Supporting Information

C18:1 improves the freeze-drying resistance of *Lactobacillus plantarum* by maintaining the cell membrane

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Supplementary Materials and Methods

Bacterial strains and culture conditions. The 12 *L. plantarum* strains tested were provided by the Shanghai Engineering Research Center of Food Microbiology, University of Shanghai for Science and Technology (Shanghai, China). The strains were as follows: AR113, AR307, AR326, AR495, AR503, AR504, AR509, AR510, AR513, AR514, AR611, and WCFS1. *L. plantarum* WCFS1 has been deposited in a culture collection belonging to the World Data Centre for Microorganism (WDCM), with the strain numbers are ATCC BAA-793 and NCIMB 8826. The 12 *L. plantarum* strains were previously sub-cultured three times in de Man, Rogosa and Sharpe (MRS) culture medium before inoculation in MRS broth at 37°C for 12 h.

Phylogenetic tree. Two universal primer pairs, 27F：5'-AGAGTTTGATCCTGGCTCA-3' and 1492R：5'-GGTTACCTTGTTACGACTT-3', were used for amplification of 16S rRNA gene sequences form the *L. plantarum* strains. The PCR amplification procedure was conducted in a S1000 thermal cycler (Bio-Rad, California, USA). The following parameters were used: a step at 95°C for 3 min; a denaturation step at 95°C for 30 s, an annealing step at 58°C for 30 s, and a primer extension step at 72°C for 40 s (10 min in the final cycle), 30 cycles; a step at 72°C for 10 min. The PCR products were subsequently sequenced by Sangon Biotech (Shanghai) Co., Ltd. The GenBank accession numbers for the 16S rRNA gene sequences of strains AR113, AR307, AR326, AR495, AR503, AR504, AR509, AR510, AR513, AR514, AR611, and WCFS1 are MK311257, MK311258, MK311259, MK311260, MK311261, MK311262, MK311263, MK311264, MK311265, MK311266, MK311267, and AL935263, respectively. The Mega software package, version 7.0, was used for construction of a phylogenetic tree based on the neighbor-joining method.

Growth curve, specific growth rate and lag phase. Bio screen C MBR (Labsystems, Finland), an automated optical density-monitoring system, was used to monitor the growth curve of *L. plantarum* strains. The cultures after inoculation in MRS broth at 37°C for 12 h were diluted (1:100 v/v) with fresh MRS both. Then, a portion of 200 μL was incubated in honeycomb sterile plates. The plates were covered and monitored in a Bioscreen C at 18°C for 48 h, 24°C for 24 h, 30°C for 18 h, and 37°C for 12 h. The optical density at 600 nm (OD_{600}) was measured every two hours. The measurements of growth curve for every strain were done in triplication. The specific growth rate and the length of lag phase were determined using DMFit 3.5 software (Norwich Research Park, Norwich, United Kingdom) by Baranyi & Roberts.[1]

Membrane FA composition. After inoculation in MRS broth at 37°C for 12 h, the cultures were diluted (1:100) into fresh MRS broth and incubated at 18°C for 48 h, 24°C for 24 h, 30°C for 18 h, and 37°C for 12 h. The cells were then collected by centrifugation (at 7378 × g for 5 min), and then successively washed twice in sterilized saline water.

The FAs in the cell membrane were extracted from concentrated cells. The composition of FAs was determined mainly based on the method described by Sasser.[2] After finishing saponification, methylation, extraction, and base washing, the upper phase solvent was collected and transferred into GC-MS vials for FA composition analysis.

The FA composition analysis was performed on a GC-MS system (ISQ 7000, ThermoFisher Scientific Inc., USA) equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, USA). Helium was delivered at a flow rate of 1 mL/min as a carrier gas. The injection of 1 μL sample was done in splitless mode at 250°C into the GC inlet. The oven temperature was held at 100°C for 1 min, then increased from 100 to 200°C at 5°C/min and

held at 200°C for 5 min, followed by increasing from 200 to 250°C at 5°C/min and held at 250°C for 5 min. A transfer line was set at 250°C; the ion source temperature was set at 220°C. The mass detector was operated in electron impact mode at an ionizing voltage of 70 eV. The quantification of the FA composition was confirmed using a mass selective detector for collecting the data in the range of 20 to 400 amu and comparing the MS spectra with a mass spectrum library search (NIST 1.6) and the retention times with those of known standards (Supelco, PA, USA). The extraction and analysis of the FA composition of each strain was performed in triplicat.

Sample preparation. Cultures (2 mL, OD_{600}=1) during the logarithmic phase of growth (18°C for 48 h, 24°C for 24 h, 30°C for 18 h, and 37°C for 12 h) were harvested by centrifugation at 7378 × g for 5 min. The cultures were then successively washed twice in sterilized saline water, and centrifuged to collect bacteria mass. Then, the bacteria mass was resuspended in sterilized 1 mL sucrose (100 g/L), sorbitol (100 g/L), or PBS (pH 6.5). The suspension (10^9 CFU/mL) was prepared and loaded into penicillin bottles (5 mL).

Freeze-drying. The penicillin bottles containing suspensions were freeze-dried using a Labconco FreeZone (Labconco, Kansas City, MO, USA) at a collector temperature of -80°C at 20 Pa. The samples were frozen at -40°C for 3 h, and then dried from -40 to -30°C at 1°C/min. After 800 min of sublimation, the secondary drying step (holding for 2 h) was initiated from -30 to 20°C at 1°C/min.

The survival rates of *L. plantarum* after freezing, drying, and freeze-drying. Total viable cell counts were determined prior to freezing, after freezing for 3 h, and after the freeze-drying process. The frozen cells were thawed at room temperature before the total cell count analysis.

Freeze-dried cells were resuspended in 0.9 mL sterilized saline water. All samples (0.1 mL) were serially diluted in sterilized saline water (9 g/L NaCl), plated on MRS agar plates, and then anaerobically incubated at 37°C. The cell counts were determined after anaerobic incubation at 37°C for 48 h. The survival rates (%) after freezing, drying, and freeze-drying were determined and expressed as $N_1/N_0 \times 100\%$, $N_2/N_1 \times 100\%$, and $N_2/N_0 \times 100\%$, respectively, where N_0, N_1, and N_2 were, respectively, the numbers of viable cells before, after the freezing for 3h, and after freeze-drying process. The measurements of viable cell counts were repeated three times at each temperature.

