Real-time detection of riboflavin production by *Lactobacillus plantarum* strains and tracking of their gastrointestinal survival and functionality *in vitro* and *in vivo* using mCherry labeling

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Supplementary Figure S1. Riboflavin calibration curve. Correlation of riboflavin concentration and fluorescence. Serial dilutions of a riboflavin solution in CDM medium lacking riboflavin at 10 mg/mL were used to determine its fluorescence emission at a wavelength of 520 nm after excitation at a wavelength of 440 nm.
Supplementary Figure S2. Calibration curve of viable cells. After removal of culture supernatants, serial dilutions of bacterial cultures resuspended in PBS at 1 x 10^{10} cfu/mL determined by plating were diluted and the emission of the mCherry fluorescence expressed in the bacteria was measured at a wavelength of 610 nm after excitation at a wavelength of 587 nm.
Supplementary Figure S3. DNA sequence of the RFN regions of the *L. plantarum* strains. The identical sequences of the wild-type strains (wild-type) as well as of those of their derivatives are depicted. Nucleotides with yellow background indicate the mutations detected in the riboflavin-overproducing derivatives.
Supplementary Figure S4. Detection of riboflavin production by *L. plantarum* M5MA1[pCR12] and M5MA1-B2[pCR12] during growth. Bacteria were grown in CDM medium without riboflavin, or supplemented with either riboflavin or FMN both at a concentration of 2 μg/mL. The growth of cultures (blue) was monitored by measurement of OD$_{480}$. Fluorescence emission of riboflavin or FMN (green) was recorded at 520 nm after excitation at a wavelength of 440 nm.
Supplementary Table S1. Detection of SCFA and ammonium concentration in the vessels of the BFBL

| Compound  | Sample | R1       | R2       | R3       |
|-----------|--------|----------|----------|----------|
| Acetate   | Stab   | 37.56±7.95 | 49.72±8.79 | 55.41±12.57 |
|           | Test   | 39.95±7.07 | 48.87±6.35 | 54.01±13.22 |
| Propionate| Stab   | 10.95±3.70 | 17.65±4.16 | 16.27±4.72 |
|           | Test   | 10.08±2.22 | 17.42±v   | 16.99±4.75 |
| Butyrate  | Stab   | 2.18±1.32  | 4.13±0.86  | 2.98±1.39  |
|           | Test   | 2.63±1.25  | 4.16±0.85  | 3.00±1.11  |
| Lactate*  | Stab   | 1.91±0.17  | 0.92±0.15  | 0.73±0.12  |
|           | Test   | 1.51±0.24  | 0.83±0.05  | 0.77±0.09  |
| Formate   | Stab   | 0.62±0.04  | 0.47±0.03  | 0.43±0.02  |
|           | Test   | 0.76±0.17  | 0.50±0.04  | 0.45±0.02  |
| Ammonium  | Stab   | 6.70±0.32  | 11.37±0.98 | 13.64±0.20 |
|           | Test   | 6.81±0.82  | 11.08±1.11 | 13.41±1.78 |

*One inoculum only in R1, with stabilization value 0.79±0.03 and 0.67±0.15 during the test period. No changes in SCFA and ammonium values (Student's t-test) between estabilization (Stab) and test periods.