

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

Dong-Ju Chen¹ · Li-Hua Yan¹ · Qian Li¹ · Cai-jiao Zhang¹ · Chuan-Ling Si^{2,3} ·
Zhong-Yuan Li¹ · Ya-Jian Song¹ · Hao Zhou¹ · Tong-Cun Zhang¹ ·
Xue-Gang Luo¹

Received: 21 December 2016/Revised: 2 May 2017/Accepted: 21 July 2017/Published online: 12 December 2017
© The Korean Society of Food Science and Technology and Springer Science+Business Media B.V. 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 (*LP8198*), a novel probiotics strain. Polymerase chain reaction results showed that there was a conserved linoleate isomerase (LIase) gene in *LP8198*, 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 *LP8198*.

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

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 PPAR γ 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 (*LP8198*), 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-

✉ 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

CLA and *t*10, *c*12-CLA in the fermentation of *LP8198* 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 $-80\text{ }^{\circ}\text{C}$ until further use. The strain of *Lactobacillus plantarum* CGMCC8198 (*LP8198*) 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% $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, and 0.005% $\text{MnSO}_4\cdot \text{H}_2\text{O}$ (pH 6.2 ± 1) under anaerobic conditions at $30\text{ }^{\circ}\text{C}$ for 24 h.

Total RNA extraction and RT–PCR

Prior to extraction of the total RNA of *LP8198* using a Trizol reagent, the seeds were ground in liquid nitrogen. Then $2\text{ }\mu\text{g}$ 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 $95\text{ }^{\circ}\text{C}$ followed by 30 cycles of denaturation ($95\text{ }^{\circ}\text{C}$), annealing ($52\text{ }^{\circ}\text{C}$), and elongation ($72\text{ }^{\circ}\text{C}$) for 20 s by using 1.25 U of Taq DNA polymerase (TransGen Biotech, BJ, CA). Besides, an additional $72\text{ }^{\circ}\text{C}$ 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 $95\text{ }^{\circ}\text{C}$ for 10 s and $60\text{ }^{\circ}\text{C}$ 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: *L*Iase, 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 $45\text{ }^{\circ}\text{C}$. 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 $45\text{ }^{\circ}\text{C}$ 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 $0.22\text{ }\mu\text{m}$ Minisart filter (Agilent) and stored in the dark at $-20\text{ }^{\circ}\text{C}$ before use.

Fermentation of *ATB*-seed oil by *LP8198*

LP8198 was inoculated (1%) in MRS broth with or without 0.5 mg mL^{-1} *ATB*-seed oil and then incubated anaerobically at $30\text{ }^{\circ}\text{C}$ 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 $4\text{ }^{\circ}\text{C}$ 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- CH_3OH at $65\text{ }^{\circ}\text{C}$ for 30 min, and then 1 mL $\text{BF}_3\text{-CH}_3\text{OH}$ was added to the reaction liquid at $70\text{ }^{\circ}\text{C}$ 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 $-20\text{ }^{\circ}\text{C}$.

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; $60\text{ m} \times 320\text{ }\mu\text{m} \times 0.25\text{ }\mu\text{m}$) with the following temperature program: initial temperature $50\text{ }^{\circ}\text{C}$, increased to $200\text{ }^{\circ}\text{C}$ at $10\text{ }^{\circ}\text{C}/\text{min}$ and $230\text{ }^{\circ}\text{C}$ at $2\text{ }^{\circ}\text{C}/\text{min}$, and then raised at $8\text{ }^{\circ}\text{C}/\text{min}$ to $270\text{ }^{\circ}\text{C}$ and held for 15 min. Besides, the inlet temperature was $270\text{ }^{\circ}\text{C}$ with constant flow of

Table 1 The Linoleate isomerase gene sequences from 9 strains

Gene Name	Sequence
Linoleate isomerase	<p>ATGGTTAAAAGTAAAGCAATTATGATTGGT GCCGGCTATCAAATATGGCTGGCGGCTACTTGATTCAAGATGGTCAATTGG GATGGTAAGGACATCACATTCTATGGTGTGATATGCACGGTGCCAATGATGG TGGTGCCACGACTGATTTTACTAATGAGTATTTGGAAATAAGCAATCAATCCGATGG CTAACACGACTGGTATGTTGCCCGGGTGGTCGGATGCTGAATTAACCGGACGT ACGTTGACTTAATGGATTTATTGGACCGGATCCATCGTTAACTGAACCGGGG ATGACGGCGCCGAAGATACCGGIGATTTTGTATGCGAAAACATCGGACGTATGA TATTGCCCGCTTGATGCAGGGTGGTAAAGGCATTAATATGCTGGTAAAGTTAG GATTCAAATAAAGGATCGGACTTTGCTGACTAAGTTGATTAATGATGCCAGAT AGTGAAGAAAACGAAGCTCGACAACGTTTTCGATTGCTGAGTACTTCAAGGATGA TCCGCATATGTTCCAAAACGAAATTTCTGGTATATGTGGGAAAACAACCTTTGCCT TTAGAACGCAAAGCTCTGCTCAAAGAACTCGCGCGTTACATGCATCAAATGATT TATGAATTTACAAAATTTGAACACTTAGTTGGTGTGCAACCGGACGCGTTACAA TCAATTCGAAAGCATGATTTTGGCATTAAATTAAGTACTTTGCAAGGGCAAGGTG TGACTTTCAATTGATAATAAGATTGTTAAGGATTTGGCAATTTAAAGACACGCCA ATGCAAGAAGAAATTCGGTACTGGCTTAGTCAATGAGGATGCGCAGACTGG CGAAACGGAAGAAGTTGAAAGTTGATGAGGACACAGCGGTGATCTTCACTAACG GTTCAATTAACCGATTCTGCAACGATGGTGAATACAACACGCGCTGCTCTGAA AATATGGATTATGGTGTAGTGTAGTTTGGGAAAGAGGCTACTGACGCGTT CTATACTTAGGGACGCCAGATAAGTTCTTCAACGATCGGGAATGCTAGCGAAT GGGTCAGCTTACCGTTGACGACTAAAGATCAATTTATCTTAAATGAATCGTT CGGATCACCAACCCAGGAAACCGGGAATGCGTTGAACTCCTTCTTATCAACTAC GCCAATACGCCGTTGAACCAAAGGATGTTAATATGTCGATCGTGGTGGACC ACCAACCACTTTACGACACAGCAACCAAACGAAACAGTTCTGTGGGGCTAC TTCTTGTATCCACGGCTCAAGGTGAGTTTGTAAACAAGCCGTATATCAAGAT GACGGTAAGGAAATGGCTCAAGAAATTAATGGTCAACTTTCCAAAGTAGATC CGGTCCAGGCAATTAAGGACAAGGAAAAGGAAATTTGGACAGTATTGTG AACAAATATCCGGTATACATGCCATATGCTTCCGCACTTTTAATAACCCGGC TAACTCTGATCGGCCAGAACTTACCAAGCACTCAACGAACTTAGCCCTTA CGGGTGAATTTGCGGAACAACCATACAGATGATCTTACCGGAACAGAGTGG GTCCGCTGTGGTGGATGCGCTTATCACTTGTGGGGTCCCAATGGATAA CTTGGTCAAGACACCCAGGTACGATAAGGATCCAAAGACCTTGTCTCAAGGCAAC TAAGAAGATGTTGATTA</p>

