Supplementary materials for:

**Investigation of The Lactic Acid Bacteria in Kazak Cheese and Their Contributions to Cheese Fermentation**

**Jie Li┼, Qian Huang┼, Xiaochun Zheng****, Zhengkai Ge, Ke Lin, Dandan Zhang, Yu Chen,** **Bin Wang****\*, Xuewei Shi\***

Food College, Shihezi University, Shihezi 832000, Xinjiang Uygur Autonomous Region, P. R. China.

\* Corresponding authors

E-mail addresses: B. W.: binwang0228@shzu.edu.cn; X. S.: shixuewei@shzu.edu.cn

Tel.: 86-0993-2058093

┼ These authors contributed equally to this work.

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# Supplementary tables

## **Table S1.** Similarity alignment of gene sequence

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Size (bp) | Identification | Similarity (%)  | Accession number |
| B1 | 1,059 | *Lactobacillus casei* strainNWAFU1544 | 97.68 | MG551218.1 |
| B2 | 1,151 | *Lactobacillus casei* strainNWAFU1544 | 98.68 | MG551218.1 |
| B3 | 1,252 | *Lactobacillus casei* strainNWAFU1575 | 96.88 | MG551255.1 |
| B4 | 1,243 | *Lactobacillus casei* strainNWAFU1544 | 96.76 | MG551249.1 |
| B5 | 1,233 | *Lactobacillus paracasei* strain 05 | 97.03 | JN560834.1 |
| B6 | 1,237 | *Lactobacillus helveticus* strain hstb-6 | 97.83 | KX822707.1 |
| B7 | 1,153 | *Lactobacillus helveticus* strain LH5 | 97.28 | CP019581.1 |
| B8 | 1,102 | *Streptococcus thermophilus* strain N4L | 99.73 | LS974444.1 |
| B9 | 1,080 | *Streptococcus thermophilus* strain ST3 | 99.26 | MG815652.1 |
| B10 | 1,019 | *Lactobacillus rhamnosus* strain A5 | 98.41 | MK329243.1 |
| B11 | 1,250 | *Lactococcus lactis* strain Sourdough M5 | 97.39 | MG754635.1 |
| B12 | 1,222 | *Lactobacillus rhamnosus* strain 8 | 97.7 | MN030350.1 |
| B13 | 1,226 | *Streptococcus thermophilus* strainN4L | 97.32 | LS974444.1 |
| B14 | 1,226 | *Weissella confusa* strain RCB331 | 97.19 | KT260543.1 |
| B15 | 1,219 | *Streptococcus thermophilus* strain KLDS 3.0606 | 98.84 | EU419603.1 |
| B16 | 1,051 | *Weissella confusa* strain FB054 | 96.51 | MF945623.1 |
| B17 | 1,178 | *Lactococcus lactis* strain Sourdough M8 | 98.16 | MG754628.1 |
| B18 | 1,160 | *Lactococcus gravieae* strain def2 | 99.10 | MH198321.1 |
| B19 | 1,169 | *Leuconostoc lactis* strain A4584 | 98.80 | MN441494.1 |
| B20 | 1,211 | *Leuconostoc lactis* strain KLDS 5.0604 | 98.75 | EU419606.1 |
| B21 | 1,217 | *Leuconostoc lactis* strain A4584 | 97.81 | MN441494.1 |
| B22 | 1,050 | *Lactobacillus plantarum* strain CP2 | 99.52 | MN244500.1 |

## **Table S2.** Identification and enzyme activity of LAB (U/mL)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Identification | Number | Protease activity | Lipase activity | β-galactosidase activity |
| *Streptococcus thermophilus* | B8 | 124 ± 1.32 | 87 ± 1.13 | 162 ± 1.21 |
| *Weissella confusa* | B14 | 139 ± 1.03 | 96 ± 1.06 | — |
| *Lactobacillus helveticus* | B6 | 103 ± 0.94 | 115 ± 1.65 | — |
| *Lactococcus rhamnosus* | B10 | 120 ± 1.25 | — | 186 ± 1.17 |
| *Leuconostoc lactis* | B19 | 87 ± 0.87 | — | 157 ± 1.35 |
| *Lactobacillus paracasei* | B5 | 113 ± 1.06 | — | — |
| *Leuconostoc lactis* | B20 | 105 ± 0.95 | — | — |
| *Lactobacillus casei* | B4 | 96 ± 0.78 | — | — |
| *Lactobacillus helveticus* | B7 | — | 83 ± 0.88 | 106 ± 1.18 |
| *Lactobacillus casei* | B1 | 86 ± 0.88 | 94 ± 0.82 | 138 ± 1.33 |
| *Leuconostoc lactis* | B21 | 102 ± 1.14 | — | — |
| *Weissella confusa* | B16 | 98 ± 0.92 | 87 ± 0.91 | — |
| *Streptococcus thermophilus* | B9 | 118 ± 2.03 | 68 ± 0.76 | — |
| *Streptococcus thermophilus* | B13 | 102 ± 1.06 | 73 ± 0.95 | — |
| *Streptococcus thermophilus* | B15 | 116 ± 1.31 | — | 140 ± 1.67 |
| *Lactococcus lactis* | B11 | 113 ± 1.48 | 77 ± 0.85 | — |
| *Lactobacillus casei* | B3 | — | — | 158 ± 1.42 |
| *Lactobacillus casei* | B2 | 96 ± 0.92 | 87 ± 0.97 | 155 ± 1.75 |

