Progress in Nutrition 2019; Vol. 21, N. 3: 693-701 DOI: 10.23751/pn.v21i3.8573

Original article

Production of linoleic acid metabolites by different probiotic strains of *Lactobacillus plantarum*

Tariq Aziz[1], Abid Sarwar[1], Sam Al-Dalali[1], Zia Ud Din[2], Sarah Megrous[1], Jalal ud Din[1], Xin Zou[1], Yang Zhennai[1]

[1]Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing, China - E-mail: yangzhennai@th.btbu.edu.cn; [2]Department of Chemistry Universidade Federeal de Sao Carlos, SP, Brazil

Summary. *Objective:* To evaluate the capability of *Lactobacillus plantarum* to grow and convert polyunsaturated free fatty acids, i.e. linoleic acid (LA) into bioactive and less toxic conjugated fatty acids. *Method*: Six *L. plantarum* strains were grown in MRS medium containing LA from 1% to 10% (w/v), and the LA metabolites formed in the medium were identified and quantitated by GC-MS. *Result*: In respect to the identified LA metabolites, a total of 4 metabolites were detected including linoelaidic acid, 9,12-octadecadienoic acid (Z, Z), 9,12-Octadecadienoic acid, methyl ester, and trans,trans-9,12-octadecadienoic acid, propyl ester. Among all the six *L. plantarum* strains, the best one was *L. plantarum* 2-3 which showed maximum growth and conversion of linoleic acid to different metabolites from 1% to 10 % (w/v) of LA supplied, while *L. plantarum* 12-5 showed maximum growth and conversion at higher concentration of LA supplied and showed minimal growth and conversion at lower concentration of LA. *Conclusion*: Among all the six *L. plantarum* strains, the best one was *L. plantarum* 2-3 which showed maximum growth and conversion of LA to different metabolites.

Key words: *Lactobacillus plantarum*, linoelic acid metabolites, GC MS

Introduction

Linoleic acid (c18:2 n-6) (LA) is an essential fatty acid, and it is a key constituent of low-density lipoproteins (1). LA cannot be synthesized endogenously in animals, and hence it's only source is through dietary intake (7). Plants and algae contain sufficient amounts of the Δ12- and Δ15-desaturase enzymes, and as a result LA and α-linolenic acid are the two most widespread fatty acids found in plant tissues and oils. As a polyunsaturated fatty acid, LA can be oxidized by endogenous enzymes and reactive oxygen species in the circulation. Derivatives of LA are formed as a result of oxidation by the action of endogenous enzymes. LA and its byproducts are known to employ different biological effects, and they are involved in metabolic disorders and cancer (2-6).

Lactic acid bacteria (LAB) are the most common and important starter cultures used in fermented dairy products, and they may originate from the microflora of raw milks (e.g., bovine, ovine, caprine), but more frequently are inoculated intentionally during product manufacture. LAB are generally regarded as safe (GRAS) microorganisms that have been traditionally used in food fermentation for a long history. Traditional fermented dairy products are considered as key sources of functional microorganisms, e.g. LAB, and ingredients (8). Many LAB strains are capable of producing different bacteriocins, exopolysaccharides, fatty acids, etc., employing their useful health effects. Many LAB strains have shown various promising bioactivities on human health, including antimicrobial activity, prevention and treatment of diarrhea, relief of symptoms caused by lactose intolerance, anti-mutagenic

and anti-carcinogenic activities, and stimulation of the immune system (9). However, the uncertainties of influence from these LAB strains on the quality of functional foods and their bioactivity-keeping in the food matrix frequently hamper their application in modern food industry (10).

Lactobacilli are among the first bacteria to be described as probiotics. Strains of several *Lactobacillus* species have been confirmed to exercise a range of health promoting activities such as immunomodulation, enhancement of resistance against pathogens, reduction of blood cholesterol levels and others (11-14). One of the most extensively used LAB species is *L. plantarum*, which is homofermentative with capability of converting growth-inhibiting free polyunsaturated fatty acids, e.g. LA at a fairly higher concentration, into bioactive conjugated LA (CLA) and other less toxic free saturated fatty acids (15), though LA is also a vital growth constituent for the growth of fastidious bacteria such as *L. plantarum.*

Production of different isomers of CLA from LA or polyunsaturated fat was previously reported in numerous different microorganisms such as *Propionibacterium freudenreichii* (16), *Butyrivibrio* and LAB strains including lactobacilli, lactococci and streptococci (17-28). There were fatty acid isomerases which concertedly converted LA into a bioactive isomer of CLA, but only three bacterial linoleate isomerases derived from *Butyrivibrio fibrisolvens* (29), *Clostridium sporogenes* (30) and *Propionibacteruim acnes* (31) were biochemically categorized. The *Propionibacterium acnes* isomerase was structurally characterized as a FAD dependent protein catalyzing production of 10-trans-12-cis-CLA (31-32).