Table 1 continued

Gene Name	Sequence
<p>>gi13250483 2lemb FR732045.1 <i>Lactobacillus plantarum</i> gene for putative linoleate isomerase, strain ATCC 8014</p>	<p>GAACCTAATTAACCTATTTGGGGCGTTATTTA TGGTTAAAGAGTAAAGCAATTATGATTTGGTGCCGGGCTATCAAATATGGCTGGG CGGTCTACTTGAATCAAGATGGTCAATGGGATGGTAAGGACATCACATTTA TGGTTTGATATGCACGGTGCCAAATGGTGGTCCACGACTGATTTTACTA ATGAGTATTGGAATAAGAATCATCCGATGGATACACGACTGGGTATGTTGCC CGGGTGGTGGATGCTGAATACCAGGACGTACGTTGACTTAATGGATTTATT GGACCGGATTCATCGGTAACCTGAACCGGGGATGACGGCGCCGAAGATACGC GTGATTTTGTGCGAAACATCGGACGTATGATATTTGCCCGCTTGTATGCAGGGT GGTAAAAGCATTATTAATGCTGTAAGTTAGGATTCATTAATAAAGGATCGGAC TTTGTGACTAAGTTGATATGATGCCAGATGTAAGAAGCAAGCAAGCTCGACA ACGTTTCGATTTGCTGAGTACTTCAAGGATGATCCGCATATGTTCCAAACGAAAT TTCTGGTATATGTGGAAACAACTTTGCCTTTAGAACGCAAAAGCTCTGCTCA AGAACTGGCGGTTACATGCATCAAATGATTTATGAAATTTACACAAATTTGAAC ACTTAGTTGGTGTCAACCGGACGGTTACAATCAATCGAAAGCATGATTTTG CCATTAAATTAAGTACTTGCAGGGCAAGGTGTGACTTTCATTGATAATAAGAT TGTTAAGGATTTGGCAATTTAAAGACACGCCAATGCAAGACGAAATACGGTGA CTGGCTTAGTCAATTGAGGATGGCAGACTGGGAAACGGAAAGTTGAAAGTT GATGAGAAACAACGGGTGATCTTCACTAACGGTTCAATACCGATTCTGCAAC GATGGGTGATTACAACACCGCTGCTCTGAAATATGGATTTATGGTGTATGTTG CTAGTTTGTGGAAGAGGCTACTGAGGGTTCTATAACTTAGGGACGGCCAGAT AAGTTCTCAACGATCGGAATGCTACGGAATGGGTGAGTTTACCGTTGACGAC TAAGAAATCAATTTTCTTAAATGAAATCGTTCCGGATCACCCAGGAACCCCG GGAATGCGTTGAACTCCTTCTTATCAACTACGCCAAATACGCCGTTGAACCAA AAGGATGTTAATATGTCGATCGTGTGACCCACCACTTACGACACAG CAACCAAACGAAACAGTCTGTGGGGTACTTCTGTATCCACGGCGTCAAGG TGAGTTTGTAAACAGCCGTATATCAAGATGACGGGTAAAGGAAATGGCTCAAG AATTAATTGGTCAACTTCCAAAGTAGATCCGGTCCAGGCAATATTAAGGAC AAGGAAAGGAAATTTGGACAGTATTTGGAACAATATCCGGTATACATGCC ATATGCTCCGCACTTTTAAATAAACCGGGTAAAGTCTGATCGGCCAAGAGTCTT ACCAAACACTCAACGAACTAGCCTTTACGGGTGAATTTGCGGAACAACCCAT ACCAGATGATCTTACGGAAACAGAGTGGCGTCCGCTCTGGTGAATTTGGCGCT TATCACTTTGCTGGGGTCCCAATGGATAACTTGGTCAAGACACACGGTACGA TAAGGATCCAAAGACCTTGTCTCAAGGCAACTAAGAGATGTTTGAATTAATA ATGCTGGCTGTCAAGTGGTAAATGATGATGGCATGATTTAAACACGCTCC CCATGAAAAGATGACTTTCATAGGGAGCGTTTTTAGTGTGC</p>

Table 1 continued

Gene Name	Sequence
>gi300885403 gb HM569265.1 <i>Lactobacillus plantarum</i> linoleate isomerase (lin) gene, complete cds	ATGGTTAAAAGTAAAGCAATTATGATTTGGTGGCCGGG CTATCAAATATGGCTGGGGGCTACTTGTGATTCAAAGAGGGTCAATTGGGATGG TAAGGACATACATCTATGTTGTTGATATGCACGGTGCCAAATGATGGTGGTG CCACGACTGATTTTACCAATGAGTATTGGAATAGAAATCATCCGATGGCTAAC ACGACTGGGTATGTTCCCGGGTGGTCGGATGCTGAATACCCGGACGTACGT TGACTTAAATGGAATTTATGGACCGGATCCATCGTAACTGAAACCGGGGATGA CCGCGCCGAAAGATACGGGTGATTTTGTATGCGAAACATCGGACGTATGATATT GCGCGTTGATGCAAGGTGGTAAGGCATTAATACTGGTAAAGTTAGGATTT CAATAATAAGGATCGGACTTTGCTGACTAAGTTGATTAATGATGCCAGATAGTG AAGAAACGAAAGCTCGACAACTGTTGATTTGCTGAGTACTTCAAGGATGATCCG CATATGTTCCAAACGAAATTTCTGGTATATGTGGGAAACAACCTTTGCTTTAG AACGCAAGCTCTGCTCAAGAACTGGCGGTTACATGCATCAATGATTTATG AATTTACACAAATGAACTTAGTGTGGTGTCAACCGGACCGTTACAAATCAA TTCGAAAGCATGATTTGGCATAATTAAGTACTTTGCAAGGGCAAGGTGTGAC TTTCATTTGATAATAAGATTGTTAAGGATTTGCAATTTAAAGACACGCCAAATGC AAGACGAAATACGGTGACTGGCTTAGTCAATGAGGATGCGCAGACTGGCGAA ACGGAAAGAAAGTTGAAGTTGATGAGGACACAGCGGTGACTTTCACCTAACGGTTC AATTACCGATTCTGCAACGATGGGTGATTACAACCGCTGCTCCTGAAATA TGATTTAGGTGTTAGTGTAGTTGTTGGAAAGAGGCTACTGAGCGGTCTAT AACTAGGACGCCAGATAAGTTCTTCAACGATCGGAAATGCTAGCGAATGGGT CAGTTACCGTTGACGACTAAGAAATCATTATTCTTAAATGAAATCGTTCCGA TCACCCACCCAGGAAACCCGGGATGGTTGAACTCTTCTTATCAACTACGCCA ATTACGCCGTTGAACCAAAAGGATGTTAATATGTCGATCGTGGTGCAACCCA ACCACACTTTACGACACAGCAACCAACGAAACAGTTCTGTGGGGTACTTCT TGTATCCACGGGCTCAAGGTGAGTTGTTAACAAGCCGATATCAAGATGACG GGTAAAGAAATGGCTCAAGAAATTAATTTGGTCAAATTTCCAAAATGATCCGGG TCCAGGCAATTTAAGGACAAGGAAAGGAAATTTTGGACAGTATTTGTGAACA ATATCCGGTATACATGCCATATGCTTCCGCACTCTTTAATAACCCGGGCTAAG TCTGATCGGCCAGAAAGTTTACCAAAGCACTCAACGAACTAGCCCTTTACGGG TGAATTTGGGAAACAACCATACCAGATGATCTTCAACGGAACAGAGTCCGGTCC GCTCTGGTGGATTTGCCGTTATCAGTTTGTGGGTCGCCAAATGGATAACTTG GTCGAAGACACCACGGTACGATAAGGATCCAAAGACCTTGTCTCAAGGGCAACTAA GAAGATGTTGATTA