Figure S1 showed the neighbor-joining phylogenetic tree of the 12 L. plantarum strains. The farthest relative to this group was the group of strains WCFS1 and AR514, while the others were between the two groups (Figure S1). Growth curves of the 12 *L. plantarum* strains examined at 18, 24, 30, 37°C are shown in Figure S2. Specific growth rate and lag time of the 12 *L. plantarum* strains examined at 18, 24, 30, 37°C are shown in Table S1. The growth of bacteria can be divided into three different phases: lag phase, log phase or exponential phase, and stationary phase. When the fermentation temperature rose from 24°C to 37°C, the length of lag phase was shortened from about 8 to 3 hours. The curves then remained exponential which continues until after 24 (24°C, Figure S2b), 18 (30°C Figure S2c), or 12 (37°C, Figure S2d) hours, respectively, and later constant progressively. Specific growth rate and lag time of the 12 *L. plantarum* strains examined at same fermentation temperature were different. For example, AR307 had the greatest specific growth rate (0.25 OD_{600}/h) among all the 12 *L. plantarum* strains examined at 37°C. While AR514 did the least (0.13 OD_{600}/h), lower than the

greatest by 0.12 OD_{600}/h. AR510 had the shortest lag time of (2.64 h) among all the 12 *L. plantarum* strains examined at 37°C. While AR495 did the longest (5.00 h), higher than the shortest by 2.36 h. Clearly, specific growth rate and lag time of every *L. plantarum* strain at different fermentation temperature were also different. For example, specific growth rate of AR307 was 0.04 OD_{600}/h at 18°C, 0.11 OD_{600}/h at 24°C, 0.17 OD_{600}/h at 30°C, and 0.25 OD_{600}/h at 37°C. Lag time of AR307 was 9.82 h at 18°C, 5.71 h at 24°C, 4.27 h at 30°C, and 2.91 h at 37°C. To be precise, the specific growth rates decreased from the range of 0.12-0.25 OD_{600} per h to 0.01-0.04 OD_{600} per h, and the lengths of the lag phase were extended from the range of 2.64-5.00 h to 6.41-13.75 h from 37°C to 18°C.

The FAs composition in cell membrane of 12 *L. plantarum* strains were characterized, in order to study the physiological modifications induced by fermentation temperature. The results were summarized in Table S2. Regardless of the fermentation temperature and strains, the FAs were composed of seven FAs. The 4 main peaks were identified as C16:0, octadecanoic acid (C18:0), C18:1, and nonadecenoic acid (C19:1). Their relative percentages were between 1% and 50%, corresponding to about 90% of all FAs examined. Three minor FAs were also detected at lower relative contents: tetradecanoic acid (C14:0), hexadecenoic acid (C16:1), and octadecatrienoic acid (C18:2). The 7 FAs agreed with those reported in previous studies.[3-5] However, methylenoctadecenoic acid (cycC19:0) had been not detected.[6] It may be related to the extraction method. The relative content of FAs changed with fermentation temperature, especially the U/S ratio. The U/S ratio was between 0.4 and 2.1. Generally, the lower the fermentation temperature, the greater was the U/S ratio. When the fermentation temperature was decreased from 37°C to 18°C, the U/S ratio increased from 0.42-0.79 to 0.66-2.05.

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[2] M. Sasser, Bacterial Identification by gas chromatographic analysis of fatty acid methyl esters(GC-FAME). Tech note # 101. Internal MIDI document. Revised 2009.

[3] H. Velly, M. Bouix, S. Passot, C. Penicaud, H. Beinsteiner, S. Ghorbal, P. Lieben, F. Fonseca, *Appl. Microbiol. Biot*. **2015**, *99*(2), 907.

[4] L. Hua, W. Y. Zhao, W. Hua, Z. C. Li, A. L. Wang, *Food Bioprod. Process* **2009**, *87*(1), 56.

[5] A. ÁlvarezOrdóñez, A. Fernández, M. López, A. Bernardo, *Food Microbiol.* **2009**, *26*(3), 347.

[6] R. B. Smittle, S. E. Gilliland, M. L. Speck, W. M. Jr Walter, *Appl. Microbiol*. **1974**, *27*(4), 738.

Figure S1

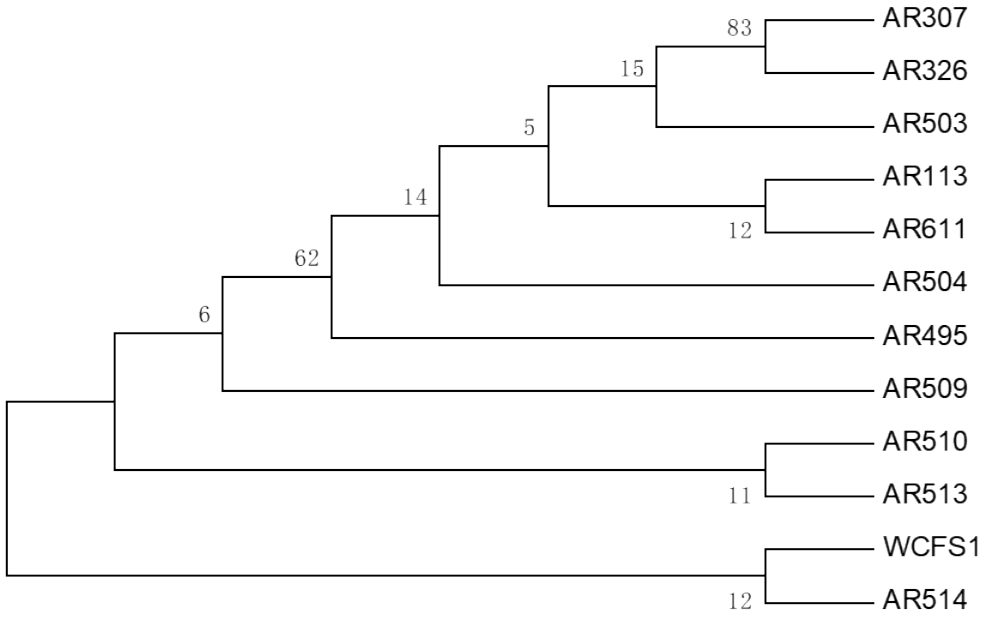


Figure S1. Neighbor-joining phylogenetic tree of the 12 *L. plantarum* strains examined based on 16S rRNA gene sequences.