L. plantarum was also shown to bio-hydrogenate LA and other polyunsaturated fatty acids to produce different fatty acids species, e.g. hydroxy fatty acids, oxo fatty acids, conjugated fatty acids and somewhat saturated trans fatty acids, as the intermediates to finally produce saturated monoenes (OA and trans-vaccenic acid). These numerous reactions were catalyzed by numerous enzymes such as hydratase (CLA HY) , dehydrogenase (CLA DH), decarboxylase (CLA-DC) and enoate reductase (CLA –ER) (20, 32-33). This saturation metabolism yields characteristic fatty acids, e.g. conjugated fatty acids and trans-fatty acids, which were known to be present in ruminant-derived foods with different physiological properties (34)

In this study, six probiotic strains of *L. plantarum* 1-2, 2-3, 3-2, 4-5, 12-4 and 12-5 were studied for production of different LA metabolites in the growth medium supplemented with different concentrations of LA. The production of LA metabolites was qualitatively and quantitatively studied by gas chromatography and mass spectrometry (GC-MS) analysis.

Materials and Methods

Microorganism and growth medium

Six strains of *L. plantarum* 1-2, 2-3, 3-2, 4-5, 12-4 and 12-5 were maintained in the culture bank of Dairy Laboratory in Beijing Technology and Business University of China. They were activated by subculturing for 3 times at 37˚C in MRS medium containing 1.0% meat extract, 1.0% tryptone, 2.0% glucose, 0.1% Tween 80, 0.5% yeast extract, 0.5% sodium acetate, 0.2% K_2HPO_4, 0.2% diammonium citrate, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.005% $MnSO_4 \cdot H2O$. The distilled water was used as solvent for dissolving the medium components, and the medium was adjusted to pH 5.5 and sterilized at 121˚C for 15 minutes. Different concentrations of LA were added to fresh MRS medium, which as inoculated with 1% of the activated culture of *L. plantarum* strains for growth and production of LA metabolites at 37˚C.

Growth of L. plantarum 1-2, 2-3, 3-2, 4-5, 12-4 and 12-5 for production of LA metabolites

L. plantarum strains were cultured in the MRS medium containing 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8% 9% and 10% (w/v) of LA. Growth of the strains was analyzed at OD 600 for the growth pattern, and 1ml sample was spread on plate count agar to determine viable counts as expressed by colony forming units (CFU) per mL. For rapid spectrophotometric determination of LA metabolites, the culture samples were analyzed at 0, 8, 16, 24, 36 and 48 h as described by the method below.

Spectrophotometric determination of LA metabolites

The culture samples were centrifuged (13,000 ×g for 5 min at 4°C), and 1 mL of the supernatant was mixed with 2 mL of isopropanol. After addition of 1.5 mL of hexane, the mixture was thoroughly vortexed in order to extract the lipids and then allowed to stand for 5 min. The hexane layer was collected and the absorbance was measured at 233 nm. The LA metabolites were extracted by using hexane/isopropanol (2:1, v/v) solution at room temperature, and the extracts were washed with distilled water and then dehydrated with anhydrous sodium sulfate (37).

Preparation of fatty acid methyl esters

For analysis by GC, the culture samples were centrifuged at 1900 rpm for 5 minutes at 4°C to remove the cells. An internal standard (C17:0, heptadecanoic acid, 98% pure; Macklin) was added to 5 ml of the supernatant fluid to give a final concentration of 0.15 g/ml. Then 5 mL of isopropanol was added and vortexed for 30 s. Subsequently 2 mL of isopropanol was added and vortexed for 30 s. Finally, 5 mL of n-hexane was added to this mixture, vortexed for 3 min, incubated for 30 min, and centrifuged at 1900 rpm for 5 min. The upper hexane layer containing fatty acid methyl esters (FAME) was collected and it was dried under a steam of liquid nitrogen (34).

GC-MS analysis

GC-MS analysis was done by using Shimadzu GC-2010 instrument coupled with a Dual Stage TMP (Ultra) mass spectrometer. The FAME sample of 2 µL was injected in a split mode, set at 10:1 split ratio at 250°C. The carrier gas was helium at a constant flow rate of 1 mL/min. The separation was conducted on a highly polar (TR-Wax MS, 30 m length × 0.25 mm i.d. × 0.25µm thickness) fused silica capillary column (Thermo Fisher Scientific). The initial oven temperature was held at 170°C for 1 min, then increased at 0.8°C/min to 200°C. The temperature of line transfer was at 250 °C, and the ion source was controlled at 200°C. The MS detector was operated in an electron ionization (EI) voltage of 70 eV under a mass scan range of 33–450 amu (m/z).

Qualification and quantitation of LA metabolites

Chemical identification was conducted by comparison of the mass spectra (MS) of the peaks with those found in the National Institute of Standard and Technology library (NIST, 2014). Quantitative analysis was conducted by their ratio peak area (RPA) to the total peak areas of total ion chromatogram. All the samples were analyzed in triplicate.

Statistical analysis

Data were statistically analyzed by ANOVA. Evaluation of the significance of differences between groups was performed with one-way ANOVA as noted in figure legends.