Table 1 continued

Gene Name	Sequence
>gi319657088 gb HQ831447.1 <i>Lactobacillus plantarum</i> strain Ip15-2-1 linoleic acid isomerase gene, complete cds	<p>ATGGTTAAAAGTAAAAGCAATTATGATTTGGTGCCGG GCTATCAAATATGGCTGGGGGCTACTTGAATCAAGAGGGTCAATGGGATG GTAAGGCACATCACAATCTATGGTGTGATATGCACGGTGCCAAATGATGGTGT GCCACGACTGATTTTACCAATGAGTATTTGGAATAAAGATCATCCGATGGCTAA CAGACTGGTATGTTGCCGGGTGGTCCGATGCTGAATACCCGGACGTACG TTGACTTAATGGATTTATGGACCGGATTCATCGGTAACCTGAAACCGGGGATG ACGGCGCCGAAGATACGCGTGAATTTGATGCGAAACATCGGACGTATGATAT TGCCCGCTTGATGCAGGGTGAAGGCAATTAATGCTGGTAAGTTAGGAT TCAATAAAGGATCGGACTTTGCTGACTAAGTTGATATGATGCCAGATAGT GAAGAAACGAAGCTCGACAACGTTTCGATTTGAGTTCAGGATCTCAAGGATGATCC GCATATGTTCCAAACGAATTTCTGGTATATGTGGGAAACAACCTTTGCTTTTA GAACGCAAGCTCTGCTCAAGAAGCTCGGGCTTACATGCATCAATGATTTAT GAATTTACACAATTTGAACACTTATGTTGGTCAACCCGGACGCTTACAAATCA ATTCGAAAGCATGATTTGGCATAAATTAAGTACTTTGCAAGGGCAAGGTGTGA CTTTCATTGATAATAAGATTGTTAAGGATTTGGCAATTTAAAGACACGCCAATG CAAGACGAAATACGGTGACTGGCTTAGTCAATGAGGATGCGCAGACTGGCGAA ACGGAAAGAAAGTTGAAGTTGATGAGGACACAGCGGTGATCTTCACTAACGGTTC AATTACCGATTCGCAACGATGGGTGATTAACAACGCTGCTCCTGAAATA TGATTAATGGTGTAGTGTAGTTGTTGGGAAAGGCTACTGAGCGGTCTAT AACTTAGGACGCCAGATAAGTTCTTCAACGATCGGAAATGTAAGCAATGGGT CAGCTTACGTTGACGACTAAGAAATCATTATTCTTAAATGAAATCGTTCGGA TCACCCAGGAAACCCGGGATGGTTGAACTCTTCTTCAACTACGCCAA TTACGCCGTTGAACCAAAAGGATGTTAATATGTCGATCGTGGTGCACCCAA CCACACTTACGACACAGCAACCAACGAAACAGTTCTGTGGGGTACTTCTT GTATCCACGGGTCAAGGTGAGTTTGTAAACAAAGCCGATATCAAGATGACGG GTAAGGAAATGGCTCAAGAAATTAATGGTCAACTTTCCAAAGTAGATCCGGGT CCAGGCAATTAAGGACAAGGAAAGGAAATTTGGACAGTATTTGTAACAA TATTCGGTATACATGCCATATGCTTCCGACTCTTAAATACCCGGGCTAAGT CTGATCGGCCAAGAGTCTTACCAAGGACTCAACGAACTAGCCTTTACGGGT GAATTTGCGGACAACCATACCAGATGATCTTCAACGGAAACAGATGCGGTCCG CTCTGGTGAATGCGCTTATCATTGCTGGGGTCCCAATGGATAACTTGG TCAAGACACCACGGTACGATAAGGATCCAAAGACCTTTGCTCAAGGCAACTAAG AAGATGTTGACTAA</p>

Table 1 continued

Gene Name	Sequence
<p>>gi3250483.1 lemb FR732048.1 <i>Lactobacillus plantarum</i> gene for putative linoleate isomerase, strain LMG 6907</p>	<p>GAACCTAAATTAACCTATTTTGGGGGGCTTATTT ATGGTTAAAAGTAAAGCAATATGATGGTGCCGGGCTATCAAATATGGCTGC GCGGCTACTTGAATCAAGATGGTCAATGGGATGGTAAGGACATCACATCT ATGGTGTGATATGCACGGTGGCAATGATGGTGGTGGCCACGACTGATTTTACC AATGATATTGGAATAAGAAATCATCCGATGGCTAACACGACTGGGTATGTTGC CCGGGTGGTCGGATGCTGAATACCGGACGTACGTTGACTTAAATGGATTTAT TGGACCGGATTCATCGGTAACTGAACCCGGGATGACGGCCGGCCGAAGATACG CGTGATTTTGTGCGGAACAATCGGACGTATGATATGGCCCGCTTGTATGCAGGG TGGTAAAGGCATTTAATGCTGGTAAAGTTAGGATCAATAATAAGGATCGGA CTTTGTGACTAAGTTGATCATGATGCCAGATAGTGAAGAAACGAAAGCTCGAC AACGTTTCGATTTGCTGACTACTTCAAGGATGATCCGCATATGTTCCAAACGAA TTCTGGTATATGTGGAAACAACCTTTGCCCTTAGAACGCAAA GCTCTGCTC AAGAACTGCGGGCTTACATGATCAAAATGATTTATGAATTTACACAATTTGAA CACTTAGTTGGTGTCAACCGGACGGTTACAATCAATTCGAAAGCATGATTTT GCCATTAATTAAGTACTTGC AAGGCAAGGTGTGACTTTTCATTGATAATAAGA TTGTTAAGGATGGCAATTTAAAGACACGCCAATGCAAGACGAAATTACGGTG ACTGGCTTAGTCAITTAGGATGGCAGACTGGCGAAACGGAAGAAGTTGAAAGT TGATGAGGACACAGCGGTGATCTTCACTAACGGTTC AATTACCGGATTTGCAA CGATGGGTGATTACAACACGCCCTGCTCTGAAATATGGATTTATGGTGTAGT GCTAGTTTGTGGAAGAAGGCTACTGAGGGTTCTATAACTTAGGGACGCCAGA TAAGTTCTCAACGATCGGAATGCTAGCGAATGGTTCAGCTTACCGTTGACGA CTAAGAAATCATTTTAAATGAAATCGTTCGGATCACCCACCCAGGAACCC GGGAA TGGGTTGAACTCCTTCTTATCAACTACGCCAATTAACGCCGTTGAACCA AAAGGATGTTAATATGTCGATCGTGGTGCACCAACCAACACCTTTACGACAC AGCAACCAACGAAACAGTTCGTGGGGCTACTTCTGTATCCACGGCGTCAA GGTGATTTGTTAACAGCCGTATATCAAGATGACGGGTAAAGGAAATGGCTCA AGAATTAATGGTCAACTTCCAAAGTAGATCCGGTCCAGGCAATATTAAGG ACAAGGAAAAGGAAATTTGGACAGTATCGTGAACAATATCCGGTATACATG CCATATGCTTCCGCACTTTAATAA CCGGGCTAAGTGTGATCGGCCAGAAGT CATAACAGATGATCTTACGGAAACAGTGGCGTCCGCTCTGGTGAGATTGCC GCTTATCCTTTTGGTGGGGTCCCAATGGATAA CTTGGTCAAGACACCCAGGTA CGATAAGGATCCAAAGACCTTGTCTCAAGGCAACTAA GAAAGATGTTTGAATTAAT TAAATGCTGGCCTGTCAGTGGTTAATGCTGATGATGGCATGATGATAAAAACGC TCCCCATGAAA GATGACTTTTCAATAGGGAGCGGTTTTAGTGTGC</p>

