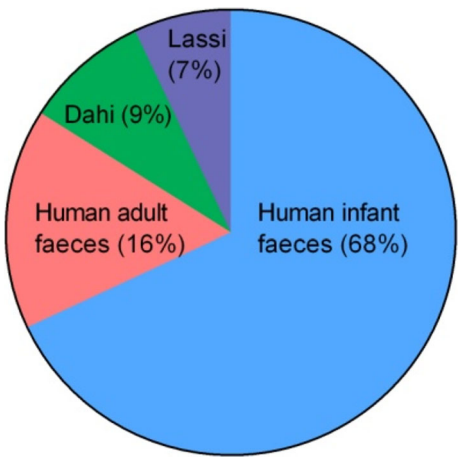

Fig. 3 Percent (%) frequency of CLA producing lactobacilli from different samples

Lactobacilli species characterization

All 57 lactobacilli were characterized for species identification (Fig. 4). Of these, 44% correspond to *L. plantarum*, *L. gasseri* (30%), *L. fermentum* (21%) and *L. salivarius* (5%). Earlier, several different CLA producing species of lactobacilli (*L. plantarum*, *L. acidophilus*, *L. casei* and *L. fermentum*) obtained from different sources were reported by several authors (Andrade et al. 2012; Puniya et al. 2008; Ando et al. 2003; Lee et al. 2007). Our results, in respect to CLA production by *L. plantarum* and *L. fermentum* are in complete with earlier findings; however, the current study indicated a major proportion of *L. gasseri* and few *L. salivarius* strains also as CLA producers. In the present study, characterization of *L. gasseri* and *L. fermentum* species as major CLA producer in addition to *L. plantarum* is might be due to involvement of more faecal samples, as up to 2 months of age *L. gasseri* (30%) was the most common lactobacilli isolated from infant feces followed by *L. fermentum* (9%) (Rubio et al. 2014).

CLA isomers analysis by GC

As the biological effects of CLA are isomers specific, thus biosynthesis must be considered prior to preparing a

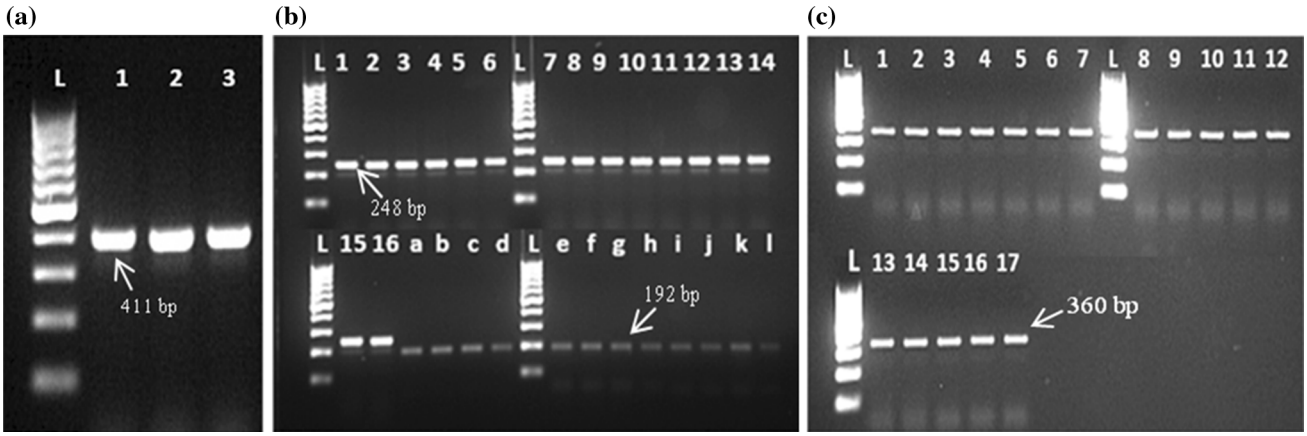

Fig. 4 PCR-amplified products of four lactobacilli species (*L*, in all representative gels from **a** to **c**, represents a 100 bp molecular size marker. In gel **a** *lanes 1–3* represent *L. salivarius* isolates by an amplicon of 411 bp, **b** *lanes 1–16* represent *L. plantarum* by 248 bp, *a–l L. fermentum* by 192 bp, **c** *lanes 1–17* indicated *L. gasseri* isolates by PCR product of 360 bp