Result and discussion

Growth pattern of *L. plantarum* strains with different concentration of LA provided *L. plantarum* strains exhibited dissimilar growth pattern of lag, stationary and declining phases at the concentrations of LA from 1% (w/v) to 10% (w/v) as shown in Figure 1A, B, C, D, E and F. For all the concentrations of LA, it was observed that all six strains grew up to 24 h, subsequently entered stationary phase till 36 h, and then the growth decreased till 48 h. There was slightly increase in the growth of the strains 1-2, 3-2 and 4-5 with the increase of the LA concentration, but with higher concentration above 6% (w/v) the growth was inhibited with significant decrease in viable count. *L. plantarum* 4-5 exhibited the least tolerance toward different concentration of LA among the six strains as shown in Figure 1D.

The O.D. values at 600 nm ranged from 0.501 at 0 h to 0.891 at 36 h. *L. plantarum* 2-3 showed the maximum and uniform growth at all concentration of LA (Figure 1B). Increase in LA concentration did not affect the growth significantly as shown by the OD value (2.337) at 24 h on 1% (w/v) LA was and that (2.996) on 10% (w/v) LA. On the other hand, strain 12-5 demonstrated opposite growth pattern as shown in Figure 1E. At lower concentration of LA, the strain did not grow well, but it displayed the maximum OD values (1.117 at 1% LA, 2.556 at 10% LA) at higher concentration.

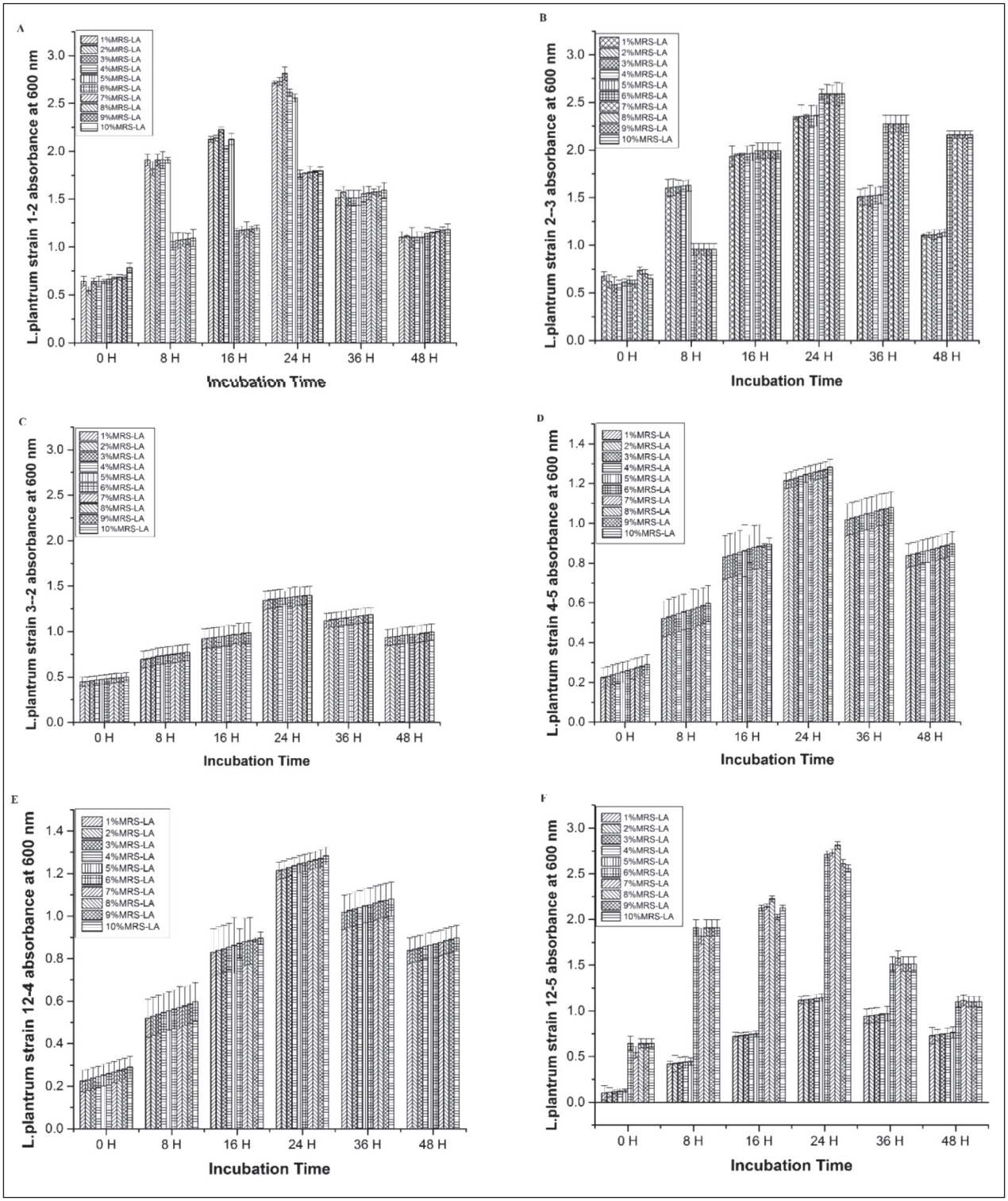

Figure 1. A, B, C, D, E, F representing the OD values of all the six strains *Lactobacillus Plantarum* at 600 nm absorbance at different incubation time and concentration of LA.