Table 1 continued

Gene Name	Sequence
>gi13250483.14 emblFR732046.1 <i>Lactobacillus plantarum</i> gene for putative linoleate isomerase, strain IMDO 130201	AGAACCTAATTACCTATTTTGGGGGGCGTTATTT ATGGTTAAAAGTAAAGCAATATGATGGTGCCGGGCTATCAAATATGGCTGC GGCGTCTACTTGATTCAGAGATGGTCAATGGGATGGTAAAGGACATCACATCTA TGGTGTGATATGCACGGTGGCAATGATGGTGGTCCACGACTGATTTTACCA ATGAGTATTGGAATAAGAATCATCCGATGGTAAACAGACTGGGTATGTTGCC CGGGTGGTGGATGCTGATACCCGGACGTGACTTAAATGGATTTATT GGACCGGATTCATCGTAACCTGAACCGGGGATGACGGCGCTGAAGATACGC GTGATTTTGGTAAACATCGGACGTATGATATGATTTGCCCGCTGATGCAGGGT GGTAAAAGCATTATTAATGCTGTAAGTTAGGATTCATATAAAGGATCGGAC TTTGTGACTAAGTTGATATGATGCCAGATGAGAAACGAAAGCTCGAC ACGTTTCGATTTGCTGATCTTCAAGGATGATCCGCATATGTTCCAAACGAAAT TTCTGGTATATGTGGAAACAACTTTGCCCTTAGAACGCAAAAGCTCTGCTCA AGAACTGGCGGCTTACATGCAATAATGATTTATGAAATTTACACAAATTTGAAAC ACTTAGTTGGTGTCAACCGGACGGTTACAATCAATTCGAAAGCATGATTTTG CCATTAAATTAAGTACTTGCAGGGCAAGGTGTGACTTTCATTGATAATAAGAT TGTTAAGGATTTGGCAATTTAAAGACACGCCAATGCAAGACGAAATACGGTGA CTGGCTTAGTCAATTGAGGATGGCAGACTGGGAAACGGAAAGATTGAAAGTT GATGAGACACAGCGGTGATCTTCACTAAACGGTTCAATACCGATTCTGCAAC GATGGGTGATTAACAACCGCTGCTCTGAAATATGGATTTATGGTGTATGTG CTAGTTTGTGGAAGAGGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGAT AAGTTCTCAACGATCGGAATGCTAGCAATGGTCAAGTTTCAAGTTGACGAC TAAGAAATCAATTTGTTTAAATGAAATCGTTCCGATCACACCCAGGAACCCG GGAATGCGTTGAACTCCTTTATCAACTACGCCAATTTACGCCGTTGAACCAA AAGGATGTTAATATGTCTATCGTGTGACACCACCACTTTACGACACA GCAACCAAAACGAAACAGTTCTGGGGCTACTTCTGTATCCACGGCGTCAAG GTGAGTTTGTAAACAAGCCGTATATCAAGATGACGGGTAAAGAAATGGCTCAA GAAATTAATGGTCAACTTCCAAAGTAGATCCGGGTCCAGGCAATATTAAGGA CAAGGAAAAGGAAATTTGGACAGTATTTGAAACAATATCCGGTATACATGC CATATGCTTCCGCACTTTTAAATAACCCGGGCTAAGTCTGATCGGCCAGAAATC TTACCAAGCACTCAACGACCTAGCCCTTACGGGTGAAATTTGGGAAACAAAC ATACCAGATGATCTTACGGAAACAGAGTGGGTCCTGCTGTTGGGATTTGCCG CTTATCACTTTGCTGGGTCCCAATGGATAACTTTGGTCAAGACACCGGTAC GATAAGGATCCAAAGACCTTGTCTCAAGGCACTAAAGAGATGTTTGTATTAAT AAATGCTGGCCTGTCAGTGGTTAATGCTGATGGCATGATTTAAAAACGCT CCCCATGAAAAGATGACTTTTCAATAGGGAGCGT

Table 1 continued

Gene Name	Sequence
<p>>gi333037510 gbJF747255.1 <i>Lactobacillus plantarum</i> strain ZS2058 putative linoleate isomerase gene</p>	<p>ATGGGGCGGTTATTTATGGTTAAAAAGTAAAG CAATTATGATGGTCCGGCTATCAAATATGGTGGGGGGTCTACTTGATT CAAGATGGTCAATGGGATGGTAAGGACATCACATCTATGGTGTGATATGCA CGGTGCCAAATGATGGTGGCCACGACTGATTTACCAATGAGATTTGGAAAT AGAATCATCCGATGGTAACACGACTGGGATGTTGCCCGGGTGGTCCGGATG CTGAATTACCGGACGTACGTTGACTTAATGGATTTATGGACCGGATCCCATC GGTAACTGAACCGGGGATGACGGCGCTGAAGATACGCGTGAATTTGATGCCGA AACATCGGACGTATGATTTGCCCGCTTGATGCAGGGTGGTAAAGGCCATTAAT AATGCTGGTAAGTTAGGATTCATAATAAAGATCGGACTTGTGCTGACTAAGTT GATCATGATGCCAGATAGTGAAGAACGAAAGCTGCACAACTTCTGATTTGCTG AGTACTTCAAGGATGATCCGCATATGTTCCAAACGAAATTTCTGGTATATGTGG GAAACAACCTTGGCTTGAACGCAAGCTCTGCTCAAGAAGCTGGGGCGTTA CATGCATCAAATGATTTATGAATTTACACAAATGAAACACTTAGTTGGTGTCA ACCGACCGGTTACAATCAATTCGAAAGCATGATTTTGGCCATTAATTAAGTAC TTGCAAGGGCAAGGTGACTTTCATTTGATTAATAGATTTAAAGGATTGCCA ATTTAAAGACACGCCAATGCAAGACGAAATTCGGTACTGGCTTAGTCAATTGA GGATGGCAGACTGGCGAAACGGAAGAGTTGAAATTTGATGAGGACACAGCGG TGATCTTCACTAACGGTTCAATACCGATTTCTGCAACGATGGGTGATTAACAAC ACGCTGCTCCTGAAAATATGGATTAATGGTGTAGTGTAGTGTGGGAAGAA GGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGATAAGTCTTCAACGATC GGAATGCTAGCGAATGGTCAAGTTCACGTTGACGACTAAGAATCATTTATTC TTAATGAAATCGTTCGGATCACCCAGGAACCCGGGAATGCGTTGAACCTC CTTCTTATCAACTAGCCAAATACGCCGTTGAACCAAAAGGATGTTAATATGT CGATCGTGTGCACCCACCACTTACGACACAGCAACCAACCAACCGAAACA GTTCTGTGGGGTACTTCTTGTATCCACGGCTCAAAGGTGAGTTTGTAAACAA GCCGTATATCAAGATACGGGTAAAGGAAATGGCTCAAGAAATTAATTTGGTCAAC TTTCCAAAGTAGATCCGGTCCAGGCAATATTAGGACAAGGAAAAGGAATTT TTGGACAGTATCGTGAACAATAATCCGGTATACATGCCATATGCTTCCGCAC CTTTAAACCCGGGCTAAGTGTGATCGGGCCAGAAAGTCTTACCAGGACTCAA CGAACCTAGCCTTACGGGTGAAATTTGGGAAACAACCATACCAGATGATCTTC ACGGAAACAGAGTCCGGTCCGCTCTGGTGAAGATTTGCCCTTATCACCTTGTCTGG GGTCCCAATGGATAACTTGGTCAAGACACCGGTACGATAAGGATCCAAAGA CCTTGCTCAAGGCAACTAAGAAGATGTTTGTAT</p>