Figure S2

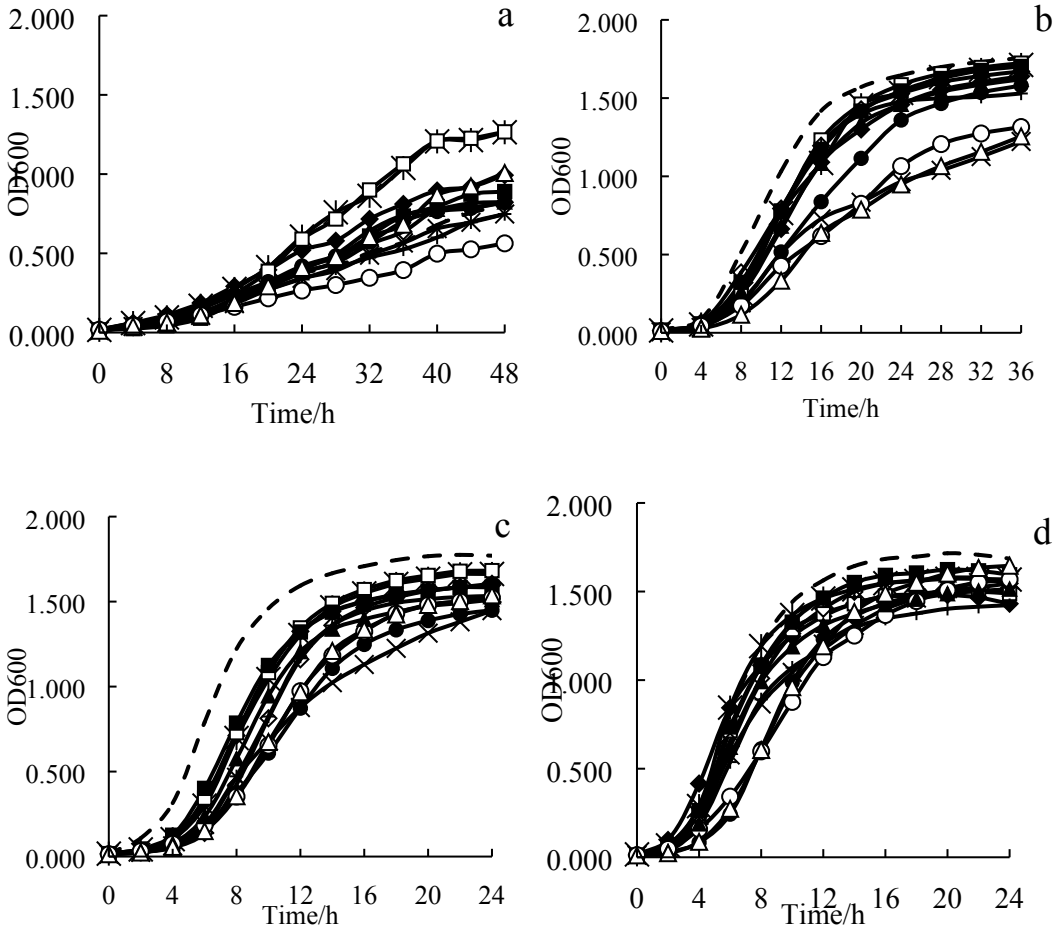
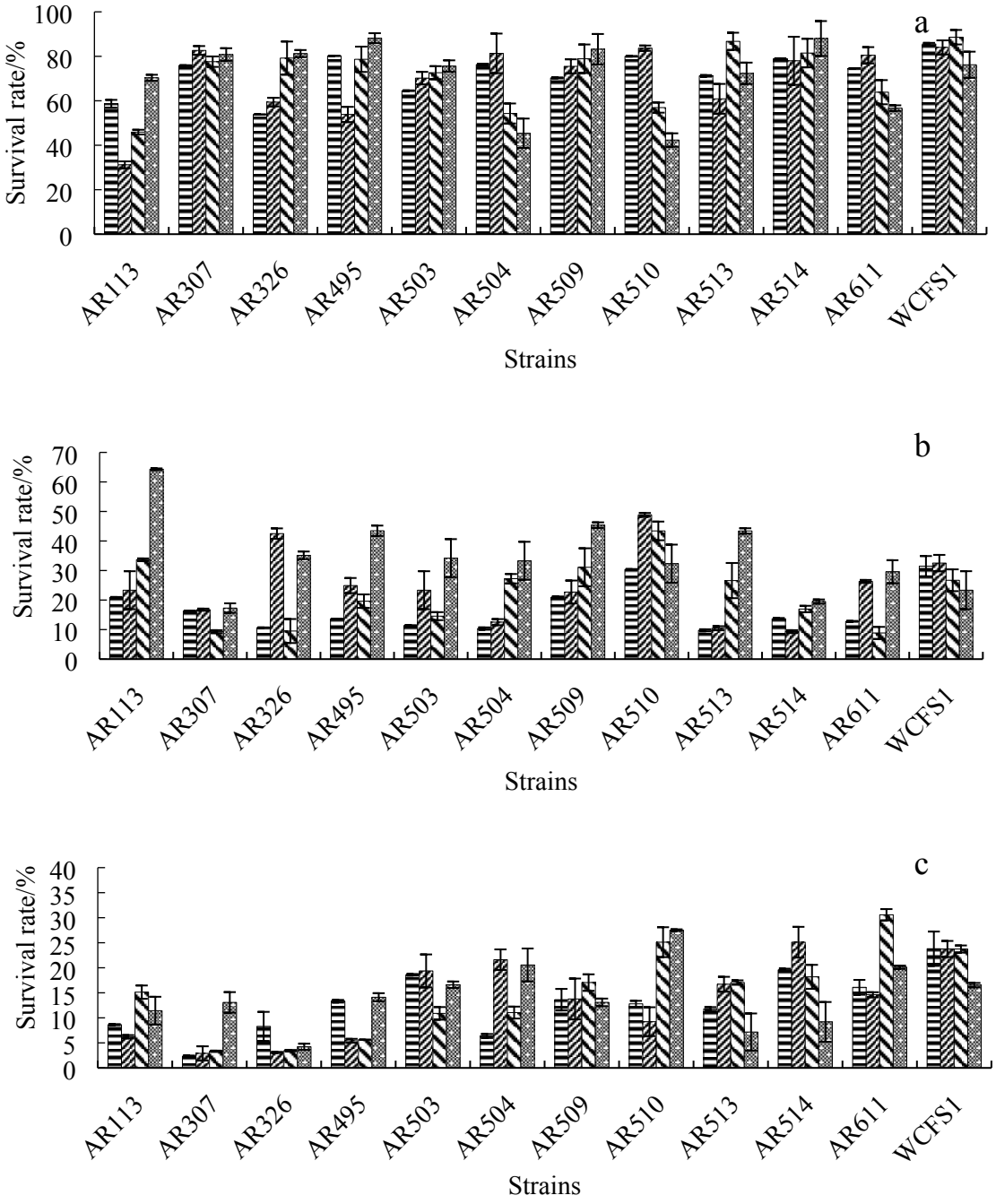


Figure S2. Growth curves of the 12 *L. plantarum* strains examined at different fermentation temperatures. a: 18°C, b: 24°C, c: 30°C, d: 37°C.