Many other LAB strains were reported to be inhibited to different extent by LA, and their tolerance to LA varied. Among five *L. plantarum* strains (lp10, lp15, lp19, lp26, and lp34), strain lp15 showed the highest tolerance upon increased levels of LA in the medium, i.e., up to 600 μg/ml (37). Among 31 *Bifidobacterium* strains screened, 3 strains belonging to the species *B. coryneforme, B. gallinarum*, and *B. saeculare* were unable to grow in the presence of LA, but the remaining *Bifidobacterium* strains grew achieving OD_{600} ranging between 1.1 and 5.7 after 24 h of growth (35). Five *L. reuteri* strains were able to grow in the presence of LA up to 1.0 mg/ml, but when addition of LA was increased to 3.0 mg/ml, growth of *L. reuteri* NCIMB 701359 and NCIMB 702656 was inhibited (22). Earlier studies also showed that even lower LA levels (25 μg/ml) could inhibit bacterial growth (16, 21, 36).

The preliminary results for capacity of different *L. plantarum* strains to convert LA to different metabolites were validated by taking supernatant of cultures in MRS medium containing LA, and the UV absorbance for conjugated double bonds was measured at λ = 233 nm (*A*233). The six strains of *L. plantarum* were found to produce different LA metabolites. Figure 2 showed that production of total LA metabolites gradually increased with the increase of LA concentration from 1% to 10% (w/v) as indicated by the increased absorption at the wavelength of 233 nm. This indicated that strains of *L. plantarum* exhibited high tolerance to LA by converting LA to less toxic LA metabolites except strain 3-2 that did not produce as compared to the rest. Variable results were observed and the range of absorbance was between 0.287 to 1.62 a.u.

Previous researcher also suggested that conversion of free LA to LA metabolites might function as a detoxification mechanism in bacteria, and a stronger LA tolerance indicated a higher productivity of LA metabolites (16, 22, 27-28). Furthermore, more LA metabolites were produced during the stationary phase of growth of all six strains, and the production reduced when the death phase started. Similar findings were also reported with other microbial producers of LA metabolites (17; 24). Significantly, it should be highlighted that the spectrophotometric method did not differentiate between isomers of CLA, since it was based on measurement of the conjugated double bond

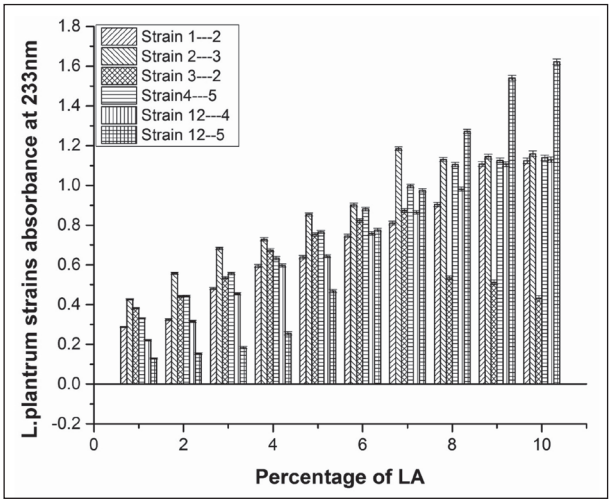

Figure 2. Representing values of the six strains *Lactobacillus Plantarum* extract isolated from MRS-LA at 233nm absorbance at concentration of LA.

in the fatty acid (37). Thus these results were further confirmed by GC-MS.

GC-MS quantification of LA metabolites produced by six L. plantarum strains

The LA metabolites produced by six strains of *L. plantarum* (1-2, 2-3, 3-2, 4-5, 12-4 and 12-5) at different concentrations of LA were quantitatively and qualitatively evaluated by GC-MS (Table 1). A total of 4 LA metabolites were produced including linoelaidic acid, 9,12-octadecadienoic acid (Z, Z), 9,12-Octadecadienoic acid, methyl ester, and trans,trans-9,12-octadecadienoic acid, propyl ester.

L. plantarum 1-2 produced linoelaidic acid at 1 % (w/v) of LA, while, beyond this concentration no linoelaidic acid was produced. Besides, the same strain produced the isomer of trans, trans-9,12-octadecadienoic acid, propyl ester with LA at 1 %, 5 % and 10 % (w/v), in which the peak area (PA) for the identified isomer increased with increasing the concentration of added LA as shown in Table 1 and Figure 3A.

L. plantarum 2-3 showed better ability to produce two metabolites of LA including trans, trans-9,12-octadecadienoic acid, propyl ester, and linoelaidic acid. The former isomer was produced with peak areas of 1135140, 3254404, and 5801121 at LA concentration of 1 %, 5 % and 10 % (w/v), respectively, indicating increased production with increasing LA concentration,

while the latter isomer was only produced at 5 % and 10 % (w/v) of added LA (Table 1 and Figure 3B).