Table 1 continued

Gene Name	Sequence
>gi325048316 emblFR732047.1 <i>Lactobacillus plantarum</i> gene for putative linoleate isomerase	<p>CAGAACCTAATTACCTAATTTGGGGGCGGT TATTTATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGGCTATCAAAATATG GCTCGGGGGTCTACTTGGATTCAAGATGGTCAATGGGATGGTAAGGACATCAC AATTTAIGGTGTGATATGCACGGTGCGAATGATGGTGGTCCAGACTGAT TTACCAATGAGTATGGGAATAAGATCATCCGATGCGTAAACAGGACTGGGTAT GTTGCCGGGGTGGTGGATGCTGAATACCGGACGTACGTTACTTAATGGA TTTATTGGACCGGATTCATCGTAACTGAAACCGGGATGACGGGGCCGGAAG ATACGGGTGATTTGATGCGAAACATCGGACGTATGATATTGCCCGTTGATG CAGGTGTTAAAGGCATTTAATGCTGTTAAGTTAGGATTCATAATAAAGGA TCGGACTTTGCTGACTAAAGTTGATCATGATGCCAGATAGTGAAGAAACGGAAGC TCGAAACGTTTCGATTTGCTGAGTACTTCAAGGATGATCCGCAATATGTTCCAA ACGAATTTCTGGTATATGTTGGAAACAACTTTGCCTTTAGAACGCAAAAGCTC TGCTCAAGAACTGCGGGCTTACATGATCAATAATGATTTAAGAAATTCACAAA TTGAACACTTAGTTGGTTC AACCGGACGGTTACAATCAATTCGAAAGGATG ATTTGGCCATTAATAAGTACTTGC AAGGCAAGGTGACTTTCATTTGATAA TAAGATTGTTAAGGATGGCAATTTAAAGACACGCCAATGCAAGACGAAATTA CGGTGACTGGCTTAGTCAATTGAGGATGCGCAGACTGGCAACCGAAAGAAAGTT GAAATTGATGAGGACACAGCGGTGATCTTCACTAACGGTTCAATACCGGATTC TGCAACGATGGGTGATTAACAACACCGCTCTCTCTGAAAATATGGATTAATGGTG TTAGTGCTAGTTTGTGGAAGAAAGGCTACTGAGCGGTTCTATAACTTAGGGACG CCAGATAAGTTCTTCAACGATCGGAATGCTAGCGAATGGTCAAGTTCACGTT GACGACTAAGAAATCAATTTAATCTTAAATGAAATCGTTCGGATCACCCACGAGG AACCCGGAAATGCGTTGAACTCCTTCTTATCAACTACGCCAATACGCCGTTG AACCAAAAGGATGTTAATATGTCGATCGTGTGTCACCAACCAACACACTTAC GACACAGCAACCAACGAAACAGTTCGTGGGGTACTTCTTGTATCCACGGC GTC AAGGTGAGTTGTTAACAAAGCGGTATATCAAGATGACGGGTAAAGAAATG GCTCAAGAATTAATGGTCAACTTCCAAAGTAGATCCGGGTCCAGGCAATAT TAAGGACAAGGAAAGAAATTTAGACAGTATCGTGAACAATATCCGGTAT ACATGCCATATGCTTCGGCACTTTAATAACCGGGCTAAGTCTGATCGGCCA ACAACCATACAGATGATTCACCGAACAAGTGCCTTACGGGTGAATTTGCGGA TTGCCGTTATCACTTTGCTGGGTCCCAATGGATAACTTGGTCAAGACACCA CGGTACGATAAGGATCCAAAGACCTTGTCTCAAGGCAACTAAGAAGATGTTTGA TTAATTAATGCTGGCCTGTCAGTGGTTAATGCTGATGATGGCATGATGATAAA AACGCTCCCAATGAAAGATGACTTTTATAGGGAGCGGTTTATAGTGT</p>

