

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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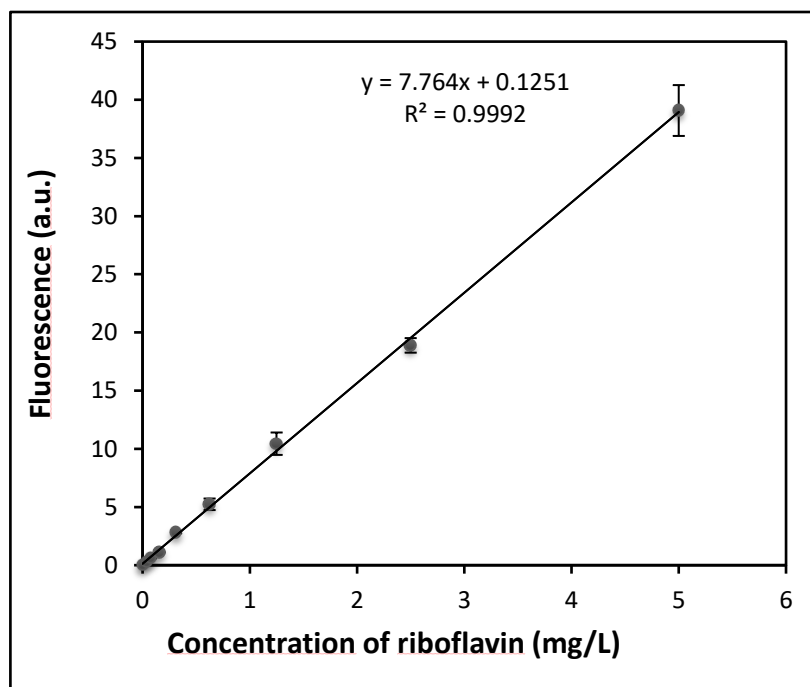
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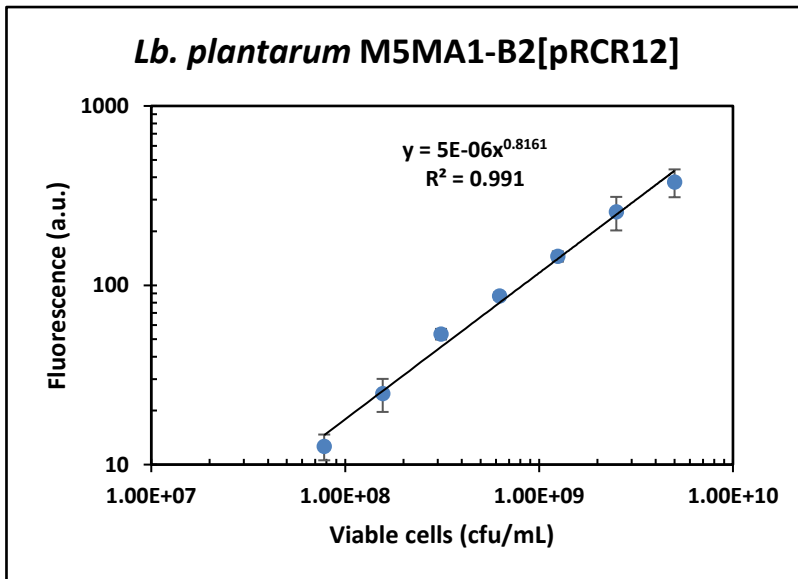
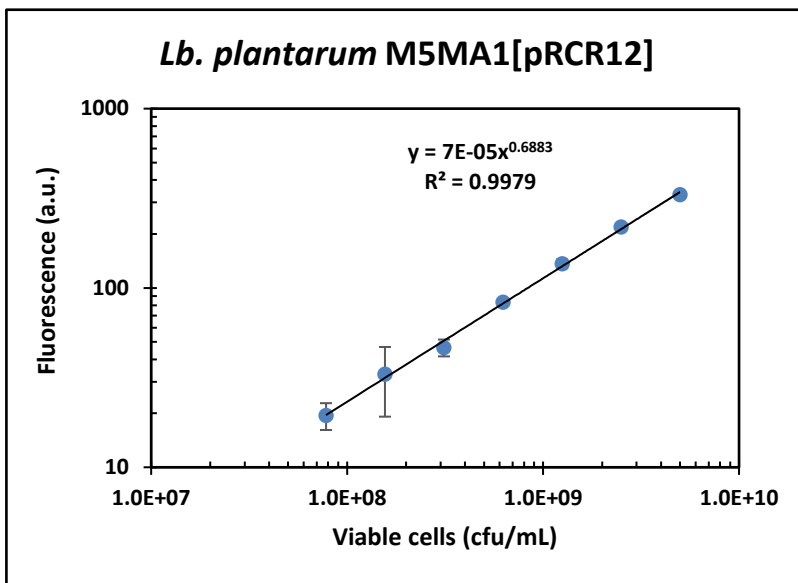
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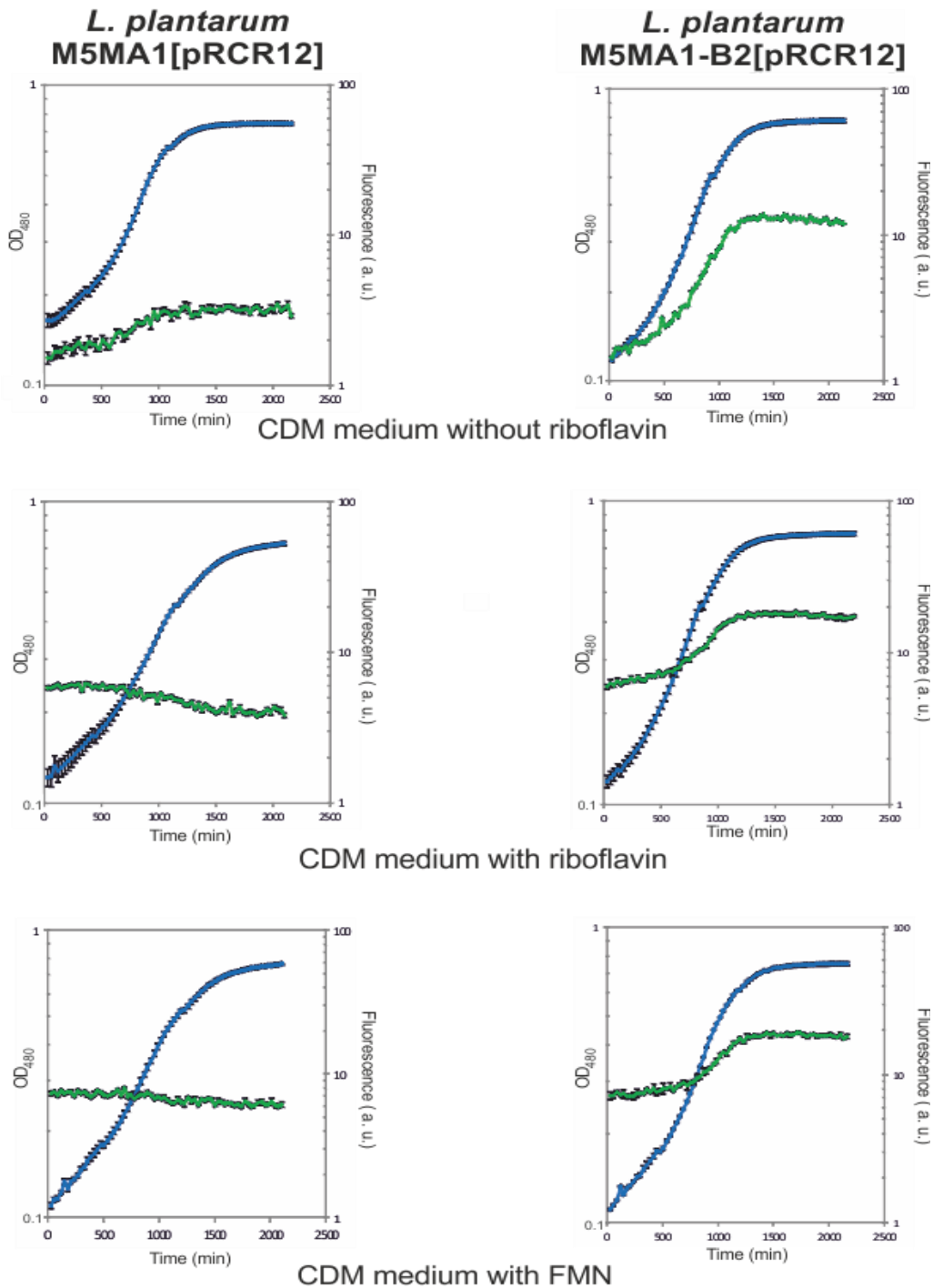
Supplementary Figure S1. Riboflavin calibration curve. Correlation of riboflavin concentration and fluorescence. Serial dilutions of a riboflavin solution in CDM medium lacking riboflavin at 10 mg/mL were used to determine its fluorescence emission at a wavelength of 520 nm after excitation at a wavelength 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 1×10^{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.

