Food Sci Biotechnol (2017) 26(6):1595–1611
https://doi.org/10.1007/s10068-017-0218-8

Bioconversion of conjugated linoleic acid by *Lactobacillus plantarum* CGMCC8198 supplemented with *Acer truncatum bunge* seeds oil

Dong-Ju Chen[1] · Li-Hua Yan[1] · Qian Li[1] · Cai-jiao Zhang[1] · Chuan-Ling Si[2,3] · Zhong-Yuan Li[1] · Ya-Jian Song[1] · Hao Zhou[1] · Tong-Cun Zhang[1] · Xue-Gang Luo[1]

Received: 21 December 2016 / Revised: 2 May 2017 / Accepted: 21 July 2017 / Published online: 12 December 2017

Abstract Conjugated linoleic acid (CLA) isomers, *c*9, *t*11-CLA and *t*10, *c*12-CLA, have been proved to exhibit excellent biomedical properties for potential use in anti-cancer applications and in reducing obesity. *Acer truncatum Bunge* (*ATB*), which is rich in unsaturated fatty acids, including oleic acid, linoleic acid, and nervonic acid, is a new resource for edible oil. In the present study, we developed a new method for producing two CLA isomers from *ATB*-seed oil by fermentation using *Lactobacillus plantarum* CGMCC8198 (*LP*8198), a novel probiotics strain. Polymerase chain reaction results showed that there was a conserved linoleate isomerase (LIase) gene in *LP*8198, and its transcription could be induced by *ATB*-seed oil. Analyses by gas chromatography–mass spectrometry showed that the concentration of *c*9, *t*11-CLA and *t*10, *c*12-CLA in *ATB*-seed oil could be increased by about 9- and 2.25-fold, respectively, after being fermented by *LP*8198.

Keywords Conjugated linoleic acid · *Acer truncatum Bunge* · *Lactobacillus plantarum* · Linoleate isomerase

✉ Xue-Gang Luo
luoxuegang@hotmail.com

1 Key Laboratory of Industrial Fermentation Microbiology of the Ministry of Education, Tianjin Key Laboratory of Industrial Microbiology, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, People's Republic of China

2 Tianjin Key Laboratory of Pulp and Paper, Tianjin University of Science and Technology, Tianjin 300457, People's Republic of China

3 State Key Laboratory of Tree Genetics and Breeding, Chinese Academy of Forestry, Beijing 100091, People's Republic of China

Introduction

Conjugated linoleic acid (CLA) is defined as a naturally occurring group of conjugated diene acid isomers derived from linoleic acid, and the most common positional and geometric isomers are those with conjugated double bonds at C10 and C12 or at C9 and C11 [1, 2]. In 1987, CLA was firstly isolated and identified in fried ground beef [3]. In all of the possible *cis* and *trans* combinations of CLAs, *c*9*, t*11-CLA and *t*10*, c*12-CLA have been implicated as the most valuable isomers with noteworthy biological activities such as anti-carcinogenic, anti-obese, anti-diabetic and anti-hypertensive activities [4]. For example, it has been reported that *c*9*, t*11-CLA could inhibit the proliferation of estrogen receptor positive breast cancer cells by hormone-mediated mitogenic pathways, and *t*10*, c*12-CLA could ameliorate disorders of glucose and lipid metabolism through PPARγ and some other signal pathways [5–8]. Therefore, how to elevate the production of *c*9*, t*11-CLA and *t*10*, c*12-CLA has become a hot spot.

Acer truncatum Bunge (*ATB*) seed oil was approved as a New Resource Food by the National Health and Family Planning Commission of the People's Republic of China in 2011, and this novel edible oil is richer with oleic acid, linoleic acid, and nervonic acid than other edible oils including rapeseed, peanut, grape and sunflower oils [9]. In addition, it was reported that the *ATB* extract might reduce weight and inhibit tumor cell proliferation by inhibiting fatty acid synthesis [10].

In this study, a conserved linoleate isomerase (LIase) gene in *Lactobacillus plantarum* CGMCC8198 (*LP*8198), a novel probiotics strain isolated in our previous study [11], was identified and analyzed. Subsequently, the effect of *ATB*-seed oil on the transcription of this LIase gene was examined via RT–PCR, and the bioconversion of *c*9, *t*11-

CLA and *t*10, *c*12-CLA in the fermentation of *LP*8198 supplemented with *ATB*-seed oil was finally detected by gas chromatography–mass spectrometry (GC–MS).

Materials and methods

Plant materials, strains, media, and growth conditions

ATB seeds were obtained from Jindao Seed Company in Yangling, Shaanxi province, in October 2013, and the seeds were stored at − 80 °C until further use. The strain of *Lactobacillus plantarum* CGMCC8198 (*LP*8198) isolated from fermented herbage was cultured in de Man, Rogosa and Sharpe (MRS) medium comprising 1% tryptone, 0.5% meat extract, 0.5% yeast extract, 2% glucose, 0.1% Tween 80, 0.2% K_2HPO_4, 0.5% sodium acetate, 0.2% triammonium citrate, 0.02% $MgSO_4 \cdot 7H_2O$, and 0.005% $MnSO_4 \cdot H_2O$ (pH 6.2 ± 1) under anaerobic conditions at 30 °C for 24 h.

Total RNA extraction and RT–PCR

Prior to extraction of the total RNA of *LP*8198 using a Trizol reagent, the seeds were ground in liquid nitrogen. Then 2 μg total RNA was reverse-transcribed using M-MLV reverse transcriptase (Promega, BJ, CA) according to the manufacturer's instructions with N6 primers (Invitrogen, BJ, CA).

Semi-quantitative PCR (semi-PCR) was performed using Applied Biosystems thermocycler (Applied Biosystems, Foster City, CA, USA). The PCR amplifications included an initial 5 min denaturation incubation at 95 °C followed by 30 cycles of denaturation (95 °C), annealing (52 °C), and elongation (72 °C) for 20 s by using 1.25 U of Taq DNA polymerase (TransGen Biotech, BJ, CA). Besides, an additional 72 °C final extension was performed for 10 min. PCR products were visualized on 2% agarose gels stained with ethidium bromide under UV transillumination. The gene of 16 s rRNA was used as an internal control to show equal loading of the cDNA samples. Besides, quantitative real-time PCR (qPCR) was further performed using a StepOne™ real-time PCR system (Applied Biosystems, Foster City, CA, USA). Bestar® SybrGreen qPCR Mastermix was obtained from DBI® Bioscience. The thermal profiles were 95 °C for 10 s and 60 °C for 1 min. Melting curve analysis was performed for each PCR to confirm the specificity of amplification. At the end of each phase, fluorescence was measured and quantified. Data was shown as a relative expression level of mRNA after normalization to 16 s rRNA. The primers for semi-PCR and qPCR analyses were as follows: LIase, 5′-CAACACGCCTGCTCCTGAA (forward), 5′-TGGGTGGTG ATCCGAACGA (reverse); 16S: AAGGCTGAAACTCAAAGG (forward), AACCCAA CAT CTCACGAC (reverse).

Lipid extraction

The oil from *ATB* seeds was extracted by the Soxhlet extraction method. Prior to lipid extraction of the seeds, all experimental material was dried for 12 h at 45 °C. About 4 g of the seed powder was placed in a 250 mL distillation flask, and 150 mL of anhydrous diethyl ether was added. Extraction was conducted at 45 °C for 12 h, and the residual solvent of the extraction was dried under nitrogen. The obtained *ATB*-seed oil was added with a 30 mg mL^{-1} stock solution containing 2/3 (w/w) Tween 80 and filter sterilized through a 0.22 μM Minisart filter (Agilent) and stored in the dark at − 20 °C before use.

Fermentation of *ATB*-seed oil by *LP*8198

*LP*8198 was inoculated (1%) in MRS broth with or without 0.5 mg mL^{-1} *ATB*-seed oil and then incubated anaerobically at 30 °C with a mixture of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen. After 24-h fermentation, the cultures were centrifuged at 5000 g for 10 min at room temperature. The lipid of the culture supernatant fluid was extracted by using a hexane/methanol (2:1, v/v) solution at room temperature and then centrifuged at 5000 g for 10 min at 4 °C after being shocked fully. The chloroform phase was finally dried under nitrogen.

GC–MS analysis of fatty acid in total lipid extracts

Fatty acids were converted to the corresponding methyl esters before GC–MS analysis. In brief, the total lipid extracts were reacted with 1 mL 0.5 M $NaOH\text{-}CH_3OH$ at 65 °C for 30 min, and then 1 mL $BF_3\text{-}CH_3OH$ was added to the reaction liquid at 70 °C for 2 min after cooling down. Subsequently, the esterified products were extracted with n-hexane by oscillating, and then a saturated NaCl aqueous solution was added to the entire mixture. After being agitated for 2 min, the fatty acid methyl esters (FAMEs) were removed from the upper layer and stored at − 20 °C.