Table 1 continued

Gene Name	Sequence
<p>>gi77819928 gb DQ227322.1 <i>Lactobacillus plantarum</i> strain AS1.555 linoleate isomerase gene</p>	<p>TTGGCGGCTTATTTATGGTTAAAAAGTA AAGCAATTATGATTGGTCCGGGTATCAAAATATGGCTCGGGGGTCTACTTGG ATTC AAGAGGGTCAATGGGATGGTAAGGACATCACATCTATGGTGTGATAT GCGCGTGC AATGATGGTGGTCCAGACTGATTTTACCAATGAGTATTTGGA ATAAGAATCATCCGATGGCTAATCGACTGGGTATGTTCCCGGGGTGGTCCG ATGCTGAATACCGGACGTACGTTGACTTAAATGGAATTAATGGACCGGATCC ATCGGTAAC TGAACCGGGATGACGGGGCCGACATACGCTGATTTTGGATG CGAAACATCGGACGTATGATTTGCCCGTTGATCGAGGTGGTAAAGGCATTT ATTAATGCTGGTAAAGTTAGGATTC AATAAAGGATCGGACTTTGCTGACTCA GTTGATTAATGATGCCAGATAGTGAAGAAACGAACTGCAACCGTTCCGATTTG CTGAGTACTTCAAGGATGATCCGATATGTTCCAAACGAAATTTCTGGTATATG TGGGAAACAACCTTTGCCTTTAGAACGCAAACTCTGCCCCAAGAACTGCGGGC TTACATGCATCAAAATGATTTATGAAATTTACAAATTTGAACTAGTGGTGGT TCAACCGGACGGTTACAATCAATTCGAAAGCATGATTTGGCCATTAATAAG TACTTGCAAGGGCAAGGTGTGACTTCCATTTGATATAAGATTTGTTAAGGATG GCAATTTAAAGACACGCCAATGCAAGACGAAATTAACGGTGACTGGCTTAGTCA TTGAGGATGCCAGACTGGCGAAA CCGGAA GAAAGTTGAAGTTGATGAGGACACA GCGGTGATCTTCACTAACGGTTCAAT ACCGATTTCTGCAACGATGGGTGATTA TAAACCGCTGCTCCTGAAAATATGGAATATGGTATAGTGTAGTGGTGGGA AGAAGGCTACTGAGCGGTTCTATAACTTAGGGACGCCAGATAAGTTCCTCAC GATCGGAATGCTAGCGAATGGGTACGCTTCA CGTTGACGACTAAGAAATCAAT ATCTTAAATGAAATCGTTCGGATCACCCAGGAAACCCGGGAATGCGTTGA ACTCCTTTATCAACTACGCCAATTA CGCCGTTGAA CCAAAGGATGTTAAT ATGTCGATCGTGGTGCACCAACCACTTTACGACACAGCAACCAACCGA AACAGTTCTGTGGGGTACTTCTGTATCCACGGCTCAAGGTGAGTTGGTGA ACAAGCCGTATATCAAGATGACGGGTAAAGGAAATGGCTCAAGAAATTAATGGTC AACTTTCCAAAGTAGATCCGGTCCAGGCAATATTAGGACAAGGAAAGGAAA TTTTGGACAGTATTGTGAACAATATCCGGTATACATGCCATATGCTTCCGCA CTCCTTAATAACCGGGCTAAGTCTGATCGGCCAGAAAGTCTTACCAAAGCATC AACGAACTTAGCCTTTACGGGTGAATTTGGGAAACAACCATACCAGATGATCT TCACGGAAACAGAGTGGTCCGCTCTGGTGAATTTGCCGTTATCACTTTGCT GGGGTCCCAATGGATAA CTTGGTCAAGACACCGGTA CGATAAGGATCCAAA GACCTTGCTCAAGGCCAACTAAGAAAGATGTTTGATTA</p>

```

10      20      30      40      50      60      70      80      90      100     110     120
1      ATGGTTAAAAGTAAAGCAATTATGATTGGTGCCGGGCTATCAAATATGGCTGCGGGCTACTTGTGATTCAGATGGTCATTGGGATGGTAAGGACATCACATTCTATGGTGTGATATG
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    CACGGTGCCAAATGATGGTGGTCCACGACTGATTTTACTAATGAGTATTGGAATAAGAATCATCCGATGGCTAACACGACTGGGTATGTTGCCCGGGGTGGTCCGGATGCTGAATTACCGG
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    ACGTAGGTTGACTTAATGGATTATTGGACCGGATCCATCGGTAACGACCGGGGATGACGGGGCCGAAGATACGCGTGTATTTGATGCGAAACATCGGACGTATGATATTGCCCGC
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    TTGATGCAGGGTGGTAAAGCCATTATTAATGCTGGTAAGTTAGGATTCATAATAAGGATCGGACTTTGCTGACTAAGTTGATTATGATGCCAGATAGTGAAGAACGAAGCTCGACAAC
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    GTTCGATTGCTGAGTACTTCAAGGATGATCCGCATATGTTCCAAACGAATTTCTGGTATATGTGGGAAACAACCTTTGCGCTTTAGAACGCAAAGCTCTGCTCAAGAARTCGCGGCTTAC
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    ATGCATCAAATGATTTATGAATTTACACAAATGAACACTTAGTTGGTGTCAACCGGACGCGTTACAAATCAATTCGAAAGCATGATTTTGCCATTAAATGAAGTACTGCAAGGGCAAGGT
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    GTGACTTTCATTGATAATAAGATTGTTAAGGATTGGCAATTTAAAGACACGCCAATGCAAGACGAAATACGGTACTGGCTTAGTCATTGAGGATGCCAGACTGGCGAAACGGAAGAA
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    GTTGAAGTTGATGAGACACAGCGGTGATCTTCACTAACCGGTTCAATACCGATTTCGCAACGATGGGTGATTACAACACGCTGCTCTGAAAATATGATTATGGTGTAGTGTAGT
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    TTGTGGAAGAAGGCTACTGAGCGGTTCTATACTTAGGGACGCGAGATAAGTTCTTCAACGATCGGAATGCTAGCGAATGGGTGAGCTTACCGTTGACGACTAAGAATCATTATTCTTA
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   AATGAATCGTTCGGATCACCCACCGAACCAGGAAACCGGGAAATCGGTTGAACCTCTTCTTATCAACTACGCCAATACCGCGTTGAACCAAAGGATGTTAATATGTCGATCGTGGTGCACCAC
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   CAACCACACTTTAGACACAGCAACCAACGAAACAGTTCTGTGGGCTACTTCTGTATCCACGGGCTCAAGGTGAGTTTGTAAACAAGCCGTATATCAAGATGACGGGTAAAGAAATG
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   GCTCAAGAAATTAATGGTCAACTTCCAAAGTAGATCCGGGTCAGGCAATATTAAGGACAAGGAAAGAAATTTGGACAGTATTGTGAACAATATCCCGGTATACATGCCATATGCT
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   TCCGCACCTTTAATAACCGGGCTAAGTCTGATCGGCCAGAAGTCTTACCAAAGCACTCAACGAACCTAGCCTTACGGGTGAATTTGCGGAACACCATACAGATGATCTTACGGAA
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   CAGAGTCCGGTCCGCTCTGGTGGATGCGCGCTTATCACTTTGCTGGGTCCTCAATGGATAACTTGGTCAAGACACCCGCTACGATAAGGATCCAAAGACCTTGTCTCAAGGCACTAAG
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   AAGATGTTGATTAA
561   K M F D *

```

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

nitrogen (N₂) 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

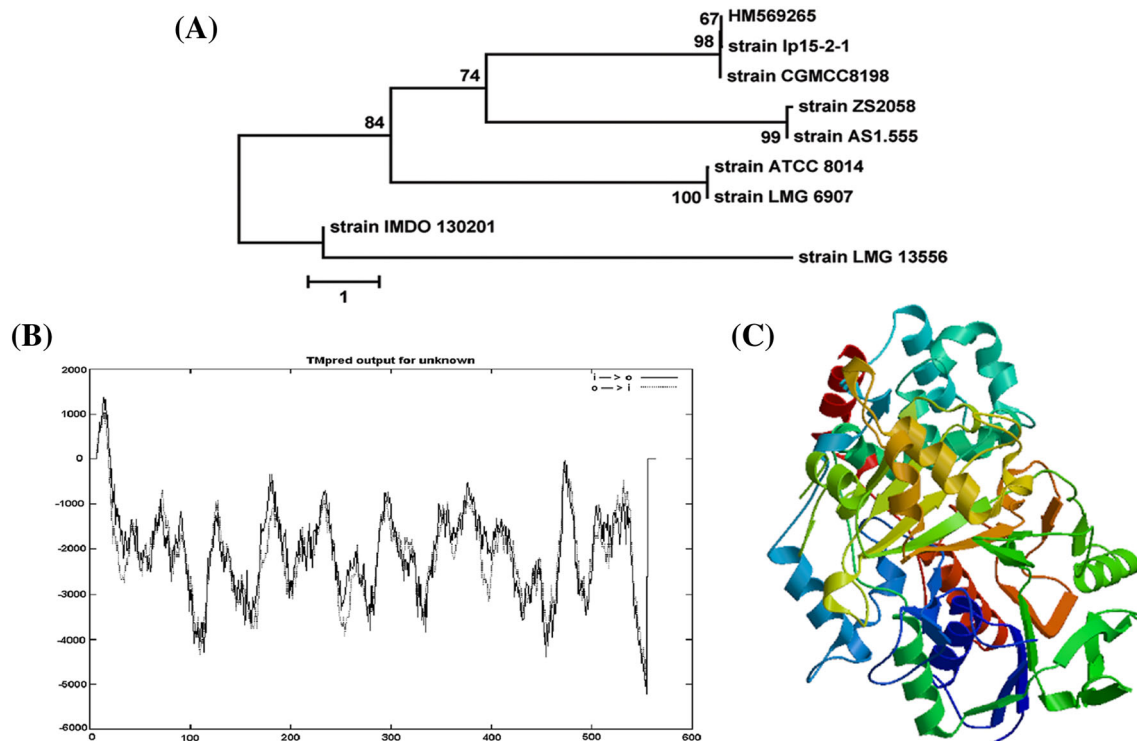


Fig. 2 Bioinformatic analysis of linoleate isomerase in *LP8198*. (A) Phylogenetic tree analysis of a new gene from *LP8198*. (B) The possible transmembrane helices structure analysis of LIase in *LP8198*. (C) Tertiary structure prediction of LIase by the SWISS-MODEL based on homology modeling