L. plantarum 3-2 showed ability to produce trans, trans-9,12-octadecadienoic acid, propyl ester, whose production was enhanced with increasing concentration of added LA, where the PA at 10 % (w/v) of added LA was 3-fold higher than its PA at 1% (w/v) LA (Table 1 and Figure 3).

L. plantarum 4-5 also produced the isomer of trans, trans-9,12-octadecadienoic acid, propyl ester, in which, its PA was high at low concentration (1%) of LA added, and decreased with increasing the amount of added LA, as well as the unique ability to produce the mentioned isomer at higher PA as showed in Table 1 and Figure 3D.

L. plantarum 12-4 produced three LA metabolites. Of them 9,12-octadecadienoic acid, methyl ester was only produced by this strain with present LA at 1 and 5 % (w/v) as listed in Table 1. On the other hand, the other two metabolites were mostly produced under different concentration of LA as shown in Table 1 and Figure 3E.

Finally, *L. plantarum* 12-5 showed ability like the other strains to produce two metabolites of LA including trans, trans-9,12-octadecadienoic acid, propyl ester, and linoelaidic acid. The PA of both the metabolites increased with the increase in added LA concentration from 1% to 10% (w/v) as listed in Table 1 and Figure 3F.

Figure 4 shows all the metabolites produced by the six strains of *L. plantarum*. Strains 1-2, 2-3 and 12-5 had the ability to produce linoledic acid and trans,trans 9,12 octadecadienoic acid propyl ester, while strain 4-5 could produce 9,12 octadecadiecnoic acid and linolelaidic acid. Strain 12-4 had the ability to produce linolelaidic acid, trans,trans 9,12 octadecadienoic acid propyl ester and 9,12 octadecadienoic acid methyl ester.

Conclusion

Among all the six *L. plantarum* strains, the best one was *L. plantarum* 2-3 which showed maximum growth and conversion of LA to different metabolites, i.e. trans, trans-9,12-octadecadienoic acid, propyl es-

Table 1. The peak area (mean + S.D) for the produced LA metabolites by six different strains

LA Metabolites/Con. of added LA	RT	1%	5%	10%
	L. plantarum 1-2			
trans, trans-9,12-Octadecadienoic acid, propyl ester	15.64	2067133±16.8	3877375±1	9.24656091±23.4
Linoelaidic acid	31.77	6069992±31.9	+	+
	L. plantarum 2-3			
trans, trans-9, 12-Octadecadienoic acid, propyl ester	15.78	1135140±13.6	3254404±27.1	5801121±35.3
Linoelaidic acid	32.22	+	2763112±19.01	5971775±37.5
	L plantarum 3-2			
trans, trans-9,12-Octadecadienoic acid, propyl ester	15.64	2104096±17.8	6470555±41.4	8249803±49.3
	L. plantarum 4-5			
trans, trans-9,12-Octadecadienoic acid, propyl ester	15.42	1837608±15.4	1867643±17.7	740970±1 1.5
9, 12-0ctadecadienoic aci d (Z,Z)	32.29	905105869±44.8	941496248±47.3	+
	L. plantarum 12-4			
trans, trans-9,12-Octadecadienoic acid, propyl ester	15.74	+	2275489±19.7	11526558±16.8
Linoelaidic acid	31.88	759959±26.2	+	16875722±23.5
9,12-Octadecadienoic acid, methyl ester	32.21	+	833754±12.9	+
	L. plantarum 12-5			
trans, trans-9, 12-Octadecadienoic acid, propyl ester	15.65	1828362±20.3	7169026±29.8	9882038±37.1
Linoelaidic acid	31.72	1885530±23.7	19559154±27.5	30808579±36.6
+ not detected				