FAMEs were analyzed by an Agilent 7890A GC with an Agilent 5975B Inert XL mass selective detector using an HP-5 column (Agilent 19091 J-416, CA; 60 m × 320 μm × 0.25 μm) with the following temperature program: initial temperature 50 °C, increased to 200 °C at 10 °C/min and 230 °C at 2 °C/min, and then raised at 8 °C/min to 270 °C and held for 15 min. Besides, the inlet temperature was 270 °C with constant flow of

Table 1 The Linoleate isomerase gene sequences from 9 strains

Gene Name	Sequence
Linoleate isomerase	ATGGTTAAAAGTAAAGCAATTATGATTGGT GCCGGGCTATCAAATATGGCTGCGGCGGTCTACTTGATTCAAGATGGTCATTGG GATGGTAAGGACATCACATTCTATGGTGTTGATATGCACGGTGCCAATGATGG TGGTGCCACGACTGATTTTACTAATGAGTATTGGAATAAGAATCATCCGATGG CTAACACGACTGGGTATGTTGCCCGGGGTGGTCGGATGCTGAATTACCGGACGT ACGTTGACTTAATGGATTTATTGGACCGGATTCCATCGGTAACTGAACCGGGG ATGACGGCGGCCGAAGATACGCGTGATTTTGATGCGAAACATCGGACGTATGA TATTGCCCGCTTGATGCAGGGTGGTAAAGGCATTATTAATGCTGGTAAGTTAG GATTCAATAATAAGGATCGGACTTTGCTGACTAAGTTGATTATGATGCCAGAT AGTGAAGAAACGAAGCTCGACAACGTTTCGATTGCTGAGTACTTCAAGGATGA TCCGCATATGTTCCAAACGAATTTCTGGTATATGTGGGAAACAACCTTTGCCT TTAGAACGCAAAGCTCTGCTCAAGAACTGCGGCGTTACATGCATCAAATGATT TATGAATTTACACAAATTGAACACTTAGTTGGTGTCAACCGGACGCGTTACAA TCAATTCGAAAGCATGATTTTGCCATTAATTAAGTACTTGCAAGGGCAAGGTG TGACTTTCATTGATAATAAGATTGTTAAGGATTGGCAATTTAAAGACACGCCA ATGCAAGACGAAATTACGGTGACTGGCTTAGTCATTGAGGATGCGCAGACTGG CGAAACGGAAGAAGTTGAAGTTGATGAGGACACAGCGGTGATCTTCACTAACG GTTCAATTACCGATTCTGCAACGATGGGTGATTACAACACGCCTGCTCCTGAA AATATGGATTATGGTGTTAGTGCTAGTTTGTGGAAGAAGGCTACTGAGCGGTT CTATAACTTAGGGACGCCAGATAAGTTCTTCAACGATCGGAATGCTAGCGAAT GGGTCAGCTTCACGTTGACGACTAAGAATCATTTATTCTTAAATGAAATCGTT CGGATCACCACCCAGGAACCCGGGAATGCGTTGAACTCCTTCTTATCAACTAC GCCAATTACGCCGTTGAACCAAAAGGATGTTAATATGTCGATCGTGGTGCACC ACCAACCACACTTTACGACACAGCAACCAAACGAAACAGTTCTGTGGGGCTAC TTCTTGTATCCACGGCGTCAAGGTGAGTTTGTTAACAAGCCGTATATCAAGAT GACGGGTAAGGAAATGGCTCAAGAATTAATTGGTCAACTTTCCAAAGTAGATC CGGGTCCAGGCAATATTAAGGACAAGGAAAAGGAAATTTTGGACAGTATTGTG AACAATATTCCGGTATACATGCCATATGCTTCCGCACTCTTTAATAACCGGGC TAAGTCTGATCGGCCAGAAGTCTTACCAAAGCACTCAACGAACCTAGCCTTTA CGGGTGAATTTGCGGAACAACCATACCAGATGATCTTCACGGAACAGAGTGCG GTCCGCTCTGGTGAGATTGCCGCTTATCACTTTGCTGGGGTCCCAATGGATAA CTTGGTCAAGACACCACGGTACGATAAGGATCCAAAGACCTTGCTCAAGGCAAC TAAGAAGATGTTTGATTAA

Table 1 continued

Gene Name	Sequence
>gi|325048312|emb|FR732045.1| *Lactobacillus plantarum* gene for putative linoleate isomerase, strain ATCC 8014	GAACCTAATTACCTATTTTGGGGGCGTTATTTA TGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGGCTATCAAATATGGCTGCG GCGGTCTACTTGATTCAAGATGGTCATTGGGATGGTAAGGACATCACATTCTA TGGTGTTGATATGCACGGTGCCAATGATGGTGGTGCCACGACTGATTTTACTA ATGAGTATTGGAATAAGAATCATCCGATGGCTAACACGACTGGGTATGTTGCC CGGGGTGGTCGGATGCTGAATTACCGGACGTACGTTGACTTAATGGATTTATT GGACCGGATTCCATCGGTAACTGAACCGGGGATGACGGCGGCCGAAGATACGC GTGATTTTGATGCGAAACATCGGACGTATGATATTGCCCGCTTGATGCAGGGT GGTAAAGGCATTATTAATGCTGGTAAGTTAGGATTCAATAATAAGGATCGGAC TTTGCTGACTAAGTTGATTATGATGCCAGATAGTGAAGAAACGAAGCTCGACA ACGTTTCGATTGCTGAGTACTTCAAGGATGATCCGCATATGTTCCAAACGAAT TTCTGGTATATGTGGGAAACAACCTTTGCCTTTAGAACGCAAAGCTCTGCTCA AGAACTGCGGCGTTACATGCATCAAATGATTTATGAATTTACACAAATTGAAC ACTTAGTTGGTGTCAACCGGACGCGTTACAATCAATTCGAAAGCATGATTTTG CCATTAATTAAGTACTTGCAAGGGCAAGGTGTGACTTTCATTGATAATAAGAT TGTTAAGGATTGGCAATTTAAAGACACGCCAATGCAAGACGAAATTACGGTGA CTGGCTTAGTCATTGAGGATGCGCAGACTGGCGAAACGGAAGAAGTTGAAGTT GATGAGGAAACAGCGGTGATCTTCACTAACGGTTCAATTACCGATTCTGCAAC GATGGGTGATTACAACACGCCTGCTCCTGAAAATATGGATTATGGTGTTAGTG CTAGTTTGTGGAAGAAGGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGAT AAGTTCTTCAACGATCGGAATGCTAGCGAATGGGTCAGCTTCACGTTGACGAC TAAGAATCATTTATTCTTAAATGAAATCGTTCGGATCACCACCCAGGAACCCG GGAATGCGTTGAACTCCTTCTTATCAACTACGCCAATTACGCCGTTGAACCAA AAGGATGTTAATATGTCGATCGTGGTGCACCACCAACCACACTTTACGACACAG CAACCAAACGAAACAGTTCTGTGGGGCTACTTCTTGTATCCACGGCGTCAAGG TGAGTTTGTTAACAAGCCGTATATCAAGATGACGGGTAAGGAAATGGCTCAAG AATTAATTGGTCAACTTTCCAAAGTAGATCCGGGTCCAGGCAATATTAAGGAC AAGGAAAAGGAAATTTTGGACAGTATTGTGAACAATATTCCGGTATACATGCC ATATGCTTCCGCACTCTTTAATAACCGGGCTAAGTCTGATCGGCCAGAAGTCTT ACCAAAGCACTCAACGAACCTAGCCTTTACGGGTGAATTTGCGGAACAACCAT ACCAGATGATCTTCACGGAACAGAGTGCGGTCCGCTCTGGTGAGATTGCCGCT TATCACTTTGCTGGGGTCCCAATGGATAACTTGGTCAAGACACCACGGTACGA TAAGGATCCAAAGACCTTGCTCAAGGCAACTAAGAAGATGTTTGATTAATTAA ATGCTGGCCTGTCAGTGGTTAATACTGATGATGGCATGATTTAAAAACGCTCC CCATGAAAGATGACTTTCATAGGGAGCGTTTTTAGTGTGC

Table 1 continued

Gene Name	Sequence
>gi|300885403|gb|HM569265.1| *Lactobacillus plantarum* linoleate isomerase (l1n) gene, complete cds	ATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGG CTATCAAATATGGCTGCGGCGGTCTACTTGATTCAAGAGGGTCATTGGGATGG TAAGGACATCACATTCTATGGTGTTGATATGCACGGTGCCAATGATGGTGGTG CCACGACTGATTTTACCAATGAGTATTGGAATAAGAATCATCCGATGGCTAAC ACGACTGGGTATGTTGCCCGGGGTGGTCGGATGCTGAATTACCGGACGTACGT TGACTTAATGGATTTATTGGACCGGATTCCATCGGTAACTGAACCGGGGATGA CGGCGGCCGAAGATACGCGTGATTTTGATGCGAAACATCGGACGTATGATATT GCCCGCTTGATGCAGGGTGGTAAAGGCATTATTAATGCTGGTAAGTTAGGATT CAATAATAAGGATCGGACTTTGCTGACTAAGTTGATTATGATGCCAGATAGTG AAGAAACGAAGCTCGACAACGTTTCGATTGCTGAGTACTTCAAGGATGATCCG CATATGTTCCAAACGAATTTCTGGTATATGTGGGAAACAACCTTTGCCTTTAG AACGCAAAGCTCTGCTCAAGAACTGCGGCGTTACATGCATCAAATGATTTATG AATTTACACAAATTGAACACTTAGTTGGTGTCAACCGGACGCGTTACAATCAA TTCGAAAGCATGATTTTGCCATTAATTAAGTACTTGCAAGGGCAAGGTGTGAC TTTCATTGATAATAAGATTGTTAAGGATTGGCAATTTAAAGACACGCCAATGC AAGACGAAATTACGGTGACTGGCTTAGTCATTGAGGATGCGCAGACTGGCGAA ACGGAAGAAGTTGAAGTTGATGAGGACACAGCGGTGATCTTCACTAACGGTTC AATTACCGATTCTGCAACGATGGGTGATTACAACACGCCTGCTCCTGAAAATA TGGATTATGGTGTTAGTGCTAGTTTGTGGAAGAAGGCTACTGAGCGGTTCTAT AACTTAGGGACGCCAGATAAGTTCTTCAACGATCGGAATGCTAGCGAATGGGT CAGCTTCACGTTGACGACTAAGAATCATTTATTCTTAAATGAAATCGTTCGGA TCACCACCCAGGAACCCGGGAATGCGTTGAACTCCTTCTTATCAACTACGCCA ATTACGCCGTTGAACCAAAAGGATGTTAATATGTCGATCGTGGTGCACCACCA ACCACACTTTACGACACAGCAACCAAACGAAACAGTTCTGTGGGGCTACTTCT TGTATCCACGGCGTCAAGGTGAGTTTGTTAACAAGCCGTATATCAAGATGACG GGTAAGGAAATGGCTCAAGAATTAATTGGTCAACTTTCCAAAGTAGATCCGGG TCCAGGCAATATTAAGGACAAGGAAAAGGAAATTTTGGACAGTATTGTGAACA ATATTCCGGTATACATGCCATATGCTTCCGCACTCTTTAATAACCGGGCTAAG TCTGATCGGCCAGAAGTCTTACCAAAGCACTCAACGAACCTAGCCTTTACGGG TGAATTTGCGGAACAACCATACCAGATGATCTTCACGGAACAGAGTGCGGTCC GCTCTGGTGAGATTGCCGCTTATCACTTTGCTGGGGTCCCAATGGATAACTTG GTCAAGACACCACGGTACGATAAGGATCCAAAGACCTTGCTCAAGGCAACTAA GAAGATGTTTGATTAA