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 *LP8198*

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 *LP8198* 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 *LP8198* 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 Server 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 *LP8198* by the TargetP 1.1 Server

Name	Length	Location	RC ^a
Linoleate isomerase	564	Secretory pathway	4

^aRC 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: $\text{diff} > 0.800$; 2: $0.800 > \text{diff} > 0.600$; 3: $0.600 > \text{diff} > 0.400$; 4: $0.400 > \text{diff} > 0.200$; 5: $0.200 > \text{diff}$. Thus, the lower the value of RC indicates the safer the prediction

ATB-seed oil induced the transcription of *LP8198* 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 *LP8198* LIase. To confirm this issue, the transcriptional level of *LP8198* 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.

c9, t11-CLA and *t10, c12*-CLA could be biotransformed by *LP8198* fermentation with *ATB*-seed oil

Accumulating evidence has demonstrated that *c9, t11*-CLA and *t10, c12*-CLA, two major isomers of CLA, have excellent biomedical properties for potential use in anti-cancer applications and for improving immunity, preventing inflammation, reducing obesity by different pathways such as Wnt/beta-catenin pathway, hormone-mediated mitogenic pathway, PPAR γ , 5-lipoxygenase (5-LOX) pathway and NF- κ B 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

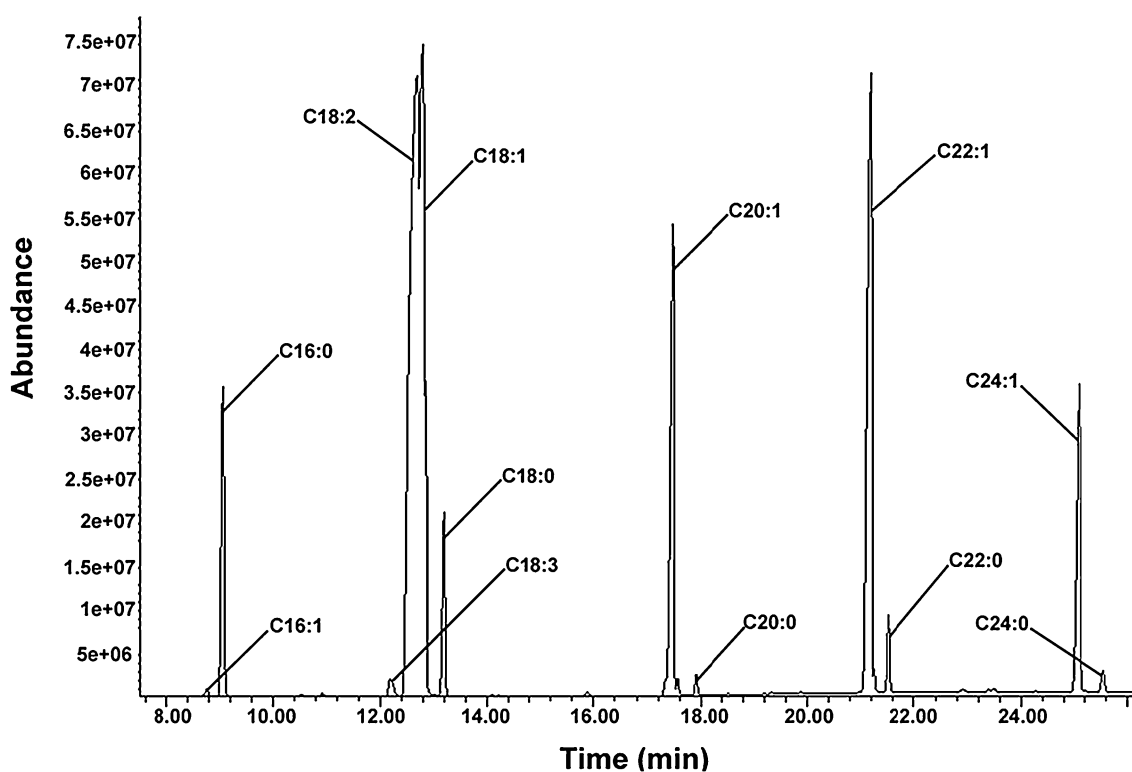


Fig. 3 GC–MS analysis of fatty acid content in *ATB*-seed oil

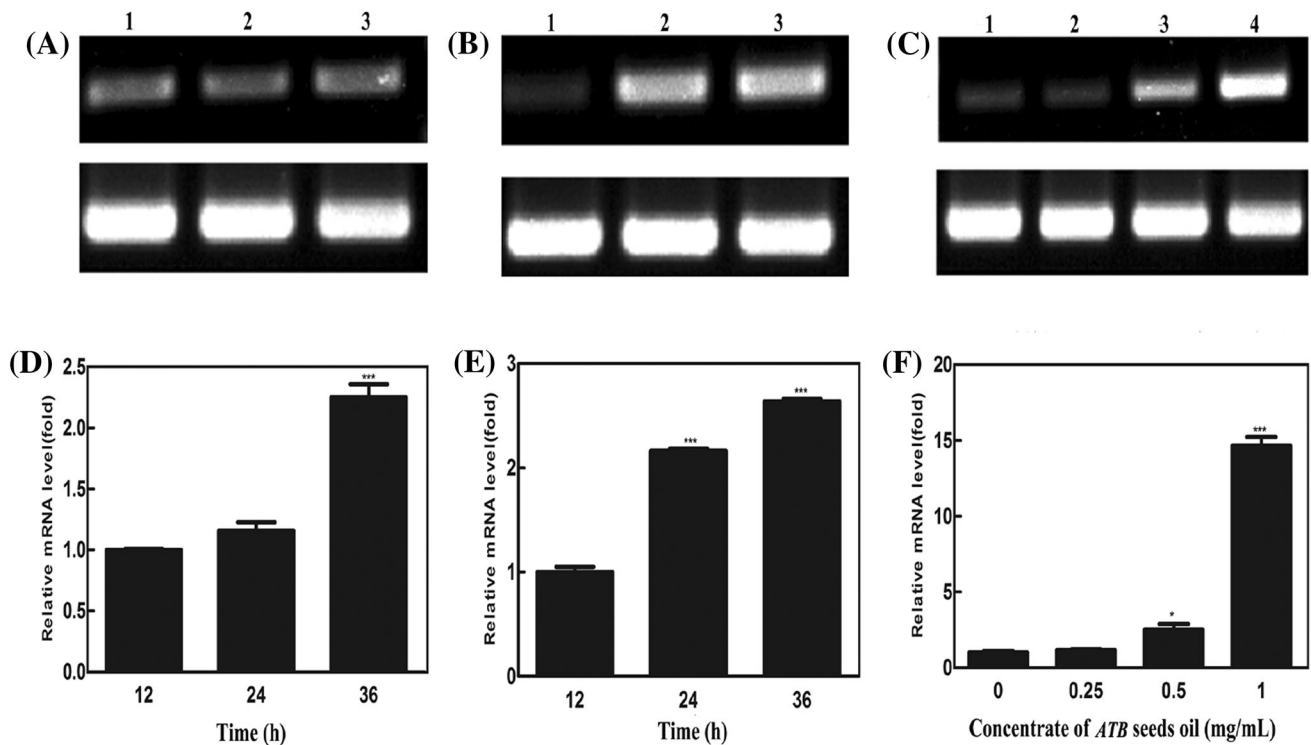


Fig. 4 The effects of *ATB*-seed oil on transcriptional level of *LP8198* Liase. (A) The semi-PCR analysis of the transcriptional level of Liase in *LP8198* without *ATB*-seed oils. Lanes 1–3 represent fermentation for 12, 24, and 36 h, respectively. (B) The semi-PCR analysis of the transcriptional level of Liase in *LP8198* 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 *LP8198* 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 *c9*, *t11*-CLA and *t10*, *c12*-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 *c9*, *t11*-CLA