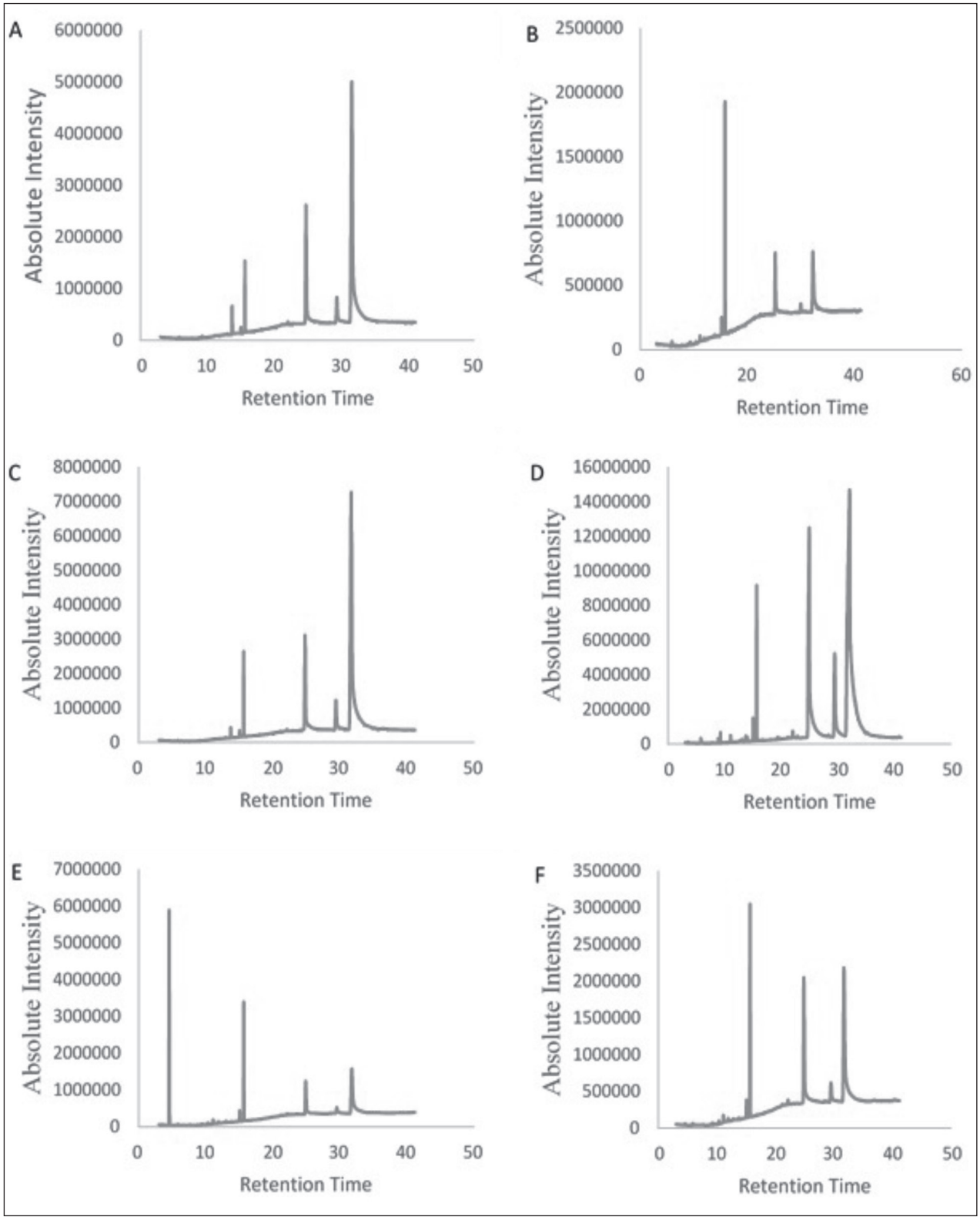

Figure 3. Total ion chromatogram of LA metabolites that produced by six strains of *L. Plantarum* with presence 10 % of LA: (A) is *L. Plantarum* 1-2; (B) is *L. Plantarum* 2-3; (C) is *L. plantarum* 3-2; (D) is *L. Plantarum* 4-5; (E) is *L. Plantarum* 12-4; (F) is *L. Plantarum* 12-5

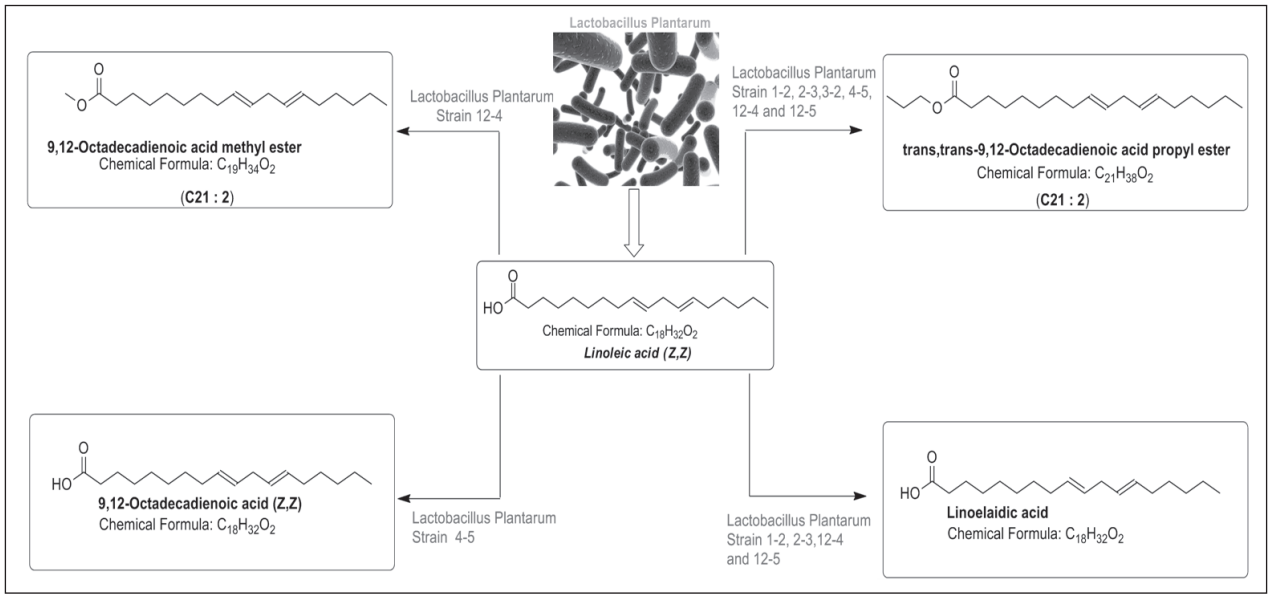

Figure 4. Showing all the metabolites produced by the six strains of *Lactobacillus Plantarum*.

ter and linoelaidic acid, with 1% to 10 % (w/v) of LA supplied. In contrast, *L. plantarum* 12-5 showed maximum growth and conversion at higher concentration of LA supplied, and it showed minimal growth and conversion at lower concentration of LA.