Table 1 continued

Gene Name	Sequence
>gi|319657088|gb|HQ831447.1| *Lactobacillus plantarum* strain lp15-2-1 linoleic acid isomerase gene, complete cds	ATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGG GCTATCAAATATGGCTGCGGCGGTCTACTTGATTCAAGAGGGTCATTGGGATG GTAAGGACATCACATTCTATGGTGTTGATATGCACGGTGCCAATGATGGTGGT GCCACGACTGATTTTACCAATGAGTATTGGAATAAGAATCATCCGATGGCTAA CACGACTGGGTATGTTGCCCGGGGTGGTCGGATGCTGAATTACCGGACGTACG TTGACTTAATGGATTTATTGGACCGGATTCCATCGGTAACTGAACCGGGGATG ACGGCGGCCGAAGATACGCGTGATTTTGATGCGAAACATCGGACGTATGATAT TGCCCGCTTGATGCAGGGTGGTAAAGGCATTATTAATGCTGGTAAGTTAGGAT TCAATAATAAGGATCGGACTTTGCTGACTAAGTTGATTATGATGCCAGATAGT GAAGAAACGAAGCTCGACAACGTTTCGATTGCTGAGTACTTCAAGGATGATCC GCATATGTTCCAAACGAATTTCTGGTATATGTGGGAAACAACCTTTGCCTTTA GAACGCAAAGCTCTGCTCAAGAACTGCGGCGTTACATGCATCAAATGATTTAT GAATTTACACAAATTGAACACTTAGTTGGTGTCAACCGGACGCGTTACAATCA ATTCGAAAGCATGATTTTGCCATTAATTAAGTACTTGCAAGGGCAAGGTGTGA CTTTCATTGATAATAAGATTGTTAAGGATTGGCAATTTAAAGACACGCCAATG CAAGACGAAATTACGGTGACTGGCTTAGTCATTGAGGATGCGCAGACTGGCGAA ACGGAAGAAGTTGAAGTTGATGAGGACACAGCGGTGATCTTCACTAACGGTTC AATTACCGATTCTGCAACGATGGGTGATTACAACACGCCTGCTCCTGAAAATA TGGATTATGGTGTTAGTGCTAGTTTGTGGAAGAAGGCTACTGAGCGGTTCTAT AACTTAGGGACGCCAGATAAGTTCTTCAACGATCGGAATGCTAGCGAATGGGT CAGCTTCACGTTGACGACTAAGAATCATTTATTCTTAAATGAAATCGTTCGGA TCACCACCCAGGAACCCGGGAATGCGTTGAACTCCTTCTTATCAACTACGCCAA TTACGCCGTTGAACCAAAAGGATGTTAATATGTCGATCGTGGTGCACCACCAA CCACACTTTACGACACAGCAACCAAACGAAACAGTTCTGTGGGGCTACTTCTT GTATCCACGGCGTCAAGGTGAGTTTGTTAACAAGCCGTATATCAAGATGACGG GTAAGGAAATGGCTCAAGAATTAATTGGTCAACTTTCCAAAGTAGATCCGGGT CCAGGCAATATTAAGGACAAGGAAAAGGAAATTTTGGACAGTATTGTGAACAA TATTCCGGTATACATGCCATATGCTTCCGCACTCTTTAATAACCGGGCTAAGT CTGATCGGCCAGAAGTCTTACCAAAGCACTCAACGAACCTAGCCTTTACGGGT GAATTTGCGGAACAACCATACCAGATGATCTTCACGGAACAGAGTGCGGTCCG CTCTGGTGAGATTGCCGCTTATCACTTTGCTGGGGTCCCAATGGATAACTTGG TCAAGACACCACGGTACGATAAGGATCCAAAGACCTTGCTCAAGGCAACTAAG AAGATGTTTGACTAA

Table 1 continued

Gene Name	Sequence
>gi|325048318|emb|FR732048.1| *Lactobacillus plantarum* gene for putative linoleate isomerase, strain LMG 6907	GAACCTAATTACCTATTTTGGGGGCGTTATTT ATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGGCTATCAAATATGGCTGC GGCGGTCTACTTGATTCAAGATGGTCATTGGGATGGTAAGGACATCACATTCT ATGGTGTTGATATGCACGGTGCGAATGATGGTGGTGCCACGACTGATTTTACC AATGAGTATTGGAATAAGAATCATCCGATGGCTAACACGACTGGGTATGTTGC CCGGGGTGGTCGGATGCTGAATTACCGGACGTACGTTGACTTAATGGATTTAT TGGACCGGATTCCATCGGTAACTGAACCGGGGATGACGGCGGCCGAAGATACG CGTGATTTTGATGCGAAACATCGGACGTATGATATTGCCCGCTTGATGCAGGG TGGTAAAGGCATTATTAATGCTGGTAAGTTAGGATTCAATAATAAGGATCGGA CTTTGCTGACTAAGTTGATCATGATGCCAGATAGTGAAGAAACGAAGCTCGAC AACGTTTCGATTGCTGAGTACTTCAAGGATGATCCGCATATGTTCCAAACGAA TTTCTGGTATATGTGGGAAACAACCTTTGCCTTTAGAACGCAAAGCTCTGCTC AAGAACTGCGGCGTTACATGCATCAAATGATTTATGAATTTACACAAATTGAA CACTTAGTTGGTGTCAACCGGACGCGTTACAATCAATTCGAAAGCATGATTTT GCCATTAATTAAGTACTTGCAAGGGCAAGGTGTGACTTTCATTGATAATAAGA TTGTTAAGGATTGGCAATTTAAAGACACGCCAATGCAAGACGAAATTACGGTG ACTGGCTTAGTCATTGAGGATGCGCAGACTGGCGAAACGGAAGAAGTTGAAGT TGATGAGGACACAGCGGTGATCTTCACTAACGGTTCAATTACCGATTCTGCAA CGATGGGTGATTACAACACGCCTGCTCCTGAAAATATGGATTATGGTGTTAGT GCTAGTTTGTGGAAGAAGGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGA TAAGTTCTTCAACGATCGGAATGCTAGCGAATGGGTCAGCTTCACGTTGACGA CTAAGAATCATTTATTCTTAAATGAAATCGTTCGGATCACCACCCAGGAACCC GGGAATGCGTTGAACTCCTTCTTATCAACTACGCCAATTACGCCGTTGAACCA AAAGGATGTTAATATGTCGATCGTGGTGCACCACCAACCACACTTTACGACAC AGCAACCAAACGAAACAGTTCTGTGGGGCTACTTCTTGTATCCACGGCGTCAA GGTGAGTTTGTTAACAAGCCGTATATCAAGATGACGGGTAAGGAAATGGCTCA AGAATTAATTGGTCAACTTTCCAAAGTAGATCCGGGTCCAGGCAATATTAAGG ACAAGGAAAAGGAAATTTTGGACAGTATCGTGAACAATATTCCGGTATACATG CCATATGCTTCCGCACTCTTTAATAACCGGGCTAAGTCTGATCGGCCAGAAGT CTTACCAAAGCACTCAACGAACCTAGCCTTTACGGGTGAATTTGCGGAACAAC CATACCAGATGATCTTCACGGAACAGAGTGCGGTCCGCTCTGGTGAGATTGCC GCTTATCACTTTGCTGGGGTCCCAATGGATAACTTGGTCAAGACACCACGGTA CGATAAGGATCCAAAGACCTTGCTCAAGGCAACTAAGAAGATGTTTGATTAAT TAAATGCTGGCCTGTCAGTGGTTAATGCTGATGATGGCATGATGTAAAAACGC TCCCCATGAAAGATGACTTTCATAGGGAGCGTTTTAGTGTGC