1 Symbol “—” stands for undetectable.

2 Data is expressed as the mean ± standard deviation and three replicate analyses (n = 3) of three replicate samples.

## **Table S3.** Construction of fingerprints of volatile compounds in cheeses

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Number | Flavor compounds | Time (min) | LrC-5 | StC | WcC | LhC |
| 1 | Ethyl acetate | 2.36 |  | 10.028\* |  | −0.909 |
| 2 | Ethanol | 2.73 | 8.140\* | 38.015\* |  | −0.981 |
| 3 | Ethyl butanoate | 4.25 |  |  | 1.278 | −0.743 |
| 4 | Hexanal | 5.20 |  |  |  | 1.424 |
| 5 | Isobutanol | 5.91 |  | −0770 |  |  |
| 6 | Isoamyl acetate | 6.25 |  | −0.474 |  | 6.954\* |
| 7 | Pentyl acetate | 7.81 |  | +1 |  |  |
| 8 | 5-Methyl-2-hexanone | 8.24 |  |  |  | −1 |
| 9 | Isoamylol | 9.63 |  |  |  | −1 |
| 10 | Ethyl hexanoate | 10.56 | 1.912 |  |  |  |
| 11 | Hexyl acetate | 12.47 | 16.959\* |  |  | 2.134 |
| 12 | Octanal | 13.156 | +1 |  |  |  |
| 13 | 2-Heptenal | 14.85 | +1 |  |  | +1 |
| 14 | Prenol | 15.00 |  |  |  | 1.492 |
| 15 | Ethyl l-lactate | 16.15 | 2.942 | 4.069\* | 1.091 | 2.490 |
| 16 | Hexanol | 16.74 |  |  |  | 4.495\* |
| 17 | Ethyl caprylate | 21.29 | 7.421\* | 1.532 | 3.783 | 6.954\* |
| 18 | Acetic acid | 22.06 | 16.137\* |  |  | 8.725\* |
| 19 | 2-Nonenal | 26.59 |  |  |  | −0.862 |
| 20 | Propanoic acid | 27.26 |  | 2.835 |  |  |
| 21 | Butanoic acid | 32.26 | +1 |  |  | +1 |
| 22 | α-Cumyl alcohol | 36.52 |  | 1.984 |  |  |
| 23 | 2-Methylpentanoic acid | 36.93 | 62.935\* |  |  | 32.762\* |
| 24 | Phenethyl acetate | 38.2 |  | +1 |  |  |
| 25 | Hexanoic acid | 40.66 |  | +1 |  |  |
| 26 | Phenylethyl alcohol | 41.00 |  | +1 |  |  |
| 27 | Heptanoic acid | 42.42 |  |  |  | 1.203 |
| 28 | 3-Butanolal | 50.80 | 1.445 |  |  |  |
| 29 | Benzoic acid | 52.14 |  |  |  | 1.871 |