functional food. All 19 lactobacilli having CLA production ≥25 μg/ml in UV screening were analysed for specific-isomers production in LA–MRS and LA–SM. Notably, significant differences in production profiles were obtained in both the fermentation medium (Table 2). Eight lactobacilli strains (HIF15, HIF27, HIF53, HIF64, HIF128, HIF133, HIF191 and HIF221) have shown the ability to produce *c*9, *t*11, *t*10, *c*12, and *t*9, t11 CLA isomers in both the mediums and thus considered as potential strains (Table 2, a representative chromatograph is presented in Fig. 5a, b). In all 19 lactobacilli *c*9, *t*11 isomer were reported as the most predominant isomer in LA–MRS and LA–SM mediums. These findings are in complete agreement with previous findings of isomer variability (Sosa-Castañeda et al. 2015; Andrade et al. 2012; Puniya et al. 2008).

Strains HIF85, HIF117, HAF13, LSI04 and LSI47 did not show the production of *t*10, *c*12 and *t*9, *t*11 isomers. On the contrary, in some strains (HIF70, HIF231, HIF247 and HAF28) we only detected the presence of *c*9, *t*11 and *t*9, *t*11 isomers and no *t*10, *c*12 isomer production. From literature we understood that the biosynthesis of *t*9, *t*11 CLA isomer by lactobacilli was a further biotransformation consequence of *c*9, *t*11 CLA (Hennessy et al. 2012). Our results emphasize that production of CLA and isomer is a highly species and strain dependent phenomenon. Lin et al. (2003) detected eight different CLA isomers (*t*8, *t*10, *t*9, *t*11, *t*10, *t*12, *t*11, *t*13, *t*8, *c*10, *c*9, *t*11, *t*10, *c*12 and *c*11, *t*13) with the enzyme extract of *L. acidophilus* CCRC 14079 with LA. Lee et al. (2007) isolated and identified an *L. plantarum* PL62 from the infant faces that have *c*9, *t*11 (26.8 μg/ml) and *t*10, *c*12 (6.4 μg/ml) CLA isomers producing potential. Ando et al. (2003) optimized *L. plantarum* JCM 1551 that produced 2.4 mg/ml of CLA and was mainly comprised of *c*9, *t*11 (21% of total CLA) and *t*9, *t*11 (79% of total CLA) CLA isomers. Similarly, Li et al. (2012) assayed the CLA production of 06 lactobacilli by employing different substrates. Recently, Terán et al. (2015) examined 64 food-grade lactobacilli for CLA isomers production and revealed that only 04 *L. plantarum* strains were able to synthesize CLA isomers from LA which is lower than the 03 *L. plantarum* strains (HIF15, HIF64, HIF128) reported in present study. In another recent study Sosa-Castañeda et al. (2015) assessed the CLA production abilities of 13 *Lactobacillus* strains out of which strain *L. fermentum* J20 produced more *c*9, *t*11 (42.63 ± 0.91 μg/ml) and *t*10, *c*12 (8.27 ± 0.64 μg/ml) CLA isomers then reported in present investigation. By contrary, the production of *c*9, *t*11(7.73 ± 0.52 μg/ml) isomer by another *L. fermentum* strain J23 is lower while that of *t*10, *c*12 (11.25 ± 0.51 μg/ml) is higher than *L. fermentum* strains reported here.

Ogawa et al. (2001) suggested that the biotransformation of LA into CLA isomers is an isomerisation effect of linoleate isomerase (LI) enzyme. Similarly, Kishino et al. (2011) affirmed that a multi-component enzymatic system encoded in the lactobacilli genome was responsible for biohydrogenation activity. Thus, the reported lactobacilli in the present study might have produced these isomers via the LI enzymatic activity. The variability noticed in CLA production might be due to varying ability of strains to synthesize the LI enzyme (Farmani et al. 2010). Furthermore, the variation in production of CLA isomers might be due to presence of different isomeric forms of LI enzyme within the strains (Farmani et al. 2010).

Overall, strain HIF15 was found as the most efficient CLA producer in terms of bioactive isomers and total CLA production. Moreover, detailed SEM examined (Fig. 6) revealed that this strain (HIF15) was a non-spore and non-capsule forming strain. However, future studies will be required to exploit the potential health effects of these potential strains in suitable animal models.