Table 1 continued

Gene Name	Sequence
>gi|325048314|emb|FR732046.1| *Lactobacillus plantarum* gene for putative linoleate isomerase, strain IMDO 130201	AGAACCTAATTACCTATTTTGGGGGCGTTATTT ATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGGCTATCAAATATGGCTGC GGCGGTCTACTTGATTCAAGATGGTCATTGGGATGGTAAGGACATCACATTCTA TGGTGTTGATATGCACGGTGCCAATGATGGTGGTGCCACGACTGATTTTACCA ATGAGTATTGGAATAAGAATCATCCGATGGCTAACACGACTGGGTATGTTGCC CGGGGTGGTCGGATGCTGAATTACCGGACGTACGTTGACTTAATGGATTTATT GGACCGGATTCCATCGGTAACTGAACCGGGGATGACGGCGGCTGAAGATACGC GTGATTTTGATGCGAAACATCGGACGTATGATATTGCCCGCTTGATGCAGGGT GGTAAAGGCATTATTAATGCTGGTAAGTTAGGATTCAATAATAAGGATCGGAC TTTGCTGACTAAGTTGATTATGATGCCAGATAGTGAAGAAACGAAGCTCGACA ACGTTTCGATTGCTGAGTACTTCAAGGATGATCCGCATATGTTCCAAACGAAT TTCTGGTATATGTGGGAAACAACCTTTGCCTTTAGAACGCAAAGCTCTGCTCA AGAACTGCGGCGTTACATGCATCAAATGATTTATGAATTTACACAAATTGAAC ACTTAGTTGGTGTCAACCGGACGCGTTACAATCAATTCGAAAGCATGATTTTG CCATTAATTAAGTACTTGCAAGGGCAAGGTGTGACTTTCATTGATAATAAGAT TGTTAAGGATTGGCAATTTAAAGACACGCCAATGCAAGACGAAATTACGGTGA CTGGCTTAGTCATTGAGGATGCGCAGACTGGCGAAACGGAAGAAGTTGAAGTT GATGAGGACACAGCGGTGATCTTCACTAACGGTTCAATTACCGATTCTGCAAC GATGGGTGATTACAACACGCCTGCTCCTGAAAATATGGATTATGGTGTTAGTG CTAGTTTGTGGAAGAAGGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGAT AAGTTCTTCAACGATCGGAATGCTAGCGAATGGGTCAGCTTCACGTTGACGAC TAAGAATCATTTGTTCTTAAATGAAATCGTTCGGATCACCACCCAGGAACCCG GGAATGCGTTGAACTCCTTCTTATCAACTACGCCAATTACGCCGTTGAACCAA AAGGATGTTAATATGTCTATCGTGGTGCACCACCAACCACACTTTACGACACA GCAACCAAACGAAACAGTTCTGTGGGGCTACTTCTTGTATCCACGGCGTCAAG GTGAGTTTGTTAACAAGCCGTATATCAAGATGACGGGTAAGGAAATGGCTCAA GAATTAATTGGTCAACTTTCCAAAGTAGATCCGGGTCCAGGCAATATTAAGGA CAAGGAAAAGGAAATTTTGGACAGTATTGTGAACAATATTCCGGTATACATGC CATATGCTTCCGCACTCTTTAATAACCGGGCTAAGTCTGATCGGCCAGAAGTC TTACCAAAGCACTCAACGAACCTAGCCTTTACGGGTGAATTTGCGGAACAACC ATACCAGATGATCTTCACGGAACAGAGTGCGGTCCGCTCTGGTGAGATTGCCG CTTATCACTTTGCTGGGGTCCCAATGGATAACTTGGTCAAGACACCACGGTAC GATAAGGATCCAAAGACCTTGCTCAAGGCAACTAAGAAGATGTTTGATTAATT AAATGCTGGCCTGTCAGTGGTTAATGCTGATGATGGCATGATTTAAAAACGCT CCCCATGAAAGATGACTTTCATAGGGAGCGT

Table 1 continued

Gene Name	Sequence
>gi|333037510|gb|JF747255.1| *Lactobacillus plantarum* strain ZS2058 putative linoleate isomerase gene	ATGGGGGCGTTATTTATGGTTAAAAGTAAAG CAATTATGATTGGTGCCGGGCTATCAAATATGGCTGCGGCGGTCTACTTGATT CAAGATGGTCATTGGGATGGTAAGGACATCACATTCTATGGTGTTGATATGCA CGGTGCCAATGATGGTGGTGCCACGACTGATTTTACCAATGAGTATTGGAATA AGAATCATCCGATGGCTAACACGACTGGGTATGTTGCCCGGGGTGGTCGGATG CTGAATTACCGGACGTACGTTGACTTAATGGATTTATTGGACCGGATTCCATC GGTAACTGAACCGGGGATGACGGCGGCTGAAGATACGCGTGATTTTGATGCGA AACATCGGACGTATGATATTGCCCGCTTGATGCAGGGTGGTAAAGGCATTATT AATGCTGGTAAGTTAGGATTCAATAATAAGGATCGGACTTTGCTGACTAAGTT GATCATGATGCCAGATAGTGAAGAAACGAAGCTCGACAACGTTTCGATTGCTG AGTACTTCAAGGATGATCCGCATATGTTCCAAACGAATTTCTGGTATATGTGG GAAACAACCTTTGCCTTTAGAACGCAAAGCTCTGCTCAAGAACTGCGGCGTTA CATGCATCAAATGATTTATGAATTTACACAAATTGAACACTTAGTTGGTGTCA ACCGGACGCGTTACAATCAATTCGAAAGCATGATTTTGCCATTAATTAAGTAC TTGCAAGGGCAAGGTGTGACTTTCATTGATAATAAGATTGTTAAGGATTGGCA ATTTAAAGACACGCCAATGCAAGACGAAATTACGGTGACTGGCTTAGTCATTGA GGATGCGCAGACTGGCGAAACGGAAGAAGTTGAAGTTGATGAGGACACAGCGG TGATCTTCACTAACGGTTCAATTACCGATTCTGCAACGATGGGTGATTACAAC ACGCCTGCTCCTGAAAATATGGATTATGGTGTTAGTGCTAGTTTGTGGAAGAA GGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGATAAGTTCTTCAACGATC GGAATGCTAGCGAATGGGTCAGCTTCACGTTGACGACTAAGAATCATTTATTC TTAAATGAAATCGTTCGGATCACCACCCAGGAACCCGGGAATGCGTTGAACTC CTTCTTATCAACTACGCCAATTACGCCGTTGAACCAAAAGGATGTTAATATGT CGATCGTGGTGCACCACCAACCACACTTTACGACACAGCAACCAAACGAAACA GTTCTGTGGGGCTACTTCTTGTATCCACGGCGTCAAGGTGAGTTTGTTAACAA GCCGTATATCAAGATGACGGGTAAGGAAATGGCTCAAGAATTAATTGGTCAAC TTTCCAAAGTAGATCCGGGTCCAGGCAATATTAAGGACAAGGAAAAGGAAATT TTGGACAGTATCGTGAACAATATTCCGGTATACATGCCATATGCTTCCGCACT CTTTAATAACCGGGCTAAGTCTGATCGGCCAGAAGTCTTACCAAAGCACTCAA CGAACCTAGCCTTTACGGGTGAATTTGCGGAACAACCATACCAGATGATCTTC ACGGAACAGAGTGCGGTCCGCTCTGGTGAGATTGCCGCTTATCACTTTGCTGG GGTCCCAATGGATAACTTGGTCAAGACACCACGGTACGATAAGGATCCAAAGA CCTTGCTCAAGGCAACTAAGAAGATGTTTGAT

Table 1 continued

Gene Name	Sequence
>gi|325048316|emb|FR732047.1| *Lactobacillus plantarum* gene for putative linoleate isomerase	CAGAACCTAATTACCTATTTTGGGGGCGT TATTTATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGGCTATCAAATATG GCTGCGGCGGTCTACTTGATTCAAGATGGTCATTGGGATGGTAAGGACATCAC ATTCTATGGTGTTGATATGCACGGTGCGAATGATGGTGGTGCCACGACTGATT TTACCAATGAGTATTGGAATAAGAATCATCCGATGGCTAACACGACTGGGTAT GTTGCCCGGGGTGGTCGGATGCTGAATTACCGGACGTACGTTGACTTAATGGA TTTATTGGACCGGATTCCATCGGTAACTGAACCGGGGATGACGGCGGCCGAAG ATACGCGTGATTTTGATGCGAAACATCGGACGTATGATATTGCCCGCTTGATG CAGGGTGGTAAAGGCATTATTAATGCTGGTAAGTTAGGATTCAATAATAAGGA TCGGACTTTGCTGACTAAGTTGATCATGATGCCAGATAGTGAAGAAACGAAGC TCGACAACGTTTCGATTGCTGAGTACTTCAAGGATGATCCGCATATGTTCCAA ACGAATTTCTGGTATATGTGGGAAACAACCTTTGCCTTTAGAACGCAAAGCTC TGCTCAAGAACTGCGGCGTTACATGCATCAAATGATTTATGAATTTACACAAA TTGAACACTTAGTTGGTGTCAACCGGACGCGTTACAATCAATTCGAAAGCATG ATTTTGCCATTAATTAAGTACTTGCAAGGGCAAGGTGTGACTTTCATTGATAA TAAGATTGTTAAGGATTGGCAATTTAAAGACACGCCAATGCAAGACGAAATTA CGGTGACTGGCTTAGTCATTGAGGATGCGCAGACTGGCGAAACGGAAGAAGTT GAAGTTGATGAGGACACAGCGGTGATCTTCACTAACGGTTCAATTACCGATTC TGCAACGATGGGTGATTACAACACGCCTGCTCCTGAAAATATGGATTATGGTG TTAGTGCTAGTTTGTGGAAGAAGGCTACTGAGCGGTTCTATAACTTAGGGACG CCAGATAAGTTCTTCAACGATCGGAATGCTAGCGAATGGGTCAGCTTCACGTT GACGACTAAGAATCATTTATTCTTAAATGAAATCGTTCGGATCACCACCCAGG AACCCGGGAATGCGTTGAACTCCTTCTTATCAACTACGCCAATTACGCCGTTG AACCAAAAGGATGTTAATATGTCGATCGTGGTGCACCACCAACCACACTTTAC GACACAGCAACCAAACGAAACAGTTCTGTGGGGCTACTTCTTGTATCCACGGC GTCAAGGTGAGTTTGTTAACAAGCCGTATATCAAGATGACGGGTAAGGAAATG GCTCAAGAATTAATTGGTCAACTTTCCAAAGTAGATCCGGGTCCAGGCAATAT TAAGGACAAGGAAAAGGAAATTTTAGACAGTATCGTGAACAATATTCCGGTAT ACATGCCATATGCTTCCGCACTCTTTAATAACCGGGCTAAGTCTGATCGGCCA GAAGTCTTACCAAAGCACTCAACGAACCTAGCCTTTACGGGTGAATTTGCGGA ACAACCATACCAGATGATCTTCACGGAACAGAGTGCGGTCCGCTCTGGTGAGA TTGCCGCTTATCACTTTGCTGGGGTCCCAATGGATAACTTGGTCAAGACACCA CGGTACGATAAGGATCCAAAGACCTTGCTCAAGGCAACTAAGAAGATGTTTGA TTAATTAAATGCTGGCCTGTCAGTGGTTAATGCTGATGATGGCATGATGTAAA AACGCTCCCCATGAAAGATGACTTTCATAGGGAGCGTTTTAGTGT