Figure S3


a
Survival rate/%
100
80
60
40
20
0
AR113
AR307
AR326
AR495
AR503
AR504
AR509
AR510
AR513
AR514
AR611
WCFS1
Strains
b
Survival rate/%
70
60
50
40
30
20
10
0
AR113
AR307
AR326
AR495
AR503
AR504
AR509
AR510
AR513
AR514
AR611
WCFS1
Strains
c
Survival rate/%
40
35
30
25
20
15
10
5
0
AR113
AR307
AR326
AR495
AR503
AR504
AR509
AR510
AR513
AR514
AR611
WCFS1
Strains

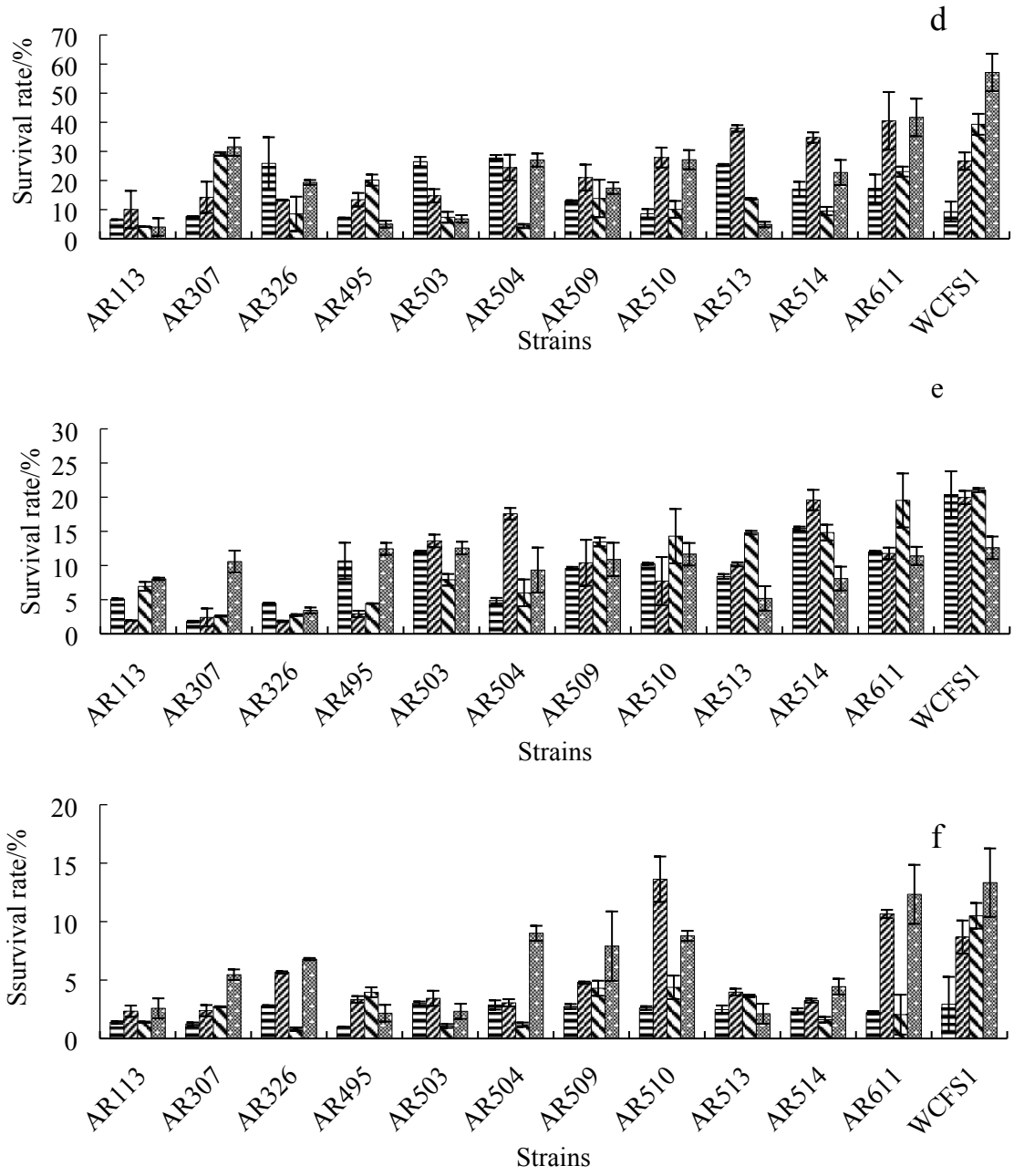


Figure S3 Survival rates of the 12 *L. plantarum* strains examined of freezing at different fermentation temperatures under the protection of 100 g/L sorbitol solution (a) and PBS buffer (b); drying at different fermentation temperatures under the protection of 100 g/L sorbitol solution (c) and PBS buffer (d); freeze-drying at different fermentation temperatures under the

protection of 100 g/L sorbitol solution (e) and PBS buffer (f). Data are presented as means ± SD (n=3). ▭ 18 °C ▨ 24 °C ▧ 30 °C ▩ 37 °C.