Table 2 GC analysis of CLA isomers from lactobacilli in MRS and skim milk medium

Linoleic acid	Concentration (0.5 mg/ml)							
Isolate (s)	CLA isomers (μg/ml)							
	MRS				Skim milk			
	*c*9, *t*11 CLA	*t*10, *c*12 CLA	*t*9, *t*11 CLA	Total CLA	*c*9, *t*11 CLA	*t*10, *c*12 CLA	*t*9, *t*11 CLA	Total CLA
L. plantarum HIF15	34.73 ± 1.58^{a}	5.29 ± 0.02^{a}	6.16 ± 0.09^{acdf}	46.18 ± 1.64^{a}	38.31 ± 0.81^{a}	5.42 ± 0.05^{ad}	8.88 ± 1.04^{a}	52.61 ± 0.93^{a}
L. plantarum HIF64	30.98 ± 1.39^{e}	5.22 ± 0.04^{a}	6.17 ± 0.12^{df}	42.37 ± 1.49^{d}	32.11 ± 3.83^{eh}	5.44 ± 0.06^{d}	7.57 ± 0.53^{e}	45.12 ± 4.34^{e}
L. plantarum HIF128	25.95 ± 3.05^{b}	4.98 ± 0.03^{c}	6.59 ± 0.07^{b}	37.52 ± 3.01^{b}	21.11 ± 0.33^{cf}	5.03 ± 0.03^{bcefg}	$6.30 \pm 0.13^{cdfgijlmno}$	32.44 ± 0.45^{cdf}
L. plantarum HIF247	$13.00 \pm 0.20^{ijkmnopqst}$	N.D.	5.36 ± 0.04^{hijklm}	18.36 ± 0.24^{hjnos}	$13.27 \pm 0.90^{iklmnoqrst}$	N.D.	$5.60 \pm 0.23^{ghijklmno}$	18.87 ± 1.03^{iklopqt}
L. plantarum HAF13	$11.49 \pm 0.33^{jklmnoqst}$	N.D.	N.D.	11.49 ± 0.33^{ilmpqs}	$13.27 \pm 0.70^{jklmnoqrst}$	N.D.	N.D.	13.27 ± 0.70^{jmnqr}
L. plantarum HAF28	11.25 ± 0.24^{klmnoqt}	N.D.	5.44 ± 0.13^{ijklm}	16.69 ± 0.36^{jns}	$13.69 \pm 0.36^{klmnoqrst}$	N.D.	5.72 ± 0.04^{jlmno}	$19.41 \pm 0.39^{klmnoqrst}$
L. plantarum HAF30	09.80 ± 0.30^{lmnoqt}	N.D.	N.D.	09.80 ± 0.30^{klmp}	$13.99 \pm 0.72^{lmnopqrst}$	N.D.	5.42 ± 0.14^{klmno}	$19.41 \pm 0.74^{lmnopqrst}$
L. plantarum LSI04	10.24 ± 0.25^{noqt}	N.D.	N.D.	10.24 ± 0.25^{mpq}	13.29 ± 0.42^{noqrst}	N.D.	N.D.	13.29 ± 0.42^{noqrst}