Table 1 continued

Gene Name	Sequence
>gi|77819928|gb|DQ227322.1| *Lactobacillus plantarum* strain AS1.555 linoleate isomerase gene	TTGGCGGCGTTATTTATGGTTAAAAGTA AAGCAATTATGATTGGTGCCGGGCTATCAAATATGGCTGCGGCGGTCTACTTG ATTCAAGAGGGTCATTGGGATGGTAAGGACATCACATTCTATGGTGTTGATAT GCGCGGTGCCAATGATGGTGGTGCCACGACTGATTTTACCAATGAGTATTGGA ATAAGAATCATCCGATGGCTAATACGACTGGGTATGTTGCCCGGGGTGGTCGG ATGCTGAATTACCGGACGTACGTTGACTTAATGGATTTATTGGACCGGATTCC ATCGGTAACTGAACCGGGGATGACGGCGGCCGACGATACGCGTGATTTTGATG CGAAACATCGGACGTATGATATTGCCCGCTTGATGCAGGGTGGTAAAGGCATT ATTAATGCTGGTAAGTTAGGATTCAATAATAAGGATCGGACTTTGCTGACTCA GTTGATTATGATGCCAGATAGTGAAGAAACGAAGCTCGACAACGTTTCGATTG CTGAGTACTTCAAGGATGATCCGCATATGTTCCAAACGAATTTCTGGTATATG TGGGAAACAACCTTTGCCTTTAGAACGCAAAGCTCTGCCCAAGAACTGCGGCG TTACATGCATCAAATGATTTATGAATTTACACAAATTGAACACTTAGTTGGTG TCAACCGGACGCGTTACAATCAATTCGAAAGCATGATTTTGCCATTAATTAAG TACTTGCAAGGGCAAGGTGTGACTTCCATTGATAATAAGATTGTTAAGGATTG GCAATTTAAAGACACGCCAATGCAAGACGAAATTACGGTGACTGGCTTAGTCA TTGAGGATGCGCAGACTGGCGAAACGGAAGAAGTTGAAGTTGATGAGGACACA GCGGTGATCTTCACTAACGGTTCAATTACCGATTCTGCAACGATGGGTGATTA TAACACGCCTGCTCCTGAAAATATGGATTATGGTGTTAGTGCTAGTTTGTGGA AGAAGGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGATAAGTTCTTCAAC GATCGGAATGCTAGCGAATGGGTCAGCTTCACGTTGACGACTAAGAATCATTT ATTCTTAAATGAAATCGTTCGGATCACCACCCAGGAACCCGGGAATGCGTTGA ACTCCTTCTTATCAACTACGCCAATTACGCCGTTGAACCAAAAGGATGTTAAT ATGTCGATCGTGGTGCACCACCAACCACACTTTACGACACAGCAACCAAACGA AACAGTTCTGTGGGGCTACTTCTTGTATCCACGGCGTCAAGGTGAGTTTGTTA ACAAGCCGTATATCAAGATGACGGGTAAGGAAATGGCTCAAGAATTAATTGGTC AACTTTCCAAAGTAGATCCGGGTCCAGGCAATATTAAGGACAAGGAAAAGGAAA TTTTGGACAGTATTGTGAACAATATTCCGGTATACATGCCATATGCTTCCGCA CTCTTTAATAACCGGGCTAAGTCTGATCGGCCAGAAGTCTTACCAAAGCACTC AACGAACCTAGCCTTTACGGGTGAATTTGCGGAACAACCATACCAGATGATCT TCACGGAACAGAGTGCGGTCCGCTCTGGTGAGATTGCCGCTTATCACTTTGCT GGGGTCCCAATGGATAACTTGGTCAAGACACCACGGTACGATAAGGATCCAAA GACCTTGCTCAAGGCAACTAAGAAGATGTTTGATTAA

```
               10        20        30        40        50        60        70        80        90       100       110       120
1     ATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGGCTATCAAATATGGCTGCGGCGGTCTACTTGATTCAAGATGGTCATTGGGATGGTAAGGACATCACATTCTATGGTGTTGATATG
1      M  V  K  S  K  A  I  M  I  G  A  G  L  S  N  M  A  A  A  V  Y  L  I  Q  D  G  H  W  D  G  K  D  I  T  F  Y  G  V  D  M

              130       140       150       160       170       180       190       200       210       220       230       240
121   CACGGTGCCAATGATGGTGGTGCCACGACTGATTTTACTAATGAGTATTGGAATAAGAATCATCCGATGGCTAACACGACTGGGTATGTTGCCCGGGGTGGTCGGATGCTGAATTACCGG
41     H  G  A  N  D  G  G  A  T  T  D  F  T  N  E  Y  W  N  K  N  H  P  M  A  N  T  T  G  Y  V  A  R  G  G  R  M  L  N  Y  R

              250       260       270       280       290       300       310       320       330       340       350       360
241   ACGTACGTTGACTTAATGGATTTATTGGACCGGATTCCATCGGTAACTGAACCGGGGATGACGGCGGCCGAAGATACGCGTGATTTTGATGCGAAACATCGGACGTATGATATTGCCCGC
81     T  Y  V  D  L  M  D  L  _  D  R  I  P  S  V  T  E  P  G  M  T  A  A  E  D  T  R  D  F  D  A  K  H  R  T  Y  D  I  A  R

              370       380       390       400       410       420       430       440       450       460       470       480
361   TTGATGCAGGGTGGTAAAGGCATTATTAATGCTGGTAAGTTAGGATTCAATAATAAGGATCGGACTTTGCTGACTAAGTTGATTATGATGCCAGATAGTGAAGAAACGAAGCTCGACAAC
121    L  M  Q  G  G  K  G  I  I  N  A  G  K  L  G  F  N  N  K  D  R  T  L  L  T  K  L  I  M  M  P  D  S  E  E  T  K  L  D  N

              490       500       510       520       530       540       550       560       570       580       590       600
481   GTTTCGATTGCTGAGTACTTCAAGGATGATCCGCATATGTTCCAAACGAATTTCTGGTATATGTGGGAAACAACCTTTGCCTTTAGAACGCAAAGCTCTGCTCAAGAACTGCGGCGTTAC
161    V  S  I  A  E  Y  F  K  D  D  P  H  M  F  Q  T  N  F  W  Y  M  W  E  T  T  F  A  F  R  T  Q  S  S  A  Q  E  L  R  R  Y

              610       620       630       640       650       660       670       680       690       700       710       720
601   ATGCATCAAATGATTTATGAATTTACACAAATTGAACACTTAGTTGGTGTCAACCGGACGCGTTACAATCAATTCGAAAGCATGATTTTGCCATTAATTAAGTACTTGCAAGGGCAAGGT
201    M  H  Q  M  I  Y  E  F  T  Q  I  E  H  L  V  G  V  N  R  T  R  Y  N  Q  F  E  S  M  I  L  P  L  I  K  Y  L  Q  G  Q  G

              730       740       750       760       770       780       790       800       810       820       830       840
721   GTGACTTTCATTGATAATAAGATTGTTAAGGATTGGCAATTTAAAGACACGCCAATGCAAGACGAAATTACGGTGACTGGCTTAGTCATTGAGGATGCGCAGACTGGCGAAACGGAAGAA
241    V  T  F  I  D  N  K  I  V  K  D  W  Q  F  K  D  T  P  M  Q  D  E  I  T  V  T  G  L  V  I  E  D  A  Q  T  G  E  T  E  E

              850       860       870       880       890       900       910       920       930       940       950       960
841   GTTGAAGTTGATGAGGACACAGCGGTGATCTTCACTAACGGTTCAATTACCGATTCTGCAACGATGGGTGATTACAACACGCCTGCTCCTGAAAATATGGATTATGGTGTTAGTGCTAGT
281    V  E  V  D  E  D  T  A  V  I  F  T  N  G  S  I  T  D  S  A  T  M  G  D  Y  N  T  P  A  P  E  N  M  D  Y  G  V  S  A  S

              970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080
961   TTGTGGAAGAAGGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGATAAGTTCTTCAACGATCGGAATGCTAGCGAATGGGTCAGCTTCACGTTGACGACTAAGAATCATTTATTCTTA
321    L  W  K  K  A  T  E  R  F  Y  N  L  G  T  P  D  K  F  F  N  D  R  N  A  S  E  W  V  S  F  T  L  T  T  K  N  H  L  F  L

             1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
1081  AATGAAATCGTTCGGATCACCACCCAGGAACCCGGGAATGCGTTGAACTCCTTCTTATCAACTACGCCAATTACGCCGTTGAACCAAAAGGATGTTAATATGTCGATCGTGGTGCACCAC
361    N  E  I  V  R  I  T  T  Q  E  P  G  N  A  L  N  S  F  L  S  T  T  P  I  T  P  L  N  Q  K  D  V  N  M  S  I  V  V  H  H

             1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320
1201  CAACCACACTTTACGACACAGCAACCAAACGAAACAGTTCTGTGGGGCTACTTCTTGTATCCACGGCGTCAAGGTGAGTTTGTTAACAAGCCGTATATCAAGATGACGGGTAAGGAAATG
401    Q  P  H  F  T  T  Q  Q  P  N  E  T  V  L  W  G  Y  F  L  Y  P  R  R  Q  G  E  F  V  N  K  P  Y  I  K  M  T  G  K  E  M

             1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430      1440
1321  GCTCAAGAATTAATTGGTCAACTTTCCAAAGTAGATCCGGGTCCAGGCAATATTAAGGACAAGGAAAAGGAAATTTTGGACAGTATTGTGAACAATATTCCGGTATACATGCCATATGCT
441    A  Q  E  L  I  G  Q  L  S  K  V  D  P  G  P  G  N  I  K  D  K  E  K  E  I  L  D  S  I  V  N  N  I  P  V  Y  M  P  Y  A

             1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560
1441  TCCGCACTCTTTAATAACCGGGCTAAGTCTGATCGGCCAGAAGTCTTACCAAAGCACTCAACGAACCTAGCCTTTACGGGTGAATTTGCGGAACAACCATACCAGATGATCTTCACGGAA
481    S  A  L  F  N  N  R  A  K  S  D  R  P  E  V  L  P  K  H  S  T  N  L  A  F  T  G  E  F  A  E  Q  P  Y  Q  M  I  F  T  E

             1570      1580      1590      1600      1610      1620      1630      1640      1650      1660      1670      1680
1561  CAGAGTGCGGTCCGCTCTGGTGAGATTGCCGCTTATCACTTTGCTGGGGTCCCAATGGATAACTTGGTCAAGACACCACGGTACGATAAGGATCCAAAGACCTTGCTCAAGGCAACTAAG
521    Q  S  A  V  R  S  G  E  I  A  A  Y  H  F  A  G  V  P  M  D  N  L  V  K  T  P  R  Y  D  K  D  P  K  T  L  L  K  A  T  K

             1690
1681  AAGATGTTTGATTAA
561    K  M  F  D  *
```