1 Symbol "+1" and "−1" indicated compounds that are formed and disappeared in the later stages of fermentation.

2 The data was calculated as (late peak area − initial peak area) / initial peak area.

3 Symbol "\*" indicated that the compound changes significantly.

## **Table S4.** Concentrations of volatile compounds in cheeses (µg/kg)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compounds |  RI | LrC | StC | WcC | LhC |
| **Alcohols** |  |  |  |  |  |
| Ethanol | 1,482 | 17.740 ± 4.190b | 290.176 ± 45.573a | 304.318 ± 46.775a | 2.322 ± 0.902b |
| Isobutanol | 1,526 | 1.542 ± 0.335c | 15.909 ± 2.072b | 54.540 ± 4.678a | 0.654 ± 0.078c |
| Isoamylol | 1,142 | — | 176.202 ± 4.972b | 819.311 ± 34.614a | 36.402 ± 2.479c |
| Pentanol | 1,265 | 33.034 ± 0.419a | 17.939 ± 1.657b | 36.204 ± 6.549a | 2.096 ± 0.225d |
| Prenol | 1,037 | 7.215 ± 0.647 | — | 19.365 ± 4.678 | — |
| Hexanol | 1,374 | 0.545 ± 0.042d | 44.454 ± 0.829b | 75.401 ± 3.742a | 19.925 ± 2.479c |
| 2-Nonen-1-ol | 1,026 | 8.891 ± 0.251b | 5.842 ± 0.373c | 17.868 ± 2.748a | 3.629 ± 0.158c |
| 2-Ethylhexanol | 974 | 2.338 ± 0.419b | 3.480 ± 0.043a | 3.461 ± 0.047a | 0.834 ± 0.008c |
| 1-Octanol | 2,157 | 0.930 ± 0.017d | 63.264 ± 1.657a | 25.539 ± 1.871b | 4.170 ± 0.135c |
| 2,3-Butanediol | 1,237 | 1.927 ± 0.168d | 153.622 ± 7.557a | 124.515 ± 6.874b | 67.778 ± 2.930c |
| 1-Nonanol | 1,743 | 6.570 ± 0.335b | 2.817 ± 0.124c | 8.232 ± 0.561a | 3.043 ± 0.902c |
| α-Cumyl alcohol | 1,174 | 0.712 ± 0.025c | 2.154 ± 0.207b | 2.994 ± 0.281a | — |
| Phenylethyl alcohol | 1,837 | 0.117 ± 0.021d | 28.090 ± 2.900b | 153.703 ± 10.291a | 11.315 ± 0.902c |
| 2-Methyloctan-3-ol | 1,275 | 0.696 ± 0.168 | 1.657 ± 0.414 | — | — |
| **Aldehydes** |  |  |  |  |  |
| Hexanal | 1,368 | 18.335 ± 4.190c | 95.206 ± 2.072a | 54.072 ± 3.742b | 15.801 ± 1.803d |
| 2-Heptenal | 1,623 | 1.265 ± 0.251c | 11.973 ± 0.829a | — | 7.889 ± 1.352b |
| Nonanal | 1,346 | 5.958 ± 0.168d | 13.092 ± 1.243c | 58.375 ± 3.742a | 17.401 ± 1.803b |
| Decanal | 1,573 | — | 12.222 ± 0.829a | 7.858 ± 0.561b | 2.637 ± 0.451c |
| Benzaldehyde | 1,355 | 0.737 ± 0.084b | — | 36.110 ± 1.054a | 0.676 ± 0.025b |
| 2-Nonenal | 1,257 | 1.274 ± 0.168 | — | — | 9.489 ± 1.127 |
| 3-Butanolal | 1,085 | 0.327 ± 0.024b | 0.456 ± 0.066b | 2.058 ± 0.187a | 0.316 ± 0.007b |
| Octanal | 1,548 | — | 2.154 ± 0.124c | 3.368 ± 0.187b | 4.688 ± 0.451a |
| **Acids** |  |  |  |  |  |
| Acetic acid | 1,758 | 246.070 ± 8.38d | 1295.185 ± 91.146b | 2137.056 ± 168.390a | 753.647 ± 56.350c |
| Propanoic acid | 1,029 | 0.570 ± 0.050d | 45.283 ± 5.414a | 28.346 ± 3.742b | 7.686 ± 1.127c |
| Isobutyric acid | 1,437 | 14.204 ± 0.168d | 134.150 ± 2.900b | 311.615 ± 8.420a | 41.406 ± 1.578c |
| Butanoic acid | 1,192 | 27.998 ± 5.028d | 185.524 ± 6.215b | 311.615 ± 5.613a | 107.786 ± 2.029c |
| 3-Methylbutanoic acid | 1,090 | 59.054 ± 7.542c | 238.844 ± 4.557b | 528.838 ± 8.420a | 62.323 ± 2.479c |
| 2-Methylpentanoic acid | 1,039 | 57.018 ± 0.503b | 19.886 ± 3.314c | 12.255 ± 0.561d | 105.532 ± 2.479a |
| Hexanoic acid | 964 | 43.358 ± 0.754d | 262.376 ± 2.900b | 400.113 ± 13.097a | 104.834 ± 2.930c |
| Heptanoic acid | 1,354 | 0.520 ± 0.024d | 5.320 ± 0.166a | 3.929 ± 0.157b | 1.082 ± 0.225c |
