Supplemental Information

Bacterial Adaptation to the Host's Diet

Is a Key Evolutionary Force Shaping

Drosophila-Lactobacillus Symbiosis

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Supplemental figure titles and legends

Bacterial adaptation to diet is a key evolutionary force shaping *Drosophila-Lactobacillus* symbiosis

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A

Ancestor Lp^NIZO2377 →

Fossil Records G1 →

Fossil Records G20 →

GENERATION 1

GENERATION 2

GENERATION 20

B

Generation Time

$Lp^{NIZO2377} = 3.20 \, h - SEM = 0.52$

Figure S1
Supplemental Figure 1 (related to Fig. 1): Rationale and schematic representation of the experimental setup for studying *L. plantarum* adaptive evolution (AE) with *Drosophila melanogaster*.

(A) The ancestor strain (*Lp*\textsuperscript{NIZO2877}) was added to 40 germ-free (GF) *Drosophila* embryos at the beginning of the first *Drosophila* generation (Generation 1). The first 15 emerging pupae were transferred to a new sterile poor nutrient diet. This allowed the bacteria associated with the pupae to propagate and colonize the new environment. The 15 adults emerged from the 15 transferred pupae, mated and females laid eggs that became the founders of the following fly generation (Generation 2). Once the eggs were laid, the adults were collected and homogenized to isolate the evolved bacteria they carry (fossil records from generation 1). Generation 2 followed the same experimental cycle as Generation 1, with the exception that no further inoculation of the ancestor strain *L. plantarum*\textsuperscript{NIZO2877} has been performed. Evolving bacteria were propagated through the transfer of the pupae during each generation. The experimental evolution lasted 20 *Drosophila* generations (313 days). Colour shading represents the evolution of the bacterial population during the experiment.

(B) 16S rRNA kinetics of *Lp*\textsuperscript{NIZO2877} in *Drosophila* Niche (*Drosophila* + Diet). The 16S rRNA gene quantification is shown in logarithmic scale. The mean generation time (h, hours) of *Lp*\textsuperscript{NIZO2877} in *Drosophila* niche ± the standard error of the mean (SEM) are reported on the graph (see Methods).
Supplemental Figure 2 (related to Fig. 2): Sequence/structural analysis of $Lp^{NIZO2877}$ Acetate kinase A (AckA) protein aligned against the AckA of $Lp^{NIZO2877}$-derived strains (FlyG2.1.8, FlyG9.2.5) evolved in *Drosophila* niche. The secondary structure of the protein is indicated in blue above the sequence alignment. Catalytic residues of the predicted active site are shown in bold blue characters. The mutation sites are highlighted in pink and green for FlyG2.1.8 and FlyG9.2.5 strains respectively. The alignment was performed using Clustal Omega and drawn with ESPript.
Figure S3
Supplemental Figure 3 (related to Fig. 3): CRISPR/Cas9 genome editing in *Lactobacillus plantarum* with a dsDNA repair template.

(A) Construction of the repair template plasmid containing the dsDNA template. Following successful construct generation, cells containing the repair plasmid were transformed with the self-targeting Cas9 plasmid, thereby killing any cells that did not incorporate the repair template into the genome.

(B) Spacer design for targeting *ackA* in *Lp*\(^{\text{NIZO2877}}\)_FlyG2.1.8. The spacer will only successfully cleave *Lp*\(^{\text{NIZO2877}}\)_FlyG2.1.8, while allowing any edited survivors to evade cleavage due to a spacer mis-match and presence of a non-PAM.

(C) Transformation results after Cas9 self-targeting with the repair template plasmid. Presence of the repair template allowed for a total of 15 survivors clones to Cas9 killing.

(D) *ackA* locus sequencing results for 10 of the survivors. Two survivors contained the un-edited *ackA* gene in *Lp*\(^{\text{NIZO2877}}\)_FlyG2.1.8, and one did not yield a PCR product (No Ampl.). Seven colonies contained the edited *ackA* sequence.

(E) Plasmid removal after editing. Successfully edited cells were passaged multiple times through non-selective media to remove the genome editing plasmids. After validation of plasmid removal, strains had their genomes sequenced and were analyzed for *in vivo* validation.
Supplemental Figure 4 (related to Fig. 4): Development of two Real-Time PCR assays for the discrimination and quantification of $Lp^{\text{NIZO2877}}$ and $Lp^{\text{NIZO2877-evolved}}$ strain FlyG2.1.8.