Fig. 1 The predicted ORF of LIase in *Lactobacillus plantarum* CGMCC8198

nitrogen (N_2) at 1 mL/min in split mode (50:1). The transfer lines were set to 280 °C, and the temperature of quadrupole and the MS ion source were 230 and 150 °C, respectively. MS detection mode was set as electron impact ionization, scanning from 35 amu to 800 amu masses. Characteristic peaks were identified by comparing with the NIST08 MS library and retention time of external *c*9, *t*11-CLA analytical standard (Sigma 16413, CA) and *t*10, *c*12-CLA analytical standard (NU-CHEK-PREP, INC. UC-61-A, USA).

The *c*9, *t*11-CLA and *t*10, *c*12-CLA standard curves were constructed using the concentration gradients of the corresponding methyl esters. The methods of methyl esterification and GC–MS were the same as above.

Bioinformatics analysis

Firstly, the amino acid sequence homology comparison was performed by NCBI BLASTP, and 9 LIase gene sequence (Table 1) alignment was analyzed by CLUSTAL-X. Subsequently, a phylogenetic tree was constructed with

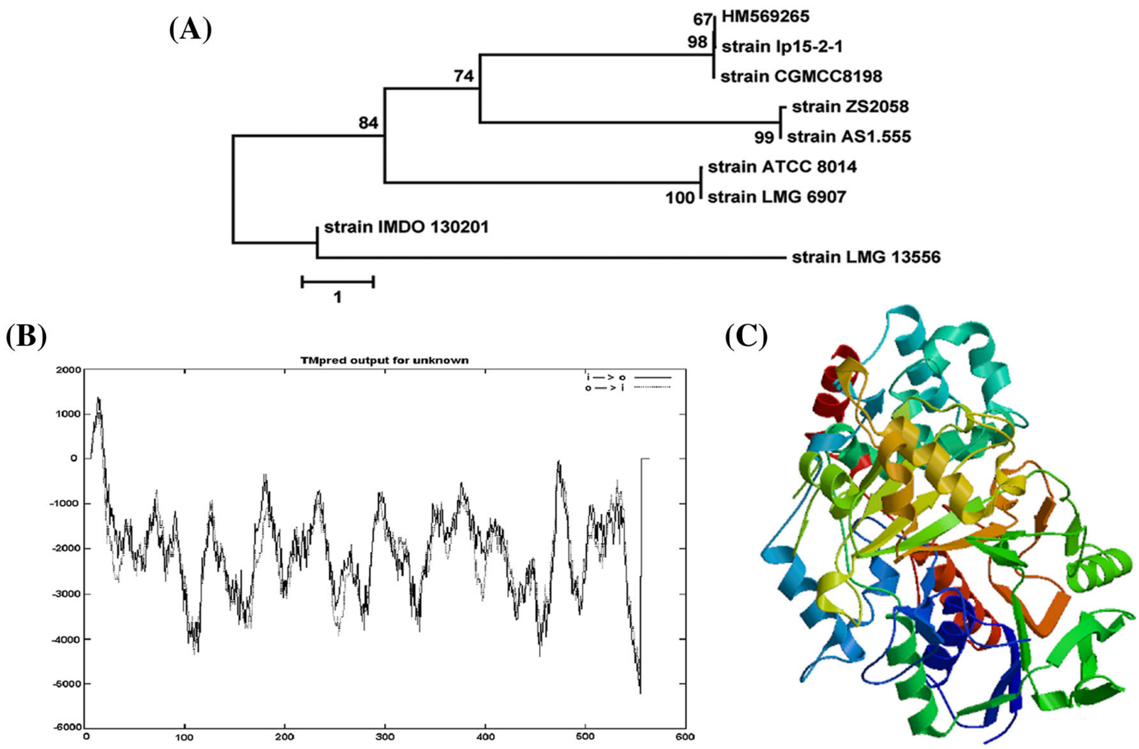

Fig. 2 Bioinformatic analysis of linoleate isomerase in *LP*8198. (**A**) Phylogenetic tree analysis of a new gene from *LP*8198. (**B**) The possible transmembrane helices structure analysis of LIase in *LP*8198. (**C**) Tertiary structure prediction of LIase by the SWISS-MODEL based on homology modeling

MEGA6. Furthermore, the subcellular localization analysis was performed using TargetP 1.1 Server (http://www.cbs.dtu.dk/services/TargetP/), the transmembrane segment prediction was performed using the TMpred Server (http://www.ch. embnet.org/software/TMPRED_form.html), and the tertiary structure of this protein was established by SWISS-MODEL (http://swissmodel.expasy.org/) and visualized by Swiss-PDB-Viewer based on homology modeling.

Statistical analysis

Statistical evaluations were performed using GraphPad PRISM 5.0, with three independent experiments. The statistics were analyzed using Student's *t* test. Differences at $P < 0.05$ were considered statistically significant.

Results and discussion

Identification, analysis, and phylogenetic analysis of LIase in *LP*8198

As shown in Fig. 1, the full-length cDNA of LIase is 1710 bp, comprising a 5′ untranslated region of 15 bp and an uninterrupted open reading frame (ORF) of 1695 bp, and the complete CDS region was submitted to GenBank by BankIt tool and acquired GenBank ID as KU555936. The predicted ORF of the cDNA encodes a protein of 564 amino acids with a molecular weight of 64.23 kDa and a theoretical pI of 5.36. Besides, it was predicted as a stable protein by the ProtParam tool (http://web.expasy.org/protparam/). Furthermore, a phylogenetic tree of the obtained LIase in *LP*8198 was constructed, and the results indicated that the gene is most closely related to the linoleic acid isomerase gene of *L. plantarum* strains lp15-2-1 and ZS2058 [Fig. 2(A)].

Further bioinformatics analyses of LIase in *LP*8198 by TargetP 1.1 Server indicated that it is a secretory pathway signal peptide (Table 2). Subsequently, the possible transmembrane helices structure performed by the TMpred Sever indicated that the N-terminal region includes 18 amino acids (from 6 th aa to 23 th aa) which were predicted as an inside to outside helices structure [Fig. 2(B)]. Besides, the tertiary structure of this protein was also established by SWISS-MODEL and visualized by Swiss-PDB-Viewer based on homology modeling [Fig. 2(C)].

Table 2 The subcellular localization prediction of LIase in *LP*8198 by the TargetP 1.1 Server

Name	Length	Location	RC[a]
Linoleate isomerase	564	Secretory pathway	4

[a]RC is a measure of the size of the difference ('diff') between the highest (winning) and the second highest output scores. There are 5 reliability classes, defined as follows: 1: diff > 0.800; 2: 0.800 > diff > 0.600; 3: 0.600 > diff > 0.400; 4: 0.400 > diff > 0.200; 5: 0.200 > diff. Thus, the lower the value of RC indicates the safer the prediction

ATB-seed oil induced the transcription of *LP*8198 LIase

Since the content of linoleic acid was up to about 34% in *ATB*-seed oil (Fig. 3), we speculated whether *ATB*-seed oil could affect the transcription of *LP*8198 LIase. To confirm this issue, the transcriptional level of *LP*8198 LIase was detected by semi-PCR and qPCR with different fermentation times and substrate concentrations. As shown in Fig. 4, the mRNA level of LIase was upregulated depending on time and dose was time-dependently and dose-dependently upregulated by *ATB*-seed oil. When the *lactobacilli* were treated by 1 mg/mL *ATB*-seed oil for 24 h, the mRNA level of LIase could attain a value nearly 15 fold of that of the control group.