| Octanoic acid | 1,503 | 16.148 ± 0.503c | — | 149.119 ± 8.420a | 37.146 ± 2.902b |
| Nonanoic acid | 1,374 | 0.520 ± 0.084b | 3.687 ± 0.207a | 5.332 ± 1.871a | 0.609 ± 0.023b |
| Decanoic acid | 1,097 | 3.143 ± 0.154d | 13.755 ± 2.041b | 26.007 ± 1.678a | 7.326 ± 1.356c |
| Benzoic acid | 1,275 | 5.799 ± 0.587c | 18.519 ± 1.249b | 38.168 ± 1.878a | 6.942 ± 1.127c |
| **Esters** |  |  |  |  |  |
| Ethyl acetate | 1,379 | 14.531 ± 2.514c | 111.488 ± 5.386b | 538.942 ± 12.162a | 19.858 ± 1.352c |
| Isobutyl acetate | 1,027 | — | 7.582 ± 1.243 | 7.858 ± 1.871 | — |
| Ethyl butanoate | 1,768 | 5.045 ± 0.335 | — | — | 4.035 ± 0.451 |
| Isoamyl acetate | 1,942 | 104.113 ± 4.190c | 358.079 ± 37.287b | 2243.423 ± 121.615a | 418.681 ± 24.743b |
| Pentyl acetate | 1,274 | 13.081 ± 1.642b | — | 70.630 ± 2.807a | 72.556 ± 2.930a |
| Ethyl hexanoate | 1,354 | 3.520 ± 0.503c | 24.029 ± 1.657b | 46.214 ± 4.678a | 47.131 ± 1.803a |
| Hexyl acetate | 1,736 | 2.564 ± 0.168c | 1.657 ± 0.414c | 16.091 ± 1.459b | 27.025 ± 2.029a |
| Ethyl l-lactate | 1,932 | 3.327 ± 0.526d | 39.731 ± 2.9b | 139.670 ± 7.484a | 17.085 ± 1.352c |
| Heptyl acetate | 1,093 | 1.089 ± 0.078d | 6.090 ± 0.249a | 4.303 ± 0.574b | 3.426 ± 0.225c |
| Ethyl caprylate | 1,127 | 45.487 ± 2.541b | 17.939 ± 2.072d | 39.478 ± 2.807c | 75.599 ± 2.254a |
| Octyl acetate | 1,425 | 0.578 ± 0.168 | — | — | 0.564 ± 0.068 |
| Butyrolactone | 1,237 | — | 2.154 ± 0.034 | — | 1.127 ± 0.676 |
| Ethyl caprate | 1,358 | — | — | — | 8.678 ± 1.127 |
| Phenethyl acetate | 1,642 | 11.472 ± 2.136c | 13.838 ± 3.362c | 215.259 ± 9.355a | 86.103 ± 15.778b |
| 5-Decanolide | 1,138 | 0.503 ± 0.059c | 13.755 ± 1.015a | 2.526 ± 0.146b | 0.947 ± 0.113c |
| 1,3-Diacetoxypropane | 1,029 | — | 1.326 ± 0.083 | — | 0.541 ± 0.088 |
| **Ketones** |  |  |  |  |  |
| Acetol | 1,528 | 0.109 ± 0.004b | 2.983 ± 0.124b | 141.354 ± 10.107a | — |
| 3-Methyl-2-hexanone | 976 | — | — | 0.164 ± 0.001 | — |
| 5-Methyl-2-hexanone | 1,069 | 16.584 ± 1.676c | 184.364 ± 4.557a | 60.620 ± 2.807b | 2.299 ± 0.451d |
| 2-Heptanone | 1,463 | 5.657 ± 0.135c | 27.924 ± 2.486a | 1.965 ± 0.094d | 8.205 ± 0.902b |
| Acetoin | 1,137 | — | 103.078 ± 1.243a | 12.723 ± 1.871b | 7.168 ± 0.120c |
| 6-Methylhept-5-en-2-one | 1,265 | — | 11.518 ± 0.414b | 12.629 ± 0.936a | 3.584 ± 0.551c |
| 2-Nonanone | 1,386 | 10.165 ± 2.745c | 46.692 ± 2.486a | 36.578 ± 2.567b | 2.029 ± 0.676d |
| 2-Undecanone | 963 | 0.712 ± 0.003b | 5.800 ± 1.203a | 5.145 ± 0.281a | — |

1 Data are expressed as the mean ± standard deviation from three replicate analyses (n = 3) of three replicate samples.

2 The different lowercase letters in each row indicated a significant difference between the samples (*P* < 0.05).

3 LrC, StC, WcC, and LhC represented samples collected at the fifth stage.

4 The symbol “—” indicated that the compound was not detected.

# Supplementary figures



## **Figure S1.** Hydrolysis halo of selected strains



## **Figure S2.** Numbers of viable bacteria in cheeses at different fermentation periods (cfu/kg)



## **Figure S3.** Construction of volatile compound fingerprints for the LABs



## **Figure S4.** Radar analysis of flavor compounds