Wild-type CGATTTCTTCGGGGCAGGGTGCAATTCCTCCGACCGACGGTAACAACGTAAGTTGAAGTCCGTGACCCGCGTGAGCGGTGGACCCAGTGCAAGTCTGGGACCGACAGTATAGTCTGGATGGGAGAAGAAAATT
 M5MA1-B2 CGATTTCTTCGGGGCAGGATGCAATTCCTCCGACCGACGGTAACAACGTAAGTTGAAGTCCGTGACCCGCGTGAGCGGTGGACCCAGTGCAAGTCTGGGACCGACAGTATAGTCTGGATGGGAGAAGAAAATT
 M9MM1-B2 CGATTTCTTCGGGGCAGGATGCAATTCCTCCGACCGACGGTAACAACGTAAGTTGAAGTCCGTGACCCGCGTGAGCGGTGGACCCAGTGCAAGTCTGGGACCGACAGTATAGTCTGGATGGGAGAAGAAAATT
 M9MG6-B2 CGATTTCTTCGGGGCAGGCTGCAATTCCTCCGACCGACGGTAACAACGTAAGTTGAAGTCCGTGACCCGCGTGAGCGGTGGACCCAGTGCAAGTCTGGGACCGACAGTATAGTCTGGATGGGAGAAGAAAATT
 M9Y2-B2 CGATTTCTTCGGGGCAGGGTGCAATTCCTCCGACCGACGGTAACAACGTAAGTTGAAGTCCGTGACCCGCGTGAGCGGTGGACCCAGTGCAAGTCTGGGACCGACAGTATAGTCTAGATGGGAGAAGAAAATT
 M9MM4-B2 CGATTTCTTCGGGGCAGGGTGCAATTCCTCCGACCGACGGTAACAACGTAAGTTGAAGTCCGTGACCCGCGTGAGCGGTGGACCCAGTGCAAGTCTGGGACCGACAGTATAGTCTGGATGGGAGAAAATAAAATT

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[pRCR12] and M5MA1-B2[pRCR12] during growth. Bacteria were grown in CDM medium without riboflavin, or supplemented with either riboflavin or FMN both at a concentration of 2 $\mu\text{g}/\text{mL}$. The growth of cultures (blue) was monitored by measurement of OD₄₈₀. 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 stabilization (Stab) and test periods.