*c*9, *t*11-CLA and *t*10, *c*12-CLA could be biotransformed by *LP*8198 fermentation with *ATB*-seed oil

Accumulating evidence has demonstrated that *c*9, *t*11-CLA and *t*10, *c*12-CLA, two major isomers of CLA, have excellent biomedical properties for potential use in anticancer applications and for improving immunity, preventing inflammation, reducing obesity by different pathways such as Wnt/beta-catenin pathway, hormone-mediated mitogenic pathway, PPARγ, 5-lipoxygenase (5-LOX) pathway and NF-κB pathway [6–8, 12–15]. Although there was tremendous potential for the application of CLA isomers, their source of human daily intake was too limited to reach the recommended dosage, 3 g/d, which would be

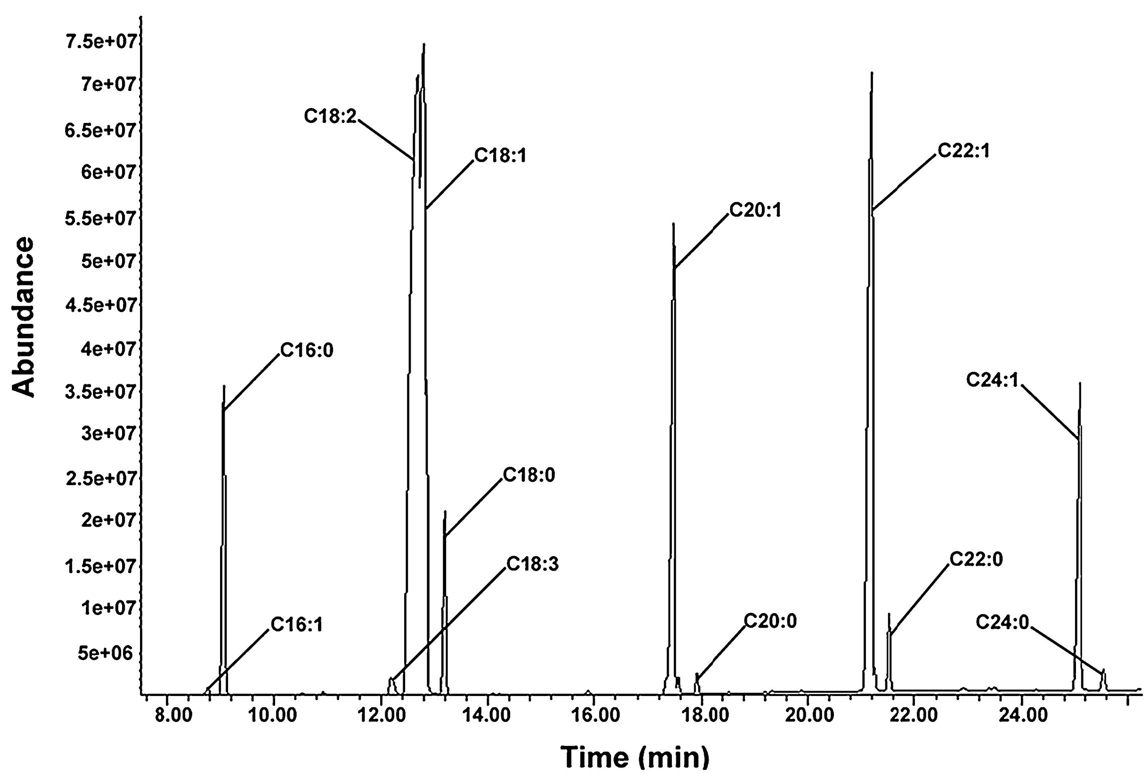

Fig. 3 GC–MS analysis of fatty acid content in *ATB*-seed oil

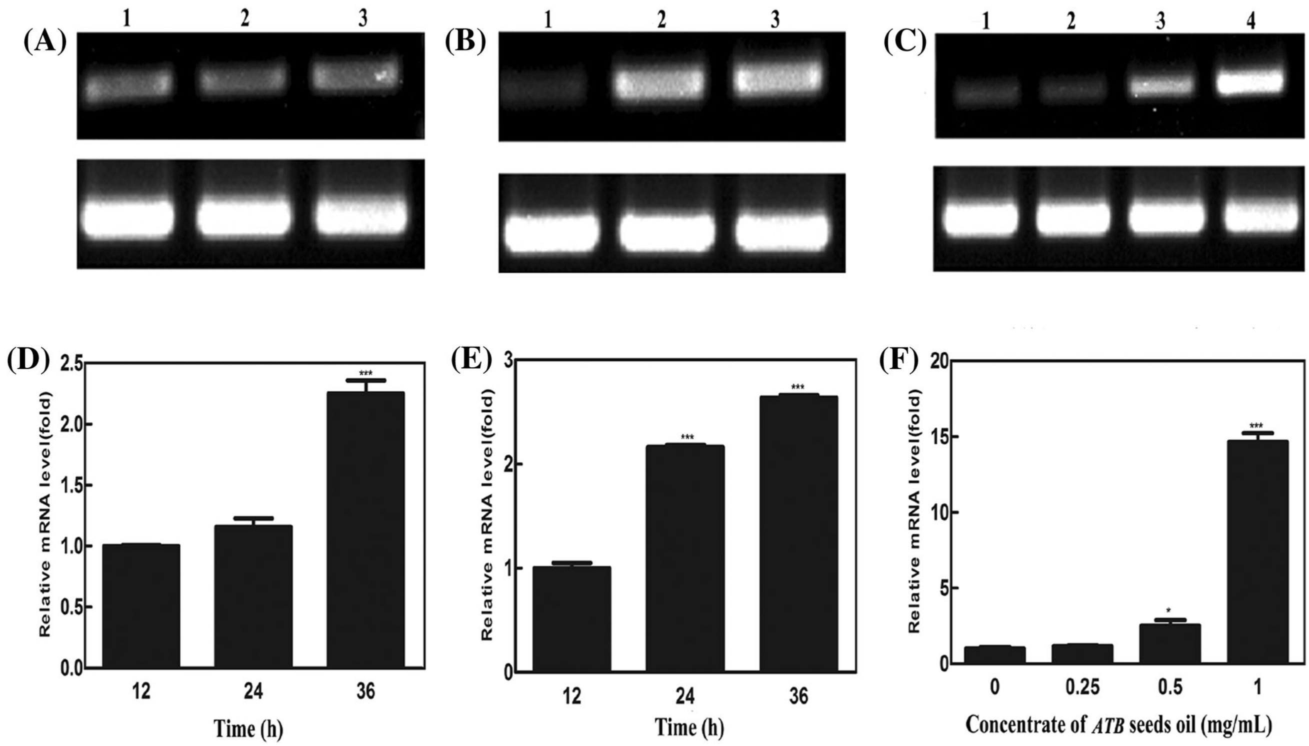

Fig. 4 The effects of *ATB*-seed oil on transcriptional level of *LP*8198 LIase. (**A**) The semi-PCR analysis of the transcriptional level of LIase in *LP*8198 without *ATB*-seed oils. Lanes 1–3 represent fermentation for 12, 24, and 36 h, respectively. (**B**) The semi-PCR analysis of the transcriptional level of LIase in *LP*8198 treated with 0.5 mg/mL *ATB*-seed oils for different times. Lanes 1–3 represent fermentation for 12, 24, and 36 h, respectively. (**C**) The semi-PCR analysis of the transcriptional level of LIase in *LP*8198 treated with different concentrations of *ATB*-seed oils for 24 h. Lanes 1–4 represent the treatments of *ATB*-seed oil at 0, 0.25, and 0.5 1 mg/mL, respectively. (**D**), (**E**), and (**F**) were detected by qPCR and the treatment was consistent with that of (**A**), (**B**), and (**C**)

required to observe health benefits in human subjects [16]. Thus, the development of production technology of these CLA isomers is very necessary.

Although CLA could be chemically synthesized, this method would produce mixed products, which might contain some unsafe ingredients [12]. Therefore, selective synthesis of CLA isomers by microbial transformation has received great interest. Nowadays, biosynthesis of CLA isomers, especially *c*9, *t*11-CLA and *t*10, *c*12-CLA, by linoleate isomerase in *Butyrivibrio fibrisolvens* and *Propionibacterium acnes*, has been well studied [17, 18]. Besides, a series of *lactobacillus* have also become the protagonist to produce *c*9, *t*11-CLA and *t*10, *c*12-CLA in the recent years. It has been reported that some *L. plantarum* strains could convert linoleic acid to *c*9, *t*11-CLA and *t*10, *c*12-CLA by LIase [18, 19]. Li and his colleagues analyzed CLA bioconversion by six *L. plantarum* strains cultured in MRS broth supplemented with sunflower oil, and the results showed that the production of CLA was increased by adding high concentration of substrate in sunflower oil, and *L. plantarum* IMAU60042 produced the highest CLA [20]. Besides, the study of Elaheh Sadat Hosseini had also shown that both sunflower oil and castor oil could be used as substrates for the production of *c*9, *t*11-CLA and *t*10, *c*12-CLA by Lactobacillus fermentation [21]. Here, to validate whether *LP*8198 could biotransform linoleic acid from *ATB*-seed oil into *c*9, *t*11-CLA and *t*10, *c*12-CLA, the concentration of these two CLA isomers in 0.5 mg/mL *ATB*-seed oil before and after *LP*8198 fermentation was detected by GC–MS. As shown in Fig. 5, according to the standard curves, the results showed that the concentration of *c*9, *t*11-CLA could be increased from 0.23 to 2.06 mg/mL by about ninefold and that of *t*10, *c*12-CLA could be increased from 1.68 to 3.79 mg/mL by about 2.25-fold.

