Supplementary Material

The appendix contains detailed experimental protocols that are essential for the reproducibility of the study but would disrupt the flow of the main text if included in the Materials and Methods section. Specifically, the detailed compositions and preparation procedures for the culture media and buffers used in this study (Table S1) and the step-by-step protocol for preparing competent cells of Lactococcus lactis NZ9000 (Text S1) are provided here. These details are crucial for researchers who wish to replicate the experiments, yet they are not central to the main narrative of the paper.

Table S1. Composition and preparation of media and buffers

Medium/Buffer	Components	Sterilization	Notes
GM17	M17 broth, 0.5% glucose	115℃, 15 min	Glucose added after sterilization
GSGM17	M17 broth, 0.5% glucose, 2% glycine, 0.5 M sucrose	115℃, 15 min	Glycine sterilized separately
Recovery Medium	M17 broth, 0.5% glucose, 0.5 M sucrose, 20 mM $MgCl_2$, 2 mM $CaCl_2$	115℃/121℃	Ice-chilled before use
Electroporation Buffer I	0.5 M sucrose, 10% glycerol	115℃, 15 min	Store at 4℃
Electroporation Buffer II	0.5 M sucrose, 10% glycerol, 0.05 M EDTA	115℃, 15 min	Store at 4℃

Text S1. Detailed protocol for preparation of competent cells of L. lactis NZ9000.

L. lactis NZ9000 was first activated and streaked for isolation on antibiotic-free MRS solid medium. A single colony was inoculated into 100 mL GM17 broth and statically incubated at 30 °C for 24 h. Afterwards, 5 mL of the overnight culture was transferred into 100 mL fresh GM17 broth and cultured at 30 °C for another 12 h. The culture was then transferred to GSGM17 medium and incubated at 37 °C with manual shaking every 20 min until OD_{600} reached approximately 0.4, corresponding to the logarithmic growth phase (around 8 h).

The culture was chilled on ice for 10 min and aliquoted into pre-cooled 1.5 mL sterile centrifuge tubes. Cells were harvested by centrifugation at 7000 rpm for 10 min at 4 °C and the supernatant was discarded. The cell pellet was gently resuspended in ice-cold electroporation buffer I, followed by centrifugation and removal of supernatant. Next, cells were resuspended in ice-cold electroporation buffer II and incubated on ice for 15 min. The centrifugation and resuspension step using electroporation buffer II was repeated one more time. After discarding the supernatant, the final cell pellet was

resuspended in 100 μL ice-cold electroporation buffer I. The prepared competent cells were stored at −20 °C for subsequent transformation experiments.