L. plantarum 1-2, 3-2, 4-5 and 12-4 showed excellent growth pattern at minimum concentration of LA supplied as compared to *L. plantarum* 2-3 and 12-5. These results evidenced the probiotic potential of the *L. plantarum* strains and their efficiency to be used in the production of novel functional foods. However, further *in vivo* study is needed in order to confirm the role of these potential probiotic strains for promoting human health.

Acknowledgement

Financial support from Natural Science Foundation of China (No. 31571857 and No. 31871823) and Beijing Talent Cultivation Quality Construction - First-class Professional Construction (Municipal Level) - Food Science and Engineering (PXM2019_014213_000010) was kindly acknowledged.

References

1. Wang R, Kern JT, Goodfriend TL, Ball DL, Luesch H. Activation of the antioxidant response element by specific oxidized metabolites of linoleic acid.Prostaglandins Leukot. Essent. Fatty Acids. 2009; 81:53-59.
2. Mathieu P, Pibarot P, Després JP. Metabolic syndrome: the danger signal in atherosclerosis. Vasc. Health Risk Manag 2006; 2, 285-302.
3. Niki E. Lipid peroxidation: physiological levels and dual biological effects. Free Radic. Biol. Med. 2009; 47: 469-484.
4. Tavakoli Yaraki, M Karami Tehrani F. Apoptosis Induced by 13-hydroxyoctadecadienoic acid in the breast cancer cell lines, MCF-7 and MDA-MB-231. Iran. J. Basic Med. Sci. 2013;16: 653-659.
5. Vangaveti V, Baune BT, Kennedy RL. Hydroxyoctadecadienoic acids: novel regulators of macrophage differentiation and atherogenesis. Ther. Adv. Endocrinol. Metab 2010;
1: 51–60.
6. Yuan H, Li MY, Ma LT, et al. 15-Lipoxygenases and its metabolites 15(S)-HETE and 13(S)-HODE in the development of non-small cell lung cancer 2010; Thorax 65, 321-326
7. Cunnane SC, Guesnet P. Linoleic acid recommendations-a house of cards. Prostaglandins Leukot. Essent. Fatty Acids 2011; 85: 399–402.
8. Selhub EM, Logan AC, Bested AC, Fermented foods, microbiota, and mental health: ancient practice meets nutritional psychiatry. J. Physiol. Anthropol. 2014; 33, 1-12.
9. Shah NP, Functional cultures and health benefits. Int. Dairy J.2007; 17: 1262-1277.
10. Younesi E, Ayseli MT. An integrated systems-based model for substantiation of health claims in functional food development. Trends Food Sci. Tech. 2015; 41: 95-100.
11. Gill HS, Rutherfurd KJ. Viability and dose–response on the effects of the immune enhancing lactic acid bacterium Lactobacillus rhamnosus in mice.Br. J. Nutr. 2001; 86: 285-289.