(A) Real-time PCR standard curves obtained from the amplification of $Lp^{\text{NIZO2877}}$ (green) and FlyG2.1.8 (grey) strains. The graph shows the interpolated standard curves using determined threshold cycles ($C_T$) values and known template numbers for five standard samples. All points represent the mean of triplicate PCR amplifications. The respective efficiency values and curve equations are reported on the graph.

(B, C) Fluorescence amplification plots obtained from the amplification of $Lp^{\text{NIZO2877}}$ and FlyG2.1.8 strains using $Lp^{\text{NIZO2877}}$-specific (B) and FlyG2.1.8 specific (C) Real-time assays.
Figure S5
Supplemental Figure 5 (related to Fig. 5): *L. plantarum* adaptive evolution (AE) in *Drosophila* diet without *Drosophila melanogaster*.

(A) Rationale and schematic representation of the experimental setup. The ancestor (*Lp*<sup>NIZO2877</sup>) was added to sterile poor nutrient diet (Cycle 1). As soon as the microbial load reached the same value found on the 15 pupae used for propagating the bacterial population in the Niche adaptive evolution setup (10<sup>7</sup> CFU/mL of diet; Figure S1A), part of the medium was crushed and transferred to a new sterile poor nutrient diet. Fossil records were isolated from the crushed medium at the end of each cycle. Cycle 2 followed the same experimental course as Cycle 1. *L. plantarum* experimental evolution on *Drosophila* diet lasted 20 cycles. Colour shading represents the evolution of the bacterial population during the experiment.

(B) Sequence/structural analysis of *Lp*<sup>NIZO2877</sup> AckA protein aligned against AckA from *Lp*<sup>NIZO2877</sup>-derived strain (DietG20.1.2) evolved in Drosophila diet. The secondary structure of the acetate kinase A protein is indicated in blue above the sequence alignment. The key catalytic residues of the predicted active sites are shown in bold blue characters. The mutation site is highlighted in cyan. The alignment was performed using Clustal Omega and drawn with ESPript.
Supplemental Figure 6 (related to Fig. 6): Sequence/structural analysis of $Lp^{NIZO2877}$ AckA protein aligned against AckA from $Lp^{WJL}$. The secondary structure of the acetate kinase A protein is indicated in blue above the sequence alignment. The key catalytic residues of the predicted active sites are shown in bold blue characters. The mutation sites are highlighted in green. The alignment was performed using Clustal Omega and drawn with ESPript.
Supplemental tables Titles and Legends

Table S1. Bacterial strains, Related to Figures 1, 2, 3, 5. List of all *L. plantarum* strains used and sequenced in this study.
| **L. plantarum Strains** | **Description** | **Fly/Diet generation of isolation** | **Replicate** | **Accession Number** | **Reference** |
|-------------------------|----------------|-------------------------------------|---------------|----------------------|--------------|
| NIZO2877                | Isolated from Vietnamese hotdog | -                                   | -             | LHIZ00000000        | (Martino et al., 2015a) |
| WIL                     | Isolated from Drosophila melanogaster intestine | -                                   | -             | LKLZ00000000        | (Martino et al., 2015b) |
| FlyG2.1.8               | NIZO2877-evolved strain | 2                                   | 1             | PEBE00000000        | This study   |
| FlyG3.1.8               | NIZO2877-evolved strain | 3                                   | 1             | PEGI00000000        | This study   |
| FlyG7.1.6               | NIZO2877-evolved strain | 7                                   | 1             | PEGJ00000000        | This study   |
| FlyG8.1.1               | NIZO2877-evolved strain | 8                                   | 1             | PEGK00000000        | This study   |
| FlyG8.1.2               | NIZO2877-evolved strain | 8                                   | 1             | PEGL00000000        | This study   |
| FlyG9.1.4               | NIZO2877-evolved strain | 9                                   | 1             | PEGM00000000        | This study   |
| FlyG10.1.5              | NIZO2877-evolved strain | 10                                  | 1             | PEGN00000000        | This study   |
| FlyG10.1.9              | NIZO2877-evolved strain | 10                                  | 1             | PEGO00000000        | This study   |
| FlyG11.1.2              | NIZO2877-evolved strain | 11                                  | 1             | PEGP00000000        | This study   |
| FlyG11.1.6              | NIZO2877-evolved strain | 11                                  | 1             | PEGQ00000000        | This study   |
| FlyG20.1.4              | NIZO2877-evolved strain | 20                                  | 1             | PEGR00000000        | This study   |
| FlyG2.1.8Rev            | NIZO2877-evolved strain | -                                   | -             |                      | This study   |
| FlyG9.2.5               | NIZO2877-evolved strain | 9                                   | 2             | PEGS00000000        | This study   |
| FlyG11.1.6              | NIZO2877-evolved strain | 11                                  | 2             | PEGT00000000        | This study   |
| FlyG20.2.6              | NIZO2877-evolved strain | 20                                  | 2             | PEGU00000000        | This study   |
| DietG20.1.2             | NIZO2877-evolved strain | 20                                  | 1             | PEGV00000000        | This study   |
| DietG20.2.2             | NIZO2877-evolved strain | 20                                  | 2             | PEGW00000000        | This study   |