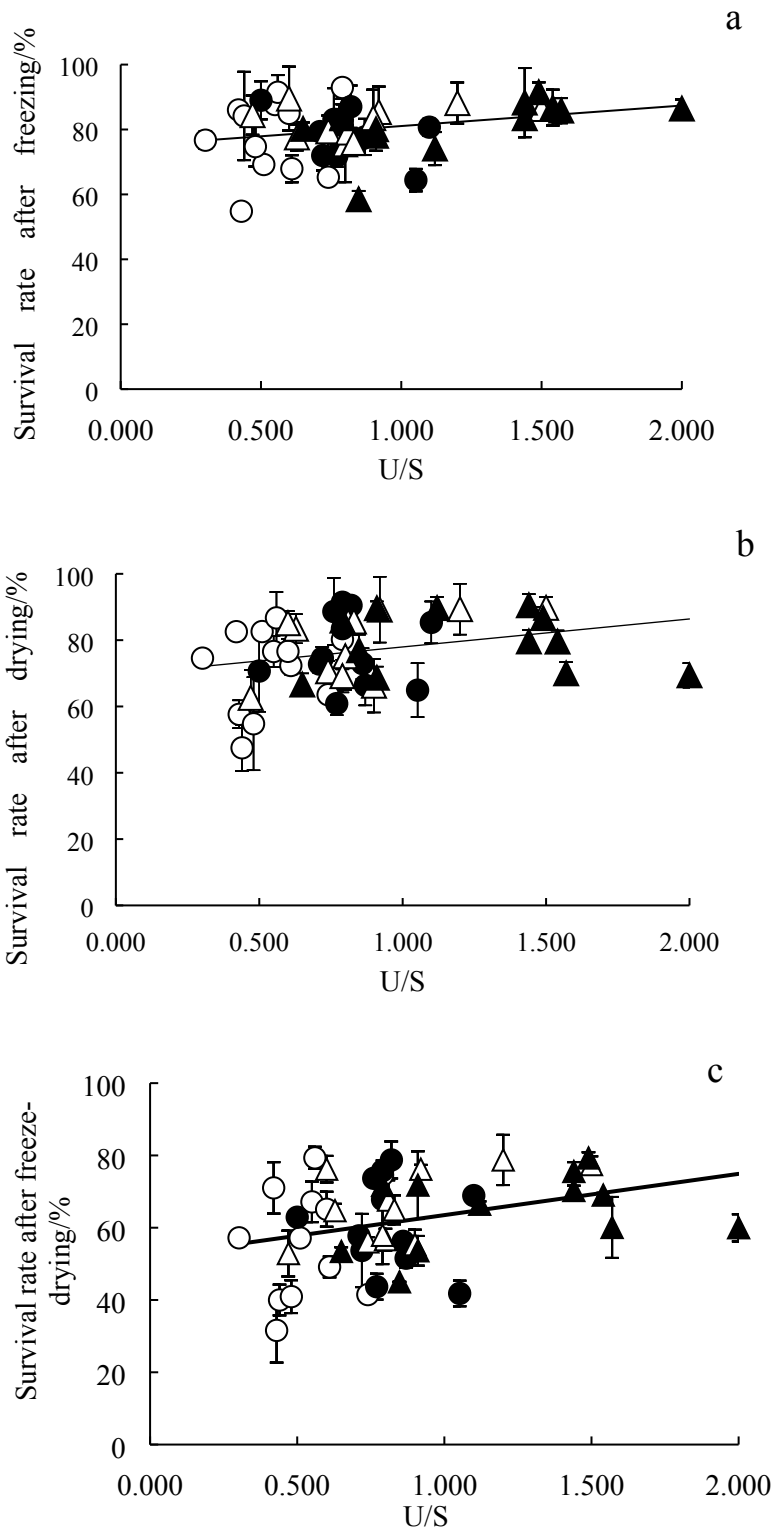


Figure S4 Relationships between survival rate of the *L. plantarum* strains examined after freezing (a), drying (b) and freeze-drying (c) and U/S at different fermentation temperatures under the protection of 100 g/L sucrose solution. Data are presented as means ± SD (n=3). The line represents a linear fit of the data. ▲18°C △24°C ● 30°C ○ 37°C.

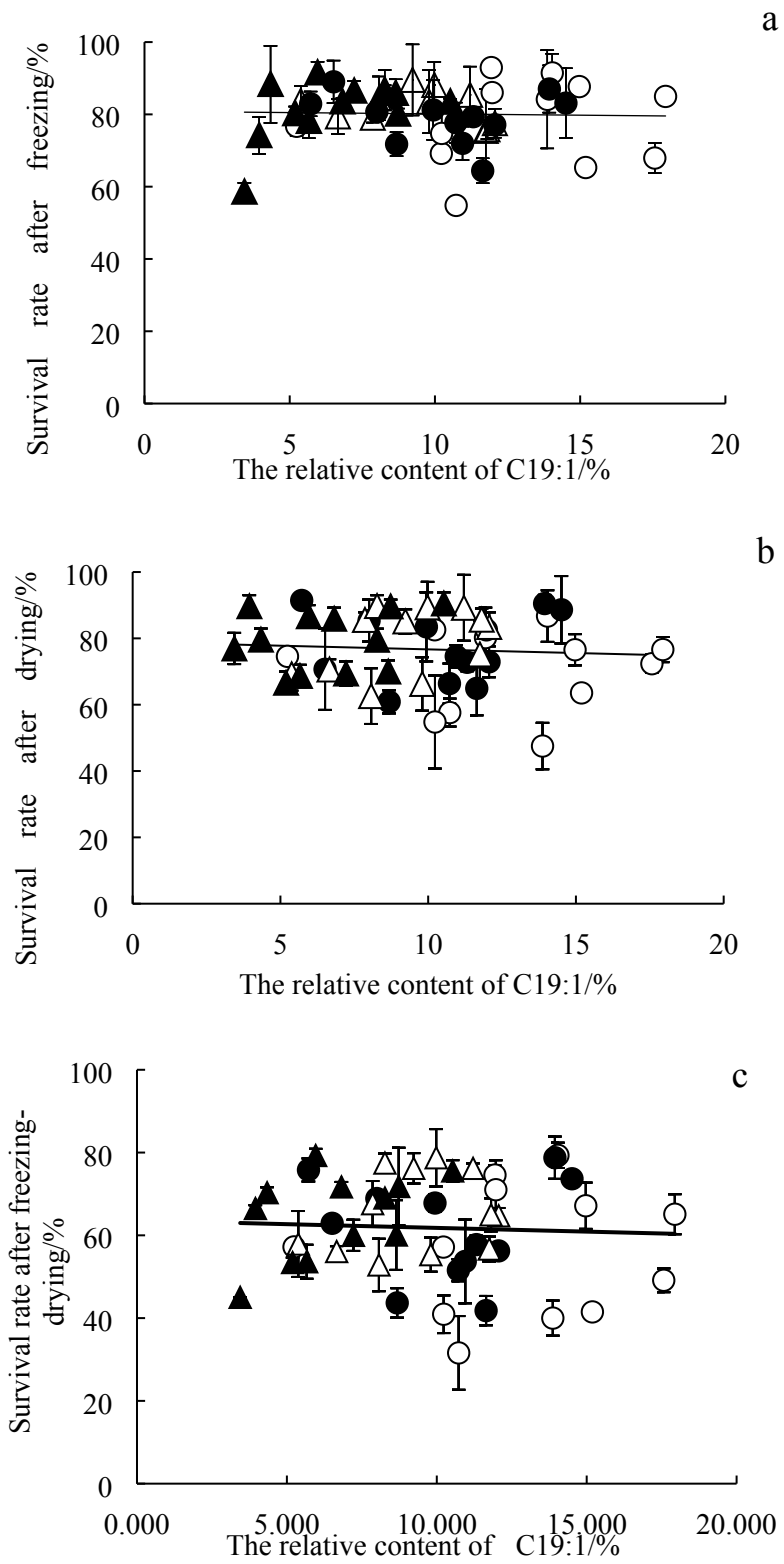


Figure S5 Relationships between survival rate of the *L. plantarum* strains examined after freezing (a), drying (b) and freeze-drying (c) and C19:1 at different fermentation temperatures under the protection of 100 g/L sucrose solution. Data are presented as means ± SD (n=3). The line represents a linear fit of the data. ▲18°C △24°C ● 30°C ○ 37°C.