12. Rosenfeldt V, Michaelsen KF, Jakobsen M, et al. Effect of probiotic Lactobacillus strains on acute diarrhea in a cohor nonhospitalized children attending day-care centers. Pediatr. Infect. Dis. J. 2002; 21: 417-419.
13. Shu Q, GillH S. Immune protection mediated by the probiotic Lactobacillus rhamnosus HN001 (DR20) against Escherichia coli O157: H7 infection in mice. FEMS Immunol. Med. Microbiol. 2002; 34: 59-64.
14. Jones ML, Chen H, Ouyang W, Metz T, Prakash S. Microencapsulated genetically engineered Lactobacillus plantarum 80 (pCBH1) for bile acid deconjugation and its implication in lowering cholesterol. J. Biomed. Biotechnol. 2004; 61-69.
15. Pariza MW, Ha YL. Antimutagenesis and Anticarcinogenesis Mechanisms II, eds Kuroda Y, Shankel D, Waters MD. Plenum, New York.1990; pp 167-170.
16. Jiang J, Bjorck L, Fonden R. Production of conjugated linoleic acid by dairy starter cultures. J. Appl. Microbiol. 1998; 85(1): 95-102.
17. Tung Y Lin, Chin Wen Lin, Chien Hsing Lee. Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid Food Chemistry. 1999; 67 :1±5.
18. Tung Y Lin. Conjugated linoleic acid concentration as affected by lactic cultures and additives Food Chemistry.2003; 69: 27±31.
19. TR Dhiman, ED Helmink, DJ Mcmahon, RL Fife, MW Pariza. Conjugated Linoleic Acid Content of Milk and Cheese from Cows Fed Extruded Oilseed. Journal of Dairy Science.1999; 82 (2): 412-419.
20. Kishino S, Ogawa J, Ando A, et al. Structural analysis of conjugated linoleic acid produced by Lactobacillus plantarum, and factors Affecting isomer production. Biosci. Biotechnol. Biochem. 2003; 67(1):179-182.
21. Kim YJ, Liu RH, Bond DR, Russell JB.Effect of linoleic acid concentration on conjugated linoleic acid production by Butyrivibrio fibrisolvens A38. Appl. Environ. Microbiol. 2000; 66 (12):5226-5230.
22. Coakley M, Ross RP, Nordgren M, Fitzgerald G, Devery R, Stanton C.Conjugated linoleic acid biosynthesis by human-derived Bifidobacterium species. J. Appl. Microbiol. 2003; 94 (1):138-145.
23. Alonso L, Cuesta EP, Gilliand SE. Production of free conjugated linoleic acid by Lactobacillus acidophilus and Lactobacillus casei of human intestinal origin. J. Dairy Sci. 2003; 86 (6):1941-1946.
24. Tung Y Lin. Conjugated linoleic acid production by cells and enzyme extract of Lactobacillus delbrueckii ssp. bulgaricus with additions of different fatty acids Food Chemistry.2006; 94: 437–441.
25. Ogawa J, Kishino S, Ando A, Sugimoto S, Mihara K, Shimizu S.Production of conjugated fatty acids by lactic acid bacteria. J. Biosci. Bioeng. 2005;100(4):355-364.
26. Chung SH, Kim IH, Park HG, et al. Synthesis of conjugated linoleic acid by human-derived Bifido bacterium breve LMC 017: utilization as a functional starter culture for milk fermentation. J. Agric. Food Chem. 2008; 56 (9):3311-3316.
27. Zeng Z, Lin J, Gong D. Identification of lactic acid bacterial strains with high conjugated linoleic acid- producing ability from natural sauerkraut fermentations. J. Food Sci.2009; 74(4): M154-M158.
28. SS Peng, MD Deng, AD Grund, RA Rosson. Purification and characterization of a membrane-bound linoleic acid isomerase from Clostridium sporogenes, Enzyme Microb Tech. 2007; 40 : 831-839.
29. KEPLER, C. R. and TOVE, S. B. Biohydrogenation of Unsaturated Fatty Acids. J. Biol. Chem. 1967; 242(24): 5686-5692.
30. A Liavonchanka, E Hornung, I Feussner, MG Rudolph, Structure and mechanism of the Propionibacterium acnes polyunsaturated fatty acid isomerase, Proceedings of the National Academy of Sciences of the United States of America. 2006; 103: 2576-2581.
31. A Liavonchanka, MG Rudolph, K Tittmann, M Hamberg, I Feussner. On the mechanism of a polyunsaturated fatty acid double bond isomerase from Propionibacterium acnes, The Journal of biological chemistry. 2009; 284: 8005-8012.
32. Kishino S, Ogawa J, Yokozeki K, Shimizu S. Linoleic acid isomerase in Lactobacillus plantarum AKU1009a proved to be a multi-component enzyme system requiring oxidoreduction cofactors, Bioscience, biotechnology, and biochemistry. 2011; 75: 318-322.
33. Kishino S, Takeuchi M, Park SB, et al. Polyunsaturated fatty acid saturation by gut lactic acid bacteria affecting host lipid composition. PNAS, 2013; 110(44): 17808–17813.
34. Shantha N, Decker EA, Henning B.Comparision of methylation methods for the quantification of Conjugated Linoleic Isomer. J. AOAC Int. 1993; 76: 664-649
35. Raimondi S, Amaretti A, Leonardi A, Quartieri A, Gozzoli C, Rossi M. Conjugated linoleic acid production by bifidobacteria: screening, kinetic, and composition. Biomed Res Int 2016; 2016: 8654317. DOI: 10.1155/2016/8654317
36. van Nieuwenhove CP, Oliszewski R, Gonzalez SN, Perez Chaia AB. Conjugated linoleic acid conversion by dairy bacteria cultured in MRS broth and buffalo milk. Lett. Appl. Microbiol. 2007; 44(5):467-474.
37. Barrett E, Ross RP, Fitzgerald GF, Stanton C. Rapid screening method for analyzing the conjugated linoleic acid production capabilities of bacterial cultures. Appl. Environ. Microbiol.2007; 73(7):2333-2337.
38. Liu P, Shen SR, Ruan H, Zhou Q, Ma LL, He GQ. Production of conjugated linoleic acids by Lactobacillus plantarum strains isolated from naturally fermented Chinese pickles. *J Zhejiang Univ Sci B*. 2011;12(11):923–930. doi:10.1631/jzus.B1100072

Correspondence:
Yang Zhennai
Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing, China
E-mail: yangzhennai@th.btbu.edu.cn