Table S1
Table S2. Summary of mutations detected across the experimental evolution of *L. plantarum*, related to Figures 1, 2, 3, 5. List of all mutations detected in the *L. plantarum* experimental evolution replicates. §Locus tag refers to *L. plantarum* reference strain WCFS1 (Kleerebezem et al., 2003). nt: nucleotide; WGS: whole genome sequencing; SS: Sanger sequencing. Mutations identified by Sanger sequencing were confirmed from alignments of both forward and reverse reads.
| Strain     | Generation/Transfer | Gene/Region | Locus Tag | Annotation | Mutation | Position in LpNIZO2877 | Method  |
|------------|---------------------|-------------|-----------|------------|----------|------------------------|---------|
| FlyG2.1.8  | Niche 1 1           | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS/SS                 |
| FlyG3.1.8  | Niche 1 1           | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS/SS                 |
| FlyG7.1.6  | Niche 1 7           | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS/SS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS/SS |
|            |                     | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS/SS |
| FlyG8.1.1  | Niche 1 8           | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS |
|            |                     | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS |
| FlyG8.1.2  | Niche 1 8           | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS |
|            |                     | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS |
| FlyG9.1.4  | Niche 1 9           | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS |
|            |                     | adhE        | lp_3662   | alcohol dehydrogenase/acetalddehyde dehydrogenase 1 nt substitution | 2268660 | WGS/SS |
|            |                     | int2        | -         | intergenic region | 1 nt substitution | 2456364 | WGS/SS |
|            |                     | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS |
| FlyG10.1.5 | Niche 1 10          | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS |
|            |                     | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS |
| FlyG10.1.9 | Niche 1 10          | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS |
|            |                     | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS |
| FlyG11.1.2 | Niche 1 11          | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS |
|            |                     | ackA        | lp_03010  | acetate kinase deletion (Δ3) | 2571613-5 | WGS |
| FlyG11.1.6 | Niche 1 11          | int1        | -         | intergenic region | 1 nt substitution | 504874 | WGS |
|            |                     | cheY        | lp_1544   | two-component system response regulator | 1 nt substitution | 1348923 | WGS |
| Niche   | Start | End | Gene | Description                          | Substitution Type | WGS/SS |
|---------|-------|-----|------|--------------------------------------|-------------------|--------|
| FlyG20.1.4 | 1    | 20  | ackA | lp_03010 acetate kinase deletion (Δ3) | 1 nt substitution | 2571613-5 WGS |
|          |       |     | patB | lp_0749 phosphate ABC transporter ATP-binding protein | 1 nt substitution | 120791 WGS/SS |
|          |       |     |       | - lp_0797 exoribonuclease II | 1 nt substitution | 177140 WGS/SS |
|          | int1  |     |       | - lp_2499 transport ATP-binding protein/permease | 1 nt substitution | 947607 WGS |
|          |       |     |       | - lp_1238 LysR family transcriptional regulator | 1 nt substitution | 110564 WGS/SS |
|          | cheY  |     |       | lp_1544 two-component system response regulator | 1 nt substitution | 1348923 WGS |
|          | int3  |     |       | - | 1 nt substitution | 1736935 WGS/SS |
|          | ackA  |     |       | lp_03010 acetate kinase deletion (Δ3) | 1 nt substitution | 2571613-5 WGS |
| FlyG9.2.5 | 2    | 9   | ackA | lp_03010 acetate kinase deletion (Δ3) | 1 nt substitution | 2571613-5 WGS |
|          |       |     | int4  | - | 1 nt substitution | 1982853 WGS/SS |
| FlyG11.2.6 | 2   | 11  | ackA | lp_03010 acetate kinase deletion (Δ3) | 1 nt substitution | 2571613-5 WGS |
|          |       |     | int4  | - | 1 nt substitution | 1982853 WGS |
| FlyG20.2.6 | 2   | 20  | cheY | lp_1544 two-component system response regulator | 1 nt substitution | 1348886 WGS/SS |
|          |       |     |       | - lp_2212 NADH-flavin reductase | 1 nt substitution | 1937136 WGS |
|          | int4  |     |       | - | 1 nt substitution | 1982853 WGS |
|          |       |     |       | - lp_0197 cell surface protein precursor, LPXTG-motif cell wall anchor | deletion (Δ6) | 2471707-12 WGS/SS |
|          | ackA  |     |       | lp_03010 acetate kinase deletion (Δ3) | 1 nt substitution | 2571613-5 WGS |
| DietG20.1.2 | 1   | 20  | ackA | lp_03010 acetate kinase deletion (Δ3) | 1 nt substitution | 2571576 WGS/SS |
| DietG20.2.2 | 2   | 20  | ackA | lp_03010 acetate kinase deletion (Δ3) | 1 nt substitution | 2571576 WGS/SS |

