**SUPPLEMENTARY**

**Antibiofilm Activity and NMR-based Metabolomic Characterization of Cell Free Supernatant of *Limosilactobacillus reuteri* DSM 17938**

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**Figure S1**. Determination of MBIC of CFS and SurE 10K through CFU counts, metabolic assay and CV assay versus *E. coli* ATCC 25922 (**A**), *P. aeruginosa* ATCC 27853 (**B**), *S. aureus* ATCC 29213 (**C**), *S. mutans* UA 159 (**D**) and *F. nucleatum* ATCC 25585 (**E**). Data are presented as the mean of three replicates of two independent experiments. The statistical comparison between control and treated samples was determined with one way ANOVA. The control was composed by media with the supplement of MRSB.



**Figure S2.** 600.17 MHz 1H NMR spectrum of CFS sample dissolved in 200 mM phosphate buffer/D2O containing TSP 1.4 mM.

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**Figure S3.** 600.17 MHz 1H NMR spectrum of SurE 10K sample dissolved in 200 mM phosphate buffer/D2O containing TSP 1.4 mM.



**Figure S4.** 1H-1H TOCSY spectrum of SurE 10K sample dissolved in 200 mM phosphate buffer/D2O containing TSP 1.4 mM.

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**Figure S5.** 1H-13C HSQC spectrum of SurE 10K sample dissolved in 200 mM phosphate buffer/D2O containing TSP 1.4 mM.



**Figure S6.** 1H-13C HMBC spectrum of SurE 10K sample dissolved in 200 mM phosphate buffer/D2O containing TSP 1.4 mM.

**Table S1.** Metabolites identified in the 600.17 MHz 1H NMR spectra of CFS and SurE 10K dissolved in 200 mM phosphate buffer/D2O containing TSP 1.4 mM. Asterisks (\*) indicate signals selected for integration.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Metabolite** | **Assignment** | **1H (ppm)** | **Multiplicity [*J*(Hz)]** | **13C (ppm)** |
|  |  |  |  |  |
| **Organic acids** |  |  |  |  |
|  |  |  |  |  |
| Lactatea,b | β-CH3 | \*1.33 | d [6.9] | 21.4 |
|  | α-CH | 4.12 | q [6.9] | 69.6 |
|  |  |  |  |  |
| Formatea | HCOOH | \*8.46 | s |  |
|  |  |  |  |  |
| **Amino acids** |  |  |  |  |
|  |  |  |  |  |
| Alaninea,b | α-CH | 3.80 |  |  |
|  | β-CH3 | \*1.49 | d [7.2] | 17.4 |
|  |  |  |  |  |
| Valinea,b | β-CH | 2.28 |  | 30.5 |
|  | γ-CH3 | 1.00 | d [7.7] | 17.8 |
|  | γ’-CH3 | \*1.05 | d [7.1] | 19.2 |
|  |  |  |  |  |
| Glycinebetainea,b | N(CH3)3+ | \*3.27 | s | 55.1 |
|  |  |  |  |  |
| Isoleucinea,b | β-CH | 1.99 |  |  |
|  | γ-CH3 | \*1.01 | d [6.9] | 15.8 |
|  |  |  |  |  |
| Leucinea,b | γ-CH | 1.74 |  |  |
|  | δ-CH3 | \*0.97 | d [6.2] | 23.2 |
|  | δ’-CH3 | 0.96 | d [6.2] | 22.3 |
|  |  |  |  |  |
| Glycinea | α-CH2 | \*3.56 | s | 42.5 |
|  |  |  |  |  |
| Phenylalaninea,b | CH-2,6 | 7.33 | m |  |
|  | CH-4 | 7.38 | m |  |
|  | CH-3,5 | \*7.43 | m |  |
|  |  |  |  |  |
| Tyrosinea,b | CH-3,5 | 7.20 | d [8.6] |  |
|  | CH-2,6 | \*6.90 | d [8.6] |  |
|  |  |  |  |  |
| Tryptophana,b | CH-4 | \*7.74 | d [7.9] |  |
|  | CH-7 | 7.55 | d [8.2] |  |
|  |  |  |  |  |
| **Miscellaneous metabolites** |  |  |  |  |
|  |  |  |  |  |
| Cholinea,b | +N(CH3)3 | \*3.21 | s |  |

ametabolite quantified in CFS; bmetabolite quantified in SurE 10K.

**Table S2.** Colorimetric CIEL\*a\*b\* parameters of analyzed samples.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **L\*** | **a\*** | **b\*** | **C\*** | **h°** | **ΔL\*** | **Δa\*** | **Δb\*** | **ΔC\*ab** | **Δhab** | **ΔE** |
| **CFS t°** | 47.91 | 6.92 | 44.25 | 44.79 | 81.11 |  |  |  |  |  |  |
| **CFS t2w** | 49.74 | 17.88 | 65.16 | 67.57 | 74.65 | 1.84Lighter | 10.96More red | 20.91More yellow | 22.78Brighter | -6.20More red | 23.68 |
| **CFS t4w** | 58.84 | 3.98 | 43.79 | 43.97 | 84.81 | 10.94Lighter | -2.95More green | -0.46More blue | -0.82Opaque | 2.87More green | 11.34 |
|  |
| **MRSB t°** | 47.37 | 7.36 | 44.76 | 45.36 | 80.66 |  |  |  |  |  |  |
| **MRSB t2w** | 48.44 | 21.43 | 68.23 | 71.51 | 72.56 | 1.06Lighter | 14.07More red | 23.47More yellow | 26.16Brighter | -8.05More red | 27.39 |
| **MRSB t4w** | 56.23 | 12.17 | 60.24 | 61.45 | 78.58 | 8.86Lighter | 4.81More red | 15.48More yellow | 16.10Brighter | -1.92More red | 18.47 |

Each reported value is the mean of four measurements. ΔE, calculated as: [(L\*2–L\*1)2 + (a\*2–a\*1)2 + (b\*2–b\*1)2]1/2, represents the overall color variation, respect to the corresponding samples at t°, used as references.