and *t10*, *c12*-CLA in the recent years. It has been reported that some *L. plantarum* strains could convert linoleic acid to *c9*, *t11*-CLA and *t10*, *c12*-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 *c9*, *t11*-CLA and *t10*, *c12*-CLA by *Lactobacillus* fermentation [21]. Here, to validate whether *LP8198* could biotransform linoleic acid from *ATB*-seed oil into *c9*, *t11*-CLA and *t10*, *c12*-CLA, the concentration of these two CLA isomers in 0.5 mg/mL *ATB*-seed oil before and after *LP8198* fermentation was detected by GC–MS. As shown in Fig. 5, according to the standard curves, the results showed that the concentration of *c9*, *t11*-CLA could be increased from 0.23 to 2.06 mg/mL by about ninefold and that of *t10*, *c12*-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 *c9*, *t11*-CLA and *t10*, *c12*-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 *c9*, *t11*-CLA and *t10*, *c12*-CLA by microbial fermentation. These findings might provide some new theoretical basis to develop a new

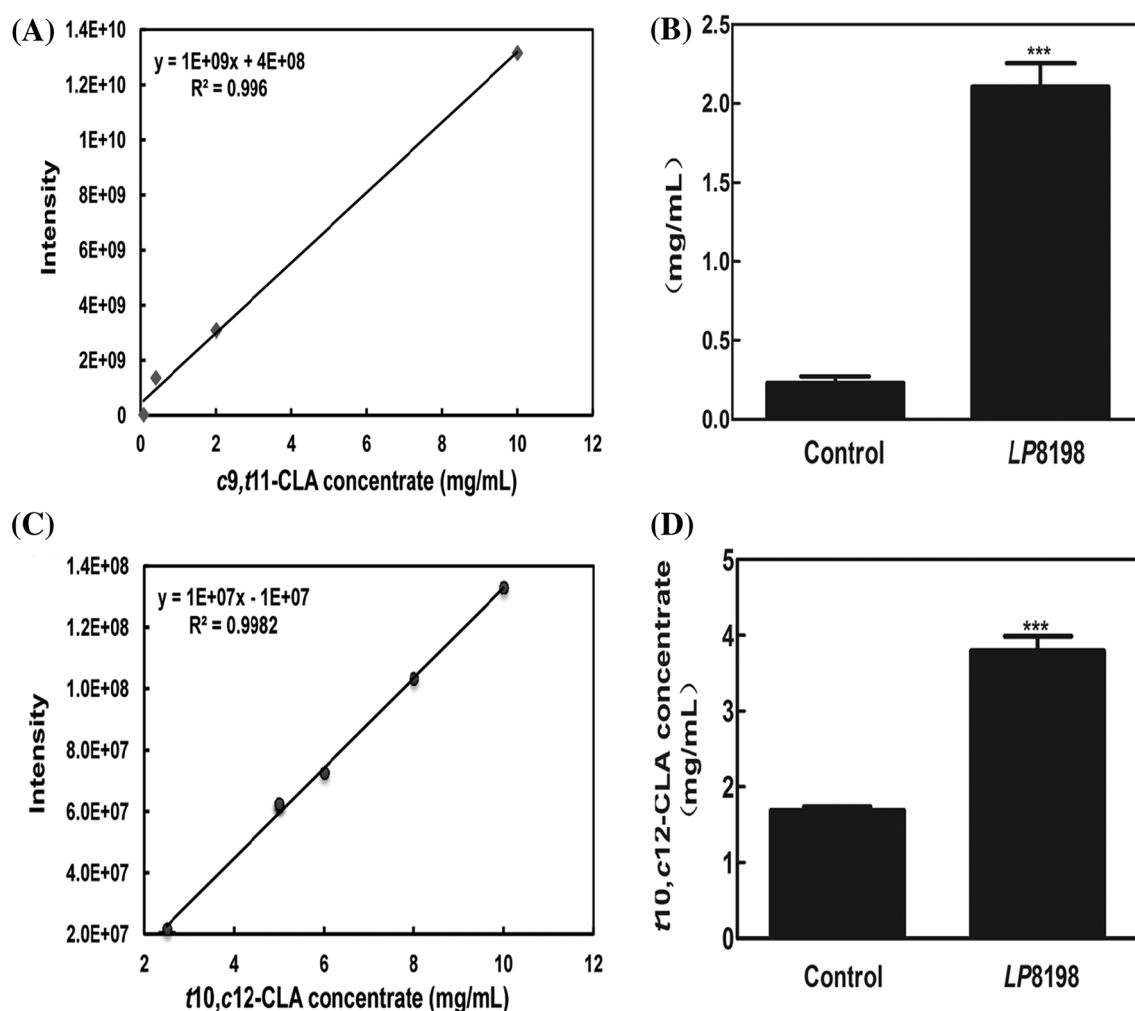


Fig. 5 The concentration of *c9*, *t11*-CLA and *t10*, *c12*-CLA in *ATB*-seed oil before and after being fermented using *LP8198*. **(A)** The standard curve of *c9*, *t11*-CLA detected by GC-MS. **(B)** The concentration of *c9*, *t11*-CLA in a 0.5 mg/mL *ATB*-seed oil emulsion

before and after being fermented using *LP8198* for 24 h. **(C)** The standard curve of *t10*, *c12*-CLA detected by GC-MS. **(D)** The concentration of *t10*, *c12*-CLA in a 0.5 mg/mL *ATB*-seed oil emulsion before and after being fermented using *LP8198*

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

- Demir AS, Talpur FN. Chemoenzymatic conversion of linoleic acid into conjugated linoleic acid. *J. Agric. Food Chem.* 58: 1646–52 (2010)
- 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)
- Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* 8: 1881–7 (1987)
- Koba K, Yanagita T. Health benefits of conjugated linoleic acid (CLA). *Obes. Res. Clin. Pract.* 8: e525–32 (2014)
- 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. Trans-10, cis-12 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).