Table S2
Table S3. Metabolomic dataset of *Drosophila* diet inoculated with Lp\(^{NIZO2877}\) and FlyG2.1.8 separately, Related to Figure 6. Table of metabolites resulted to be significantly different between Lp\(^{NIZO2877}\) - and FlyG2.1.8-associated *Drosophila* diets based on two-sided t-tests (p<0.05). Fold-changes (FC) are calculated with the ratio between means of Lp\(^{NIZO2877}\) and FlyG2.1.8 replicates for each metabolite. Metabolites with a positive FC are overrepresented in FlyG2.1.8-associated samples and those with a negative FC are underrepresented in FlyG2.1.8-associated samples. FC detail: If mean(FlyG2.1.8) > mean(Lp\(^{NIZO2877}\)), FC = mean(FlyG2.1.8)/mean(Lp\(^{NIZO2877}\)); If mean(Lp\(^{NIZO2877}\))>mean(FlyG2.1.8), FC = - mean(Lp\(^{NIZO2877}\))/mean(FlyG2.1.8)
| Metabolite           | Metabolite Class | log2 FC | Fold Change | p-value |
|---------------------|------------------|---------|-------------|---------|
| Amino Acid          |                  | -1.02   | 0.20        | 0.0119  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |
| Carbohydrate        |                  | 1.08    | 1.09        | 0.0152  |
| Amino Acid          |                  | 1.08    | 1.09        | 0.0152  |

**Note:** The table above is a subset of metabolites and their fold changes with corresponding p-values. The full dataset includes more metabolites and their respective changes. The fold changes are calculated as the ratio of the metabolite levels in the treatment group relative to the control group, and the p-values indicate the statistical significance of these changes (lower p-values indicate stronger evidence of differential expression).
| Carbohydrate | Cofactors and Vitamins | Lipids | Nucleoside Nucleotides | Other | Small Molecules | Total |
|--------------|------------------------|--------|------------------------|-------|----------------|-------|
| fructose 1,6-diphosphate/glucose 1,6-diphosphate | | | | | | |
| nicotinamide adenine dinucleotide (NAD+) | | | | | | |
| palmitoyl-oleoyl-glycerol (16:0/18:1) | | | | | | |
| oleoyl-linoleoyl-glycerol (18:1/18:2) | | | | | | |
| N-palmitoyl-sphinganine (d18:0/16:0) | | | | | | |
| 1,2-dioleoyl-GPC (18:1/18:1) | | | | | | |
| 1-palmitoleoyl-GPC (16:1)* | | | | | | |
| 1-palmitoyl-GPC (16:0) | | | | | | |
| glycerophosphoglycerol | | | | | | |
| 1-palmitoyl-GPE (16:0) | | | | | | |
| 1-stearoyl-GPC (18:0) | | | | | | |
| oleoyl ethanolamide | | | | | | |
| myo-inositol | | | | | | |
| raffinose | | | | | | |
| ribitol | | | | | | |
| myo-inositol | | | | | | |
| raffinose | | | | | | |
| ribitol | | | | | | |
| myo-inositol | | | | | | |
| raffinose | | | | | | |
| ribitol | | | | | | |
| myo-inositol | | | | | | |
| raffinose | | | | | | |
| ribitol | | | | | | |
| myo-inositol | | | | | | |
| raffinose | | | | | | |
| ribitol | | | | | | |
| myo-inositol | | | | | | |
| raffinose | | | | | | |
| ribitol | | | | | | |
| Compound                  | Lipid       | Nucleotide      | Peptide   |
|--------------------------|-------------|-----------------|-----------|
| 1-palmitoylglycerol (16:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (18:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (18:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (18:3)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (20:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (20:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (20:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (20:3)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (22:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (22:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (22:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (22:3)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (24:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (24:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (24:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (24:3)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (26:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (26:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (26:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (26:3)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (28:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (28:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (28:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (28:3)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (30:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (30:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (30:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (30:3)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (32:0)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (32:1)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (32:2)| 1,0186      | 1,0525          | 0,7021    |
| 1-palmitoylglycerol (32:3)| 1,0186      | 1,0525          | 0,7021    |