L. plantarum LSI47	12.95 ± 0.35^{rst}	N.D.	N.D.	12.95 ± 0.35^{q}	13.93 ± 0.17^{rt}	N.D.	N.D.	13.93 ± 0.17^{rt}
L. fermentum HIF27	33.14 ± 1.13^{ae}	5.07 ± 0.04^{b}	6.24 ± 0.09^{adf}	44.45 ± 1.22^{ad}	31.03 ± 1.09^{be}	5.25 ± 0.06^{abde}	7.39 ± 0.08^{bef}	43.67 ± 1.10^{be}
L. fermentum HIF53	21.37 ± 0.65^{cfh}	4.88 ± 0.01^{de}	5.88 ± 0.05^{cdefgkl}	32.13 ± 0.66^{c}	18.79 ± 0.98^{dfhpq}	4.98 ± 0.05^{cefg}	$6.25 \pm 0.11^{dfgijlmno}$	30.02 ± 1.1^{dfh}
L. ferm entum HIF191	19.57 ± 0.42^{fgh}	4.88 ± 0.02^{e}	5.78 ± 0.16^{efgkl}	30.23 ± 0.51^{eg}	18.49 ± 0.67^{fhpq}	5.04 ± 0.19^{efg}	6.76 ± 0.39^{fm}	30.29 ± 1.19^{f}
L. gasseri HIF70	15.50 ± 0.33^{ps}	N.D.	5.73 ± 0.05^{klm}	21.23 ± 0.29^{o}	17.32 ± 1.78^{pqr}	N.D.	6.05 ± 0.32^{mno}	23.37 ± 2.08^{ps}
L. gasseri HIF77	10.80 ± 0.06^{oqt}	N.D.	5.37 ± 0.06^{jlm}	16.17 ± 0.11^{ns}	14.38 ± 0.44^{opqrst}	N.D.	5.88 ± 0.111^{mno}	20.26 ± 0.50^{opst}
L. gasseri HIF85	11.72 ± 0.06^{qst}	N.D.	N.D.	11.72 ± 0.06^{pq}	16.16 ± 0.44^{qrst}	N.D.	N.D.	16.16 ± 0.44^{qrt}
L. gasseri HIF117	11.12 ± 0.54^{mnoqt}	N.D.	N.D.	11.12 ± 0.54^{lmpq}	12.63 ± 0.30^{mnorst}	N.D.	N.D.	12.63 ± 0.30^{mnqr}
L. gasseri HIF133	19.43 ± 0.27^{h}	3.82 ± 0.02^{f}	5.65 ± 0.22^{ghijklm}	28.9 ± 0.06^{g}	$15.91 \pm 0.37^{hiklmnopqrst}$	$4.81 \pm 0.09f^{g}$	5.52 ± 0.19^{hijklmno}	26.24 ± 0.59^{hps}
L. gasseri HIF221	14.28 ± 0.38^{st}	4.79 ± 0.02^{g}	5.66 ± 0.05^{lm}	24.73 ± 0.35^{r}	13.27 ± 0.47st	4.91 ± 0.05^{g}	5.82 ± 0.30^{no}	24.0 ± 0.73^{s}
L. gasseri HIF231	18.36 ± 0.61^{ghp}	N.D.	5.95 ± 0.12^{fgkl}	24.31 ± 0.50^{fr}	$16.28 \pm 0.51^{ghiklnopqrst}$	N.D.	$5.90 \pm 0.22^{ghijklmno}$	$22.18 \pm 0.37^{giklopst}$