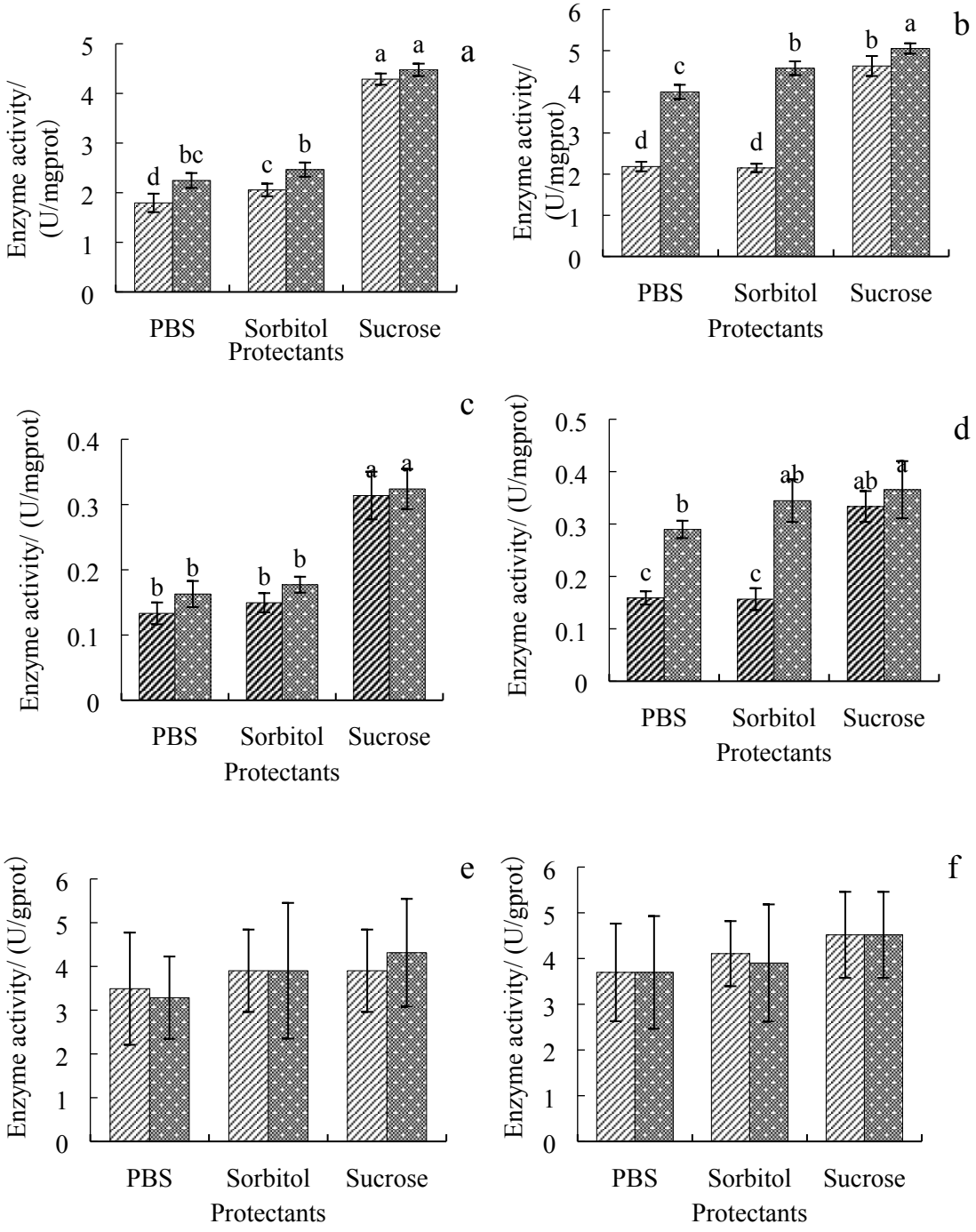


Figure S6 Effect of the C18:1 on the activity of LDH (a, b), ATPase (c, d)and PK (e, f) of *L. plantarum* after freeze-drying. A, c, e: AR307, b, d, f: WCFS1. Data are presented as means ± SD (n=3). Means with different letters differ significantly ($P < 0.05$, Duncan).

Table S1 Lag phase and specific growth rate of *L. plantarum* at four fermentation temperatures

Strains \ Temperature/°C	Lag phase/h				Specific growth rate/(OD_{600}/h)			
	18	24	30	37	18	24	30	37
AR113	10.59	6.61	4.96	4.24	0.02	0.10	0.16	0.16
AR307	9.82	5.71	4.27	2.91	0.04	0.11	0.17	0.25
AR326	11.79	6.10	4.00	3.03	0.04	0.13	0.17	0.23
AR495	6.41	5.69	5.15	5.00	0.02	0.08	0.12	0.19
AR503	7.09	4.61	4.74	2.92	0.02	0.11	0.18	0.20
AR504	10.94	5.04	4.06	2.80	0.03	0.11	0.19	0.22
AR509	6.95	4.18	2.73	2.83	0.02	0.13	0.23	0.24
AR510	7.59	3.35	4.37	2.64	0.02	0.07	0.12	0.16
AR513	13.75	9.25	5.54	3.29	0.02	0.06	0.18	0.19
AR514	13.63	9.41	4.08	3.54	0.01	0.03	0.08	0.13
AR611	10.47	7.26	4.80	3.31	0.03	0.05	0.17	0.22
WCFS1	9.86	9.40	5.75	4.60	0.03	0.04	0.15	0.17