Table S3
Table S4. Primers, Related to Figures 1, 2, 4, 5. List of DNA oligonucleotide primers used in this study.
| Name             | DNA sequence (5'–3') | Annealing t° | Reference         |
|------------------|----------------------|-------------|-------------------|
| ackA_F           | TAAGACGCAAGATACCGTG  | 62          | This study        |
| acka_R           | ACGCACAATCATCAGCTTT  | 62          | This study        |
| int1_F           | TTAAAAACATCGGCTACGGAAG | 63          | This study        |
| int1_R           | TTAATTTATCGCCCCGCAAGA | 62          | This study        |
| cheY_F           | CTCGCTCTGTATGCTTTACT | 59          | This study        |
| cheY_R           | TAACAGCACTAGCCACGTTT | 60          | This study        |
| adeE_F           | GGCTCCCTTAATTCACAAGG | 62          | This study        |
| adeE_R           | ATCCTTGAAAAGCTAACCGGG | 63          | This study        |
| int2_F           | AGCGATATCCTCTGTGAAC  | 60          | This study        |
| int2_R           | CGCGTTGTGCTAGCTAATT  | 61          | This study        |
| lp_0055_F        | GCCATGTGTGTAACGTTGC  | 61          | This study        |
| lp_0055_R        | GTGATCCAAGGGGTCCAAAT | 62          | This study        |
| pstB_F           | AAGCACAATAGGACGGCTTC | 60          | This study        |
| pstB_R           | TGCGTGATAAGCCACATCTT | 62          | This study        |
| lp_0797_F        | ATTTTCCAAAGTGATGTCG  | 63          | This study        |
| lp_0797_R        | ACTTTGATCATTTGATGAC  | 63          | This study        |
| lp_1258_F        | GGCCTTAACCGATGAATCTA | 62          | This study        |
| lp_1258_R        | GACCTGTTCCTCCGCGT    | 60          | This study        |
| int3_F           | TCTTCTACACCTTGTTTTTC | 62          | This study        |
| int3_R           | GCGAATGTCATAGTCGGGA  | 62          | This study        |
| int4_F           | GACGATTAGACTAGTCGGT  | 61          | This study        |
| int4_R           | CAGTCTAGTTTTCCACAG   | 62          | This study        |
| lp_0197_F        | CGCCCAATGTGACATGATT  | 63          | This study        |
| lp_0197_R        | CGTTGTGCTAGGATGATGG  | 63          | This study        |
| ackA2_F          | GTGAATCAGTGGGTGTTGG  | 63          | This study        |
| ackA2_R          | ACCATGATCAAAGCGGTGA  | 65          | This study        |
| int5_F           | CAACGCCGAAGTTACATGCT | 60          | This study        |
| int5_R           | GCAATCCCTGCGTTGATCATC | 62      | This study        |
| int6_F           | GTCCAGCTATTTCCACGGAT | 62          | This study        |
| int6_R           | CATACGCAAATAGGTTCCCATA | 63 | This study        |
| 16S_UniF         | GTGSTGCAYGGYTGTCGTC  | 70          | (Packey et al., 2013) |
| 16S_UniR         | ACGTCTCCTACCTCCTCCTC | 68          | (Packey et al., 2013) |
| ackA_NIZO2877_F  | CGAACGTGTCACTAAAGCCTT | 63          | This study        |
| acka_FlyG2       | GCGAACGTGTCACTAAAGAG | 62          | This study        |
| acka_R_RT        | CACCGCCAATCATCAGCTCT | 63          | This study        |