In summary, here we discovered a new *lactobacillus* strain which might produce *c*9, *t*11-CLA and *t*10, *c*12-CLA during fermentation with *ATB*-seed oil, a kind of valuable edible oil which has noteworthy health benefits and has been authorized as a New Resource Food in China [9, 10]. To the best of our knowledge, this study was applied for the first time to *ATB*-seed oil for producing *c*9, *t*11-CLA and *t*10, *c*12-CLA by microbial fermentation. These findings might provide some new theoretical basis to develop a new

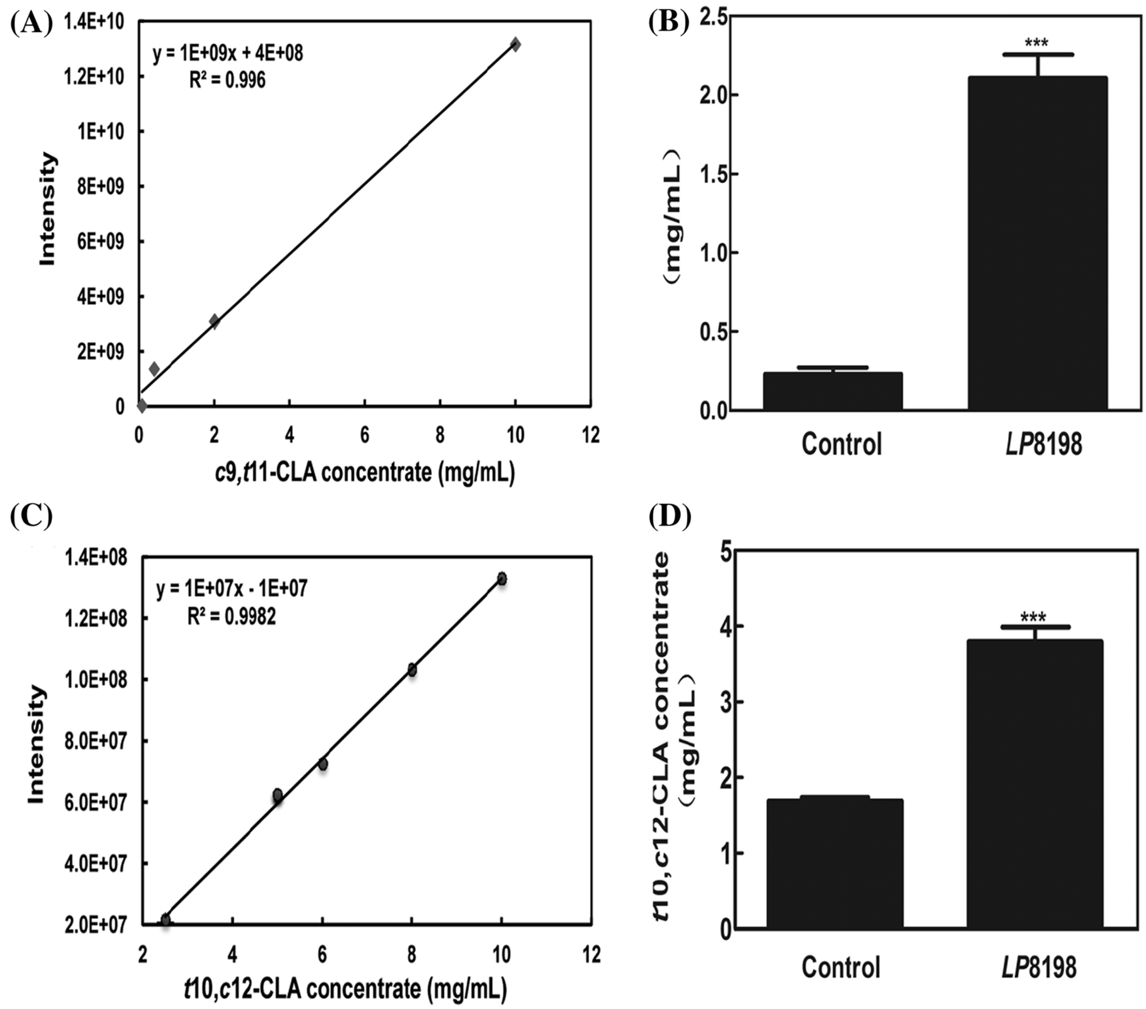

Fig. 5 The concentration of *c*9, *t*11-CLA and *t*10, *c*12-CLA in *ATB*-seed oil before and after being fermented using *LP*8198. (**A**) The standard curve of *c*9, *t*11-CLA detected by GC–MS. (**B**) The concentration of *c*9, *t*11-CLA in a 0.5 mg/mL *ATB*-seed oil emulsion before and after being fermented using *LP*8198 for 24 h. (**C**) The standard curve of *t*10, *c*12-CLA detected by GC–MS. (**D**) The concentration of *t*10, *c*12-CLA in a 0.5 mg/mL *ATB*-seed oil emulsion before and after being fermented using *LP*8198

resource for CLA isomers, and meanwhile, it also might contribute to new applications of *ATB*.

Acknowledgements This study was financially supported by the National Key R&D Program of China (2017YFD0400303), the 863 (Hi-tech research and development program of China) program under contract NO. 2012AA022108, the Tianjin Research Program of Application Foundation and Advanced Technology (14JCZDJC33200, 15JCQNJC45800), the State Key Laboratory of Tree Genetics and Breeding (Chinese Academy of Forestry) (TGB2016002), and the Program for Changjiang Scholars and Innovative Research Team in University of Ministry of Education of China (IRT1166).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Demir AS, Talpur FN. Chemoenzymatic conversion of linoleic acid into conjugated linoleic acid. J. Agric. Food Chem. 58: 1646–52 (2010)
2. Sun C, Black BA, Zhao YY, Ganzle MG, Curtis JM. Identification of conjugated linoleic acid (CLA) isomers by silver ion-liquid chromatography/in-line ozonolysis/mass spectrometry (Ag + -LC/O3-MS). Anal. Chem. 85: 7345–52 (2013)
3. Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 8: 1881–7 (1987)
4. Koba K, Yanagita T. Health benefits of conjugated linoleic acid (CLA). Obes. Res. Clin. Pract. 8: e525–32 (2014)
5. Brown JM, Boysen MS, Jensen SS, Morrison RF, Storkson J, Lea-Currie R, Pariza M, Mandrup S, McIntosh MK. Isomer-specific regulation of metabolism and PPARgamma signaling by CLA in human preadipocytes. J. Lipid. Res. 44: 1287–300 (2003)

6. Durgam VR, Fernandes G. The growth inhibitory effect of conjugated linoleic acid on MCF-7 cells is related to estrogen response system. Cancer Lett. 116: 121–30 (1997)
7. Yeganeh A, Taylor CG, Poole J, Tworek L, Zahradka P. Trans10, cis12 conjugated linoleic acid inhibits 3T3-L1 adipocyte adipogenesis by elevating beta-catenin levels. Biochim. Biophys. Acta. (2016)
8. Lehnen TE, da Silva MR, Camacho A, Marcadenti A, Lehnen AM. A review on effects of conjugated linoleic fatty acid (CLA) upon body composition and energetic metabolism. J. Int. Soc. Sports Nutr. 12: 36 (2015)
9. Zhao WH, Zhang JF, Zhe W, Zhang YX, Tian WX. The extract of leaves of *Acer truncatum Bunge*: A natural inhibitor of fatty acid synthase with antitumor activity. J. Enzyme Inhib. Med. Chem. 21: 589–96 (2006)
10. Zhao WH, Gao LF, Gao W, Yuan YS, Gao CC, Cao LG, Hu ZZ, Guo JQ, Zhang YX. Weight- reducing effect of *Acer truncatum Bunge* may be related to the inhibition of fatty acid synthase. Nat. Prod. Res. 25: 422–31 (2011)
11. Gu XC, Luo XG, Wang CX, Ma DY, Wang Y, He YY, Li W, Zhou H, Zhang TC. Cloning and analysis of bile salt hydrolase genes from *Lactobacillus plantarum* CGMCC No. 8198. Biotechnol. Lett. 36: 975–83 (2014)
12. Kelley NS, Hubbard NE, Erickson KL. Conjugated linoleic acid isomers and cancer. J. Nutr. 137: 2599–607 (2007)
13. Rosberg-Cody E, Stanton C, O'Mahony L, Wall R, Shanahan F, Quigley EM, Fitzgerald GF, Ross RP. Recombinant lactobacilli expressing linoleic acid isomerase can modulate the fatty acid composition of host adipose tissue in mice. Microbiology 157: 609–15 (2011)
14. Lee K, Paek K, Lee HY, Park JH, Lee Y. Antiobesity effect of trans-10,cis-12-conjugated linoleic acid-producing *Lactobacillus plantarum* PL62 on diet-induced obese mice. J. Appl. Microbiol. 103: 1140–6 (2007)
15. Shokryzadan P, Rajion MA, Meng GY, Boo LJ, Ebrahimi M, Royan M, Sahebi M, Azizi P, Abiri R, Jahromi MF. Conjugated Linoleic Acid: A Potent Fatty Acid Linked to Animal and Human Health. Crit. Rev. Food Sci. Nutr: 0 (2015)
16. Mushtaq S, Heather Mangiapane E, Hunter KA. Estimation of cis-9, trans-11 conjugated linoleic acid content in UK foods and assessment of dietary intake in a cohort of healthy adults. Br. J. Nutr. 103: 1366–74 (2010)
17. Gorissen L, Leroy F, De Vuyst L, De Smet S, Raes K. Bacterial production of conjugated linoleic and linolenic Acid in foods: a technological challenge. Crit. Rev. Food Sci. Nutr. 55(11): 1561–74 (2015)
18. Yang B, Chen H, Gu Z, Tian F, Ross RP, Stanton C, Chen YQ, Chen W, Zhang H. Synthesis of conjugated linoleic acid by the linoleate isomerase complex in food-derived lactobacilli. J. Appl. Microbiol. 117: 430–9 (2014)
19. Niu XY, Chen W, Tian FW, Zhao JX, Zhang H. Bioconversion of conjugated linoleic acid by resting cells of *Lactobacillus plantarum* ZS2058 in potassium phosphate buffer system. Acta Meteorol. Sin. 47: 244–8 (2007)
20. Li H, Liu Y, Bao Y, Liu X, Liu X, Zhang H. Conjugated linoleic acid conversion by six *Lactobacillus plantarum* strains cultured in MRS broth supplemented with sunflower oil and soymilk. J Food Sci 77: M330–6 (2012).
21. Hosseini, E.S, Kermanshahi, R.K, Hosseinkhani, S, Shojaosadati, S.A, Nazari, M. Conjugated linoleic acid production from various substrates by probiotic *Lactobacillus plantarum*. Ann. Microbiol. 65: 27–32 (2015).