N.D. values not determined after adjusting from blank. Data were calculated as mean ± SD of three values

HIF isolates obtained from human infant feces, *HAF* human adult feces, *LSI* Lassi, *DHI* Dahi

[abcd] Same column bearing different superscripts differ significantly ($P < 0.05$)

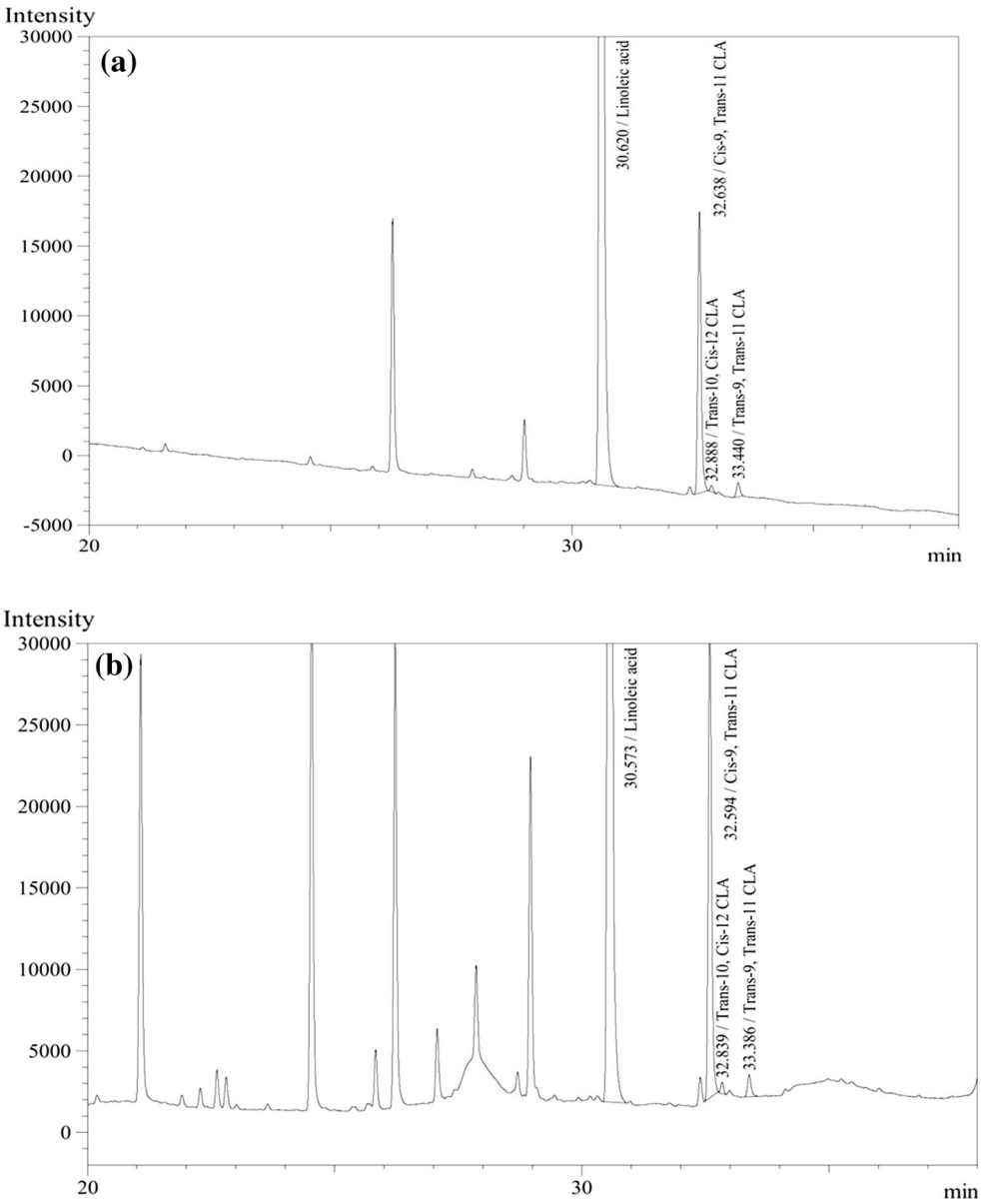

Fig. 5 Gas chromatograms of *L. plantarum* HIF15 in **a** MRS broth and **b** reconstituted SM supplemented with 0.5 mg/ml of free LA, after 48 h of incubation. *c*9, *t*11, *t*10, *c*12 and *t*9, *t*11 CLA isomers peak (RTs) were observed after 32.63, 32.86 and 33.40 min time intervals, respectively

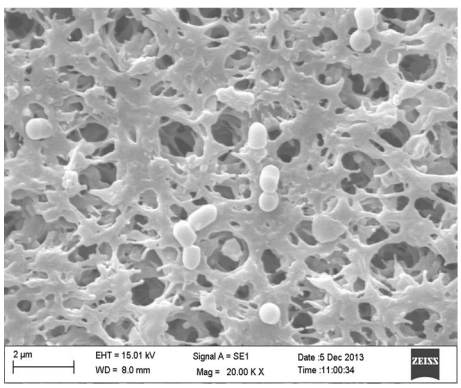

Fig. 6 Scanning electron micrograph (SEM) of high CLA producer *L. plantarum* HIF15

Conclusion

The study suggests that different species of lactobacilli from human faces and dairy samples of Indian origin have the potential to produce bioactive isomers of CLA in a highly species and strain-dependent manner. Three lactobacilli species (*L. plantarum*, *L. fermentum* and *L. gasseri*) were reported as high CLA producer. From here strain HIF15 could be utilized for the genesis of newer CLA-enriched functional foods or as probiotics to promote the continuous synthesis of these bioactive isomers at in situ in the human gut. This will not only boost the dairy industry but simultaneously satisfying the consumers' need for functional foods. However, further studies are required to validate these finding in suitable animal models for

potential health effects in vivo. Furthermore, studies are warranted to elucidate the molecular mechanism and enzyme network of CLA biosynthesis and production.

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