Table S4
Table S5. Plasmids used in this work, Related to Figure 3. List of plasmids used to engineer \( Lp^{\text{NIZO2877}} \) with CRISPR-Cas9.
| Plasmid       | Description                                                                 | Resistance | Source                  | Stock  |
|---------------|-----------------------------------------------------------------------------|------------|-------------------------|--------|
| pJP005        | RecT protein under a nisin-inducible promoter, without nisK and nisR genes  | Cm         | Van Pijkeren and Britton, 2012 | CB653  |
| pMSP3545      | Gram-positive bacterial shuttle vector for nisin-controlled inducible expression | Emr        | Addgene-CN44888          | CB6794 |
| pCas9         | Plasmid containing Streptococcus pyogenes Cas9 and its tracrRNA              | Amp        | Addgene-CN 42874         | CB6372 |
| p3545Cas9     | Shuttle vector containing S. pyogenes Cas9 and its tracrRNA                  | Emr        | This work                | CB6777 |
| p3545Cas9RSR  | Shuttle vector containing S. pyogenes Cas9, tracrRNA, and a repeat-spacer-repeat array for targeting | Emr        | This work                | CB6798 |
| p3545Cas9ackA | Cas9 shuttle vector targeting the acetate kinase gene in NIZO/G2             | Emr        | This work                | CB6799 |
| CB711         | pJP005 vector with repair template for the ackA target                      | Emr        | This work                | CB711  |

Table S5
Table S6. Oligonucleotides used to engineer \( Lp^{\text{NIZO2877}} \) with CRISPR-Cas9, Related to Figure 3.
| Shorthand | Name                     | Sequence                                                                 |
|-----------|--------------------------|--------------------------------------------------------------------------|
| oRL1      | pCas9.Gibson.fwd         | GATGATAAGCTGTCAAAAACATGAGAAATTCTTCTTACGAAATCTATCCTGTGGAGCTTAG            |
| oRL2      | pCas9.Gibson.rev         | ATTTTTAGGATAAACTTCTGCCCCACCTTTTTCTCGACTCTAGCTGTGACTC                   |
| oRL3      | pMSP3545.Gibson.fwd      | ATTGATTGGATGTCAGTAGGAGGTGACTGAAAGTGGGGCGAGAGTTATTCTCAA                  |
| oRL4      | pMSP3545.Gibson.rev      | CCTACTAAGCTCCACAGAGTAGATTTCCTATGAAAGTTATTCTCTGTTGAGACGCTTACTC          |
| oRL5      | gBlockRSR.Gibson.fwd     | TTGGTGCCAAGAAACGCTGACTGTGTCAGCTGACGAGTTAGCTGATCA                      |
| oRL6      | gBlockRSR.Gibson.rev     | GGAGGACACTCACATGCGGTACAGGATGAGTTGACTGACG                                 |
| oRL7      | Acet.Kin.pJP005.f        | ATTTACTAGTGGTTTTTTTCTATGATGACGCTCT                                   |
| oRL8      | Acet.Kin.pJP005.r        | TCACGACGCTCCACAAGAGCTACTATGAGGAAAG                                    |
| oRL9      | pJP005.seq.rev           | TGATTGCTTCTATGCAAAGGCAA                                                |
| oRL10     | pJP005.seq.fwd           | ATTGCTAGAAGGATTTCTGCTAAAGG                                            |
| oRL11     | Acet.Kin.Outer.fwd       | GGAGGAGGACACGAAAGCC                                                   |
| oRL12     | Acet.Kin.Outer.rev       | TGGCGCGTCAAAAAGCGTTTGGTTG                                              |
| oRL13     | G2.Reversion.sgRNA.fwd   | CCACGGCGGAACGGCGTTATCGGTCAAAAAGGTTTTTAGACGCTAGTGGCTGGTTTGGGGCAACATTCCAA |
| oRL14     | G2.Reversion.sgRNA.rev   | GGCGGCTGCCGATGCGTTTTTTTGGGACCATTCCAAAAACGCTAGTGGCTTAAAACACTTTTAGTGACGTCG |

Table S6