Table S2 Fatty acids composition of *L. plantarum* strains

Strains	T[a]/°C	Fatty acids							
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C19:1	U/S
AR113	18	2.28±0.01^{efghijk}	29.24±2.14^{qr}	8.14±1.24^{a}	2.16±1.14^{qr}	51.67±1.87^{a}	1.86±0.04^{efghijkl}	4.64±0.12^{pqr}	1.98±0.29^{a}
	24	1.67±0.27^{jklm}	41.3±0.23^{fghijklm}	3.82±0.14^{hijklmn}	11.12±0.47^{fghijkl}	33.74±0.54^{efg}	1.42±0.4^{hijkl}	6.93±0.21^{lmnopqr}	0.85±0.02^{efghijkl}
	30	2.00±0.32^{ghijklm}	44.91±2.64^{defghij}	6.65±0.52^{bcd}	4.94±5.2^{opqr}	26.68±1.53^{ijkl}	0.54±0.2^{l}	11.45±2.15^{defghij}	0.87±0.04^{efghij}
	37	3.18±0.35^{cde}	56.04±3.48^{a}	2.29±0.51^{nop}	11.09±0.49^{fghijkl}	11.07±1.40^{p}	2.66±1.13^{bcdefghi}	13.66±4.56^{cdefg}	0.42±0.09^{r}
AR307	18	1.59±0.02^{jklm}	42.3±0.02^{efghijkl}	3.51±0.39^{hijklmno}	7.7±0.63^{klmnop}	33.77±0.06^{efg}	1.18±0.16^{kl}	9.96±2.14^{hijklmn}	0.94±0.02^{defg}
	24	1.81±0.45^{hijklm}	46.19±1.04^{cdefgh}	2.64±0.62^{mnopq}	16.92±1.78^{bcd}	21.52±0.22^{lm}	2.43±0.23^{cdefghijk}	8.49±0.34^{hijklmno}	0.54±0.01^{lmnopq}
	30	1.38±0.02^{klm}	41.29±0.67fg^{hijklm}	2.64±0.12^{mnopq}	12.42±0.49^{efghij}	26.73±0.11^{ijkl}	1.61±0.1g^{hijkl}	13.94±1.05^{cdef}	0.82±0.04^{fghijklm}
	37	4.54±0.47^{b}	41.38±1.83^{fghijklm}	4.18±0.22^{ghijklm}	6.32±1.46^{mnopq}	21.20±2.94^{lm}	3.97±0.20^{ab}	11.93±0.54^{cdefgh}	0.79±0.11^{ghijklmn}
AR326	18	1.86±0.00^{hijklm}	45.14±2.69^{defghij}	5.64±0.19^{defg}	14.23±5.93^{defg}	26.16±6.45^{ijkl}	2.75±0.36^{bcdefgh}	5.19±0.59^{opqr}	0.66±0.18^{ghijklmnopqr}
	24	1.35±0.4^{klm}	46.48±1.49^{defghijk}	2.24±0.03^{nopq}	20.33±1.86^{ab}	19.46±1.66^{mn}	2.05±0.74^{cdefghijk}	8.07±0.52^{jklmnop}	0.47±0.06^{opqr}
	30	1.61±0.25^{jklm}	44.83±0.76^{defghijk}	2.43±0.11^{nopq}	11.62±0.40^{efghijk}	26.62±0.24^{ijkl}	1.94±0.14^{defghijk}	10.95±1.21^{efghij}	0.72±0.04^{ghijklmnopqr}
	37	2.19±0.23^{fghijkl}	48.85±2.24^{bcd}	2.52±0.41^{nopq}	19.41±0.44^{abc}	12.04±0.7^{p}	3.018±0.02^{bcdef}	11.97±1.65^{cdefgh}	0.42±0.04^{r}
AR495	18	1.81±0.50^{ijklm}	35.1±5.26^{nop}	3.4±0.16^{ijklmnop}	4.06±0.48^{pqr}	47.92±5.58^{ab}	3.37±0.11^{abc}	4.34±0.39^{qr}	1.47±0.38^{b}
	24	3.43±0.08^{c}	41.19±0.72^{fghijkl}	4.85±0.06^{efghi}	12.73±1.52^{defghij}	29.22±2.21^{fghij}	1.93±0.24^{defghijk}	6.66±0.12^{mnopqr}	0.75±0.07^{ghijklmnop}
	30	2.42±0.13^{defghij}	42.67±2.15^{efghijkl}	3.43±0.28^{ijklmnop}	11.37±1.1^{efghijk}	29.58±0.94^{fghij}	1.85±0.11^{efghijkl}	8.68±0.14^{hijklmno}	0.77±0.03^{ghijklmno}
	37	2.01±0.14^{ghijklm}	47.71±1.69^{bcde}	1.68±0.38^{q}	20.36±2.4^{ab}	14.29±3.4^{nop}	3.23±0.29^{abcd}	10.73±0.73^{fghijk}	0.43±0.09^{qr}
AR503	18	1.7±0.22^{jklm}	36.37±0.3^{mnop}	6.12±0.93^{cde}	1.53±0.03^{r}	45.14±2.07^{bc}	1.34±0.1^{ijkl}	8.27±0.02^{ijklmno}	1.54±0.05^{b}
	24	1.49±0.06^{jklm}	42.64±1.21^{efghijkl}	2.79±0.01^{lmnopq}	11.57±1.77^{efghijk}	28.43±0.53^{fghij}	1.33±0.03^{ijkl}	11.75±0.05^{cdefghi}	0.8±0.02^{fghijklmn}
	30	1.33±0.12^{klm}	45.83±1.84^{bcdefghi}	2.44±0.23^{nopq}	11.3±0.88^{efghijkl}	26.07±1.99^{ijkl}	1.72±0.29^{fighijkl}	11.32±1.14^{efghij}	0.71±0.08^{ghijklmnopqr}

Table S2 Fatty acids composition of *L. plantarum* strains

Strains	T/°C	Fatty acids							
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C19:1	U/S
AR504	37	3.33±1.39^{cd}	45.36±0.76^{defghij}	4.91±2.66^{efghi}	8.93±0.38^{ijklmno}	19.35±1.98^{mno}	2.93±1.11^{bcdefg}	15.19±7.52^{abc}	0.74±0.05^{ghijklmnopq}
	18	1.72±0.11^{jklm}	40.11±2.56^{jklmn}	3.19±0.49^{jklmnopq}	14.09±4.28^{defg}	31.16±6.28^{fghi}	2.91±0.37^{bcdefg}	6.82±0.32^{mnopqr}	0.8±0.22^{fghijklmn}
	24	1.65±0.38^{jklm}	42.8±0.66^{efghijkl}	3.71±0.47^{hijklmn}	7.67±0.37^{klmnop}	31.17±0.51^{fghi}	1.78±0.16^{fghijkl}	11.21±0.13^{efghij}	0.92±0.00^{defgh}
	30	2.51±0.6^{cdefghij}	44.23±0.05^{defghijk}	5.85±1.09^{cdef}	9.6±3.51^{hijklmn}	31.26±2.19^{fghi}	1.27±0.14^{jkl}	10.72±0.28^{fghijk}	0.87±0.05^{efghij}
AR509	37	2.82±0.82^{cdefgh}	43.85±1.43^{defghijk}	2.38±0.65^{nopq}	22.75±1.05^{a}	10.96±0.24^{p}	3.37±0.27^{abc}	13.87±0.19^{cdef}	0.44±0.01^{pqr}
	18	2.82±0.92^{cdefghi}	37.86±5.72^{lmno}	7.64±0.85^{ab}	6.58±1.17^{mnop}	38.01±5.32^{de}	3.14±1.86^{bcde}	3.95±1.16^{r}	1.13±0.25^{de}
	24	2.24±0.65^{efghijk}	42.69±3.05^{efghijkl}	3.82±0.28^{hijklmn}	10.99±0.82^{fghijkl}	32.49±2.03^{fgh}	2.4±1.43^{cdefghijk}	5.38±1.58^{opqr}	0.79±0.05^{ghijklmn}
	30	1.58±0.58^{jklm}	52.07±0.88^{ab}	1.99±0.1^{opq}	13.24±0.75^{defghi}	22.03±1.07^{klm}	2.59±0.83^{cdefghij}	6.51±0.75^{nopqr}	0.5±0.02^{nopqr}
AR510	37	5.94±0.26^{a}	56.33±1.64^{a}	7.25±0.87^{abc}	3.81±0.14^{pqr}	14.46±0.26^{nop}	2.00±1.35^{defghijk}	10.22±0.43^{ghijklm}	0.51±0.05^{mnopqr}
	18	2.98±0.43^{cdefg}	32.82±0.62^{opq}	8.44±0.85^{a}	4.3±0.87^{pqr}	43.83±0.5^{bc}	1.67±0.09^{fghijkl}	5.96±0.62^{opqr}	1.49±0.01^{b}
	24	2.08±0.04^{fghijklm}	40.46±0.44^{ijklm}	4.27±0.42^{ghijkl}	12.78±0.55^{defghij}	30.78±0.94^{fghi}	1.78±0.42^{fghijkl}	7.86±0.00^{jklmnopq}	0.81±0.03^{fghijklm}
	30	2.27±0.08^{efghijk}	43.41±0.25^{efghijk}	4.54±0.45^{fghijk}	9.39±0.13^{hijklmn}	27.35±0.8^{hijk}	1.77±0.03^{fghijkl}	9.94±0.20^{hijklmn}	0.79±0.02^{ghijklmn}
AR513	37	2.32±0.2^{efghijk}	45.22±2.56^{defghij}	3.25±0.13^{jklmnopq}	14.61±0.28^{def}	14.44±1.79^{nop}	2.6±0.17^{cdefghij}	17.57±0.82^{ab}	0.61±0.07^{hijklmnopqr}
	18	2.08±0.04^{fghijklm}	40.46±0.44^{ijklm}	4.27±0.42^{ghijkl}	12.78±0.55^{defghij}	30.78±0.94^{fghi}	1.78±0.42^{fghijkl}	7.86±0^{jklmnopq}	0.81±0.03^{fghijklm}
	24	1.68±0.04^{jklm}	39.4±0.58^{klmn}	3.37±0.67^{ijklmnop}	11.43±1.28^{efghijk}	32.9±0.55^{efgh}	1.42±0.12^{hijkl}	9.8±0.56^{hijklmn}	0.91±0.07^{efghi}
	30	1.54±0.21^{jklm}	45.77±3.62^{defghi}	2.99±0.37^{klmnopq}	8.65±2.85^{jklmno}	34±5.92^{ef}	1.34±0.45^{ijkl}	5.72±0.84^{opqr}	0.8±0.22^{fghijklmn}
AR514	37	2.01±0.25^{fghijklm}	51.31±2.41^{bc}	2.77±0.3^{lmnopq}	11.01±2.23^{fghijkl}	15.65±0.15^{nop}	2.29±0.13^{cdefghijk}	14.97±0.04^{abcd}	0.55±0.00^{klmnopqr}
	18	1.09±0.11^{m}	32.24±1.24^{pq}	3.05±0.06^{klmnopq}	5.64±0.42^{nopqr}	46.81±4.09^{ab}	2.52±0^{cdefghijk}	8.65±0.63^{hijklmno}	1.57±0.12^{b}
	24	1.6±0.02^{jklm}	32.59±1.73^{pq}	4.75±0.63^{efghij}	11.36±0.88^{efghijk}	38.04±1.3^{de}	1.68±0.04^{fghijkl}	9.98±0.63^{hijklmn}	1.2±0.13^{cd}

Table S2 Fatty acids composition of *L. plantarum* strains

Strains	T/°C	Fatty acids							
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C19:1	U/S
AR514	30	2.17±0.56^{fghijkl}	36.77±0.5^{mnop}	4.16±0.58^{ghijklm}	8.65±1.44^{jklmno}	38.32±0.66^{de}	1.93±0.25^{defghijk}	8±0.01^{jklmnop}	1.1±0.07^{def}
	37	2.00±0.12^{ghijklm}	43.13±5.68^{efghijkl}	1.86±0.06^{pq}	15.64±0.05^{cde}	13.93±0.84^{op}	2.91±0.01^{bcdefg}	10.23±0.61^{ghijklm}	0.48±0.07^{opqr}
AR611	18	1.8±0.35^{jklm}	32.21±0.51^{pq}	5.07±0.92^{efgh}	6.98±1.21^{lmnop}	41.24±1.8^{cd}	2.16±0.1^{cdefghijk}	10.53±1.34^{fghijkl}	1.44±0.02^{bc}
	24	1.87±0.36^{hijklm}	41.24±0.19^{ghijklm}	3.42±0.49^{ijklmnop}	11.44±0.04^{efghijk}	28.81±0.38^{fghij}	1.41±0.06^{hijkl}	11.81±0.22^{cdefghi}	0.83±0.02^{efghijkl}
	30	1.19±0.06^{lm}	45.59±0.79^{defghij}	2.67±0.0^{mnopq}	9.94±1.71^{ghijklm}	24.92±0.58^{ikl}	1.17±0.21^{kl}	14.51±0.5^{bcde}	0.76±0.03^{ghijklmno}
	37	2.18±0.58^{fghijkl}	46.79±2.19^{cdef}	2.91±0.7^{lmnopq}	13.72±0.24^{defgh}	13.24±1.05^{p}	3.22±1^{abcd}	17.94±1.37^{a}	0.6±0.04^{ijklmnopqr}
WCFS1	18	1.67±0.08^{jklm}	26.02±5.13^{r}	6.05±0.12^{cdef}	3.55±1.97^{pqr}	46.63±0.81^{ab}	2.54±0.04^{cdefghij}	7.22±1.11^{klmnopqr}	2.05±0.48^{a}
	24	1.86±0.00^{hijklm}	36.37±0.3^{mnop}	4.76±0.99^{efghij}	1.53±0.03^{r}	45.14±2.07^{bc}	1.34±0.1^{ijkl}	8.27±0.02^{ijklmno}	1.5±0.09^{b}
	30	2.05±0.77^{fghijklm}	40.92±1.83^{hijklm}	3.74±0.37^{hijklmn}	10.91±2.21^{fghijkl}	28.11±1.52^{ghij}	2.2±0.42^{cdefghijk}	12.06±0.43^{cdefgh}	0.86±0.04^{efghijk}
	37	3.02±0.36^{cdef}	47.44±1.02^{bcde}	3.77±0.17^{hijklmn}	13.51±0.39^{defgh}	13.77±1.03^{p}	4.46±0.56^{a}	14.04±1.12^{cdef}	0.56±0.04^{jklmnopqr}

[a]T: temperature. Data are presented as means ± SD (n=3). Means with different letters differ significantly in the same column for the same FA ($P < 0.05$).

Table S3 Effect of C18:1 on glass transition temperatures of AR113

Protectants	PBS	Sorbitol	Sucrose
Normal	-41	-40.5	-33.3
C18:1	-40.7	-40	-33.2

Table S4 Effect of C18:1 on collapse temperatures of AR113

Protectants	PBS	Sorbitol	Sucrose
Normal	-39.8	-39	-31
C18:1	-39.6	-39.2	-30.5