**Supporting information**

*In situ* spectroscopic analysis of *Lactobacillus rhamnosus* GG flow on abiotic surface reveals a role for nutrients in biofilm development

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Figure SI1. ATR-FTIR spectra of nutritive media before (dash dot) and after 2.5 (dash) and 8 hours (solid) of LGG incubation. Sterile Millli-Q water was used as a reference for all spectra. Offsets of spectra are used for clarity

The spectra of the filtered nutritive media after 2.5 and 8 hours of LGG incubation are shown in Figure SI1. After 2.5 hours, new bands at 1237, ~1129 and 1042 cm‑1 appeared in spectra of MRS/10 and AOAC/10 media. These bands were more intense in the spectra of these media recorded after 8 hours. They were assigned to νCO combined with δOH, ρCH3 combined with νCO, and νC–CH3, respectively, from lactic acid and lactate salt (Figure SI2) (Cassanas et al. 1991)). The occurrence of bands at 1416 cm­-1 and 1727 cm‑1 in MRS/10 and AOAC/10, respectively, showed that the lactate and the lactic acid forms were both present in the media, albeit in different ratios (Figure SI1). From the spectra, and in accordance with the measured values of pH, the acidic form was more prominent in AOAC/10. The spectrum of filtered mTSB/10 was almost not changed with respect to the initial record neither after 2.5 nor after 8 hours of incubation (Figure SI1). The non-significant changes in the spectra are in accordance with the stable optical density measured for LGG suspensions in mTSB/10.



Figure SI2. ATR-FTIR spectra of 0.1 M aqueous solution of sodium L-lactate recorded at pH equal to 2.0, 3.7 (pKa), and 6.1. Assignments were made in accordance with Cassanas et al. (Cassanas et al. 1991)







Figure SI3. Evolution of infrared spectra in ATR mode during the flow of LGG suspension for 2.5 hours (from bottom to up) in 10-fold diluted MRS, AOAC, and mTSB media obtained at 10 minutes interval.

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Figure SI4. Evolution of integrated intensities of the ATR-FTIR bands corresponding to proteins, as derived from the amide II band region at 1592–1486 cm-1, nucleic acids + phospholipids (NA + PL,1271–1188 cm-1), and polysaccharides + nucleic acids + phospholipids (PS + NA + PL, 1189–956 cm-1) during formation of LGG biofilm over 24 hours in MRS/10, AOAC/10, and mTSB/10 after initial 2.5 hours of flow in same media.

Table SI1. Assignments of principal infrared vibrational bands of 1800–900 cm-1 region of the ATR-FTIR spectra of LGG in different nutritive media after 2.5 hours of inoculation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| IR wavenumbers (cm-1) | | | Assignments | |
| LGG in  1/10 MRS | LGG in  1/10 AOAC | LGG in  1/10 mTSB |
| 1749 | 1745 |  | νC=O | Esters from lipids |
| 1712 | 1715 | 1715 | νC=O | Carboxylic acids |
| 1649 | 1648 | 1655 | Amide I\* (Disordered/α-helix) | Proteins |
| 1631 (sh) | 1630 (sh) | 1634 (sh) | Amide I\* (β-sheets) | Proteins |
| 1570–1516 | 1572–1518 | 1568–1515 | Amide II\*\* | Proteins |
| 1455 | 1456 |  | δaCH3 | Lactic acid |
|  |  | 1449 | δCH2 | Lipids |
| 1417 | 1418 |  | νsCOO− | Amino acids, teichoic acids, lactic acid |
| 1394 | 1393 | 1396 | νsCOO− | Proteins |
| 1316–1274 | 1313–1283 | 1311–1284 | Amide III\*\*\* | Proteins |
| 1243 | 1240 | 1242 | νaPO2− | Phosphodiester, phospholipids, nucleic acids |
| 1220 | 1221 | 1219 | νaPO2−, δCH (ring) | Ribose, phospholipids |
| 1172–1152 | 1172–1151 | 1171–1153 | νsC–OH, νC–O | Proteins, carbohydrates, esters |
| 1119 | 1122 | 1119 | νC–O | DNA, RNA |
| 1084 | 1084 | 1083 | νsPO2− | Phosphodiester, phospholipids, nucleic acids |
| 1055 | 1056 | 1052 | νsC–O–C, νsP–O–C (R–O–P–O–R') | Polysaccharides |
| 1038 | 1041 | 1033 | νC–O | Polysaccharides, RNA ribose |
| 998 | 988 | 993 | Uracil ring | RNA |
| 968 | 974 | 970 | νC–C, νP–O–P, Ribose–phosphate skeletal motions | DNA |
| 916 | 916 | 915 | Ribose–phosphate skeletal motions | DNA |

\*νC=O with the minor contribution from νC–N, overlapped with the signal of δH2O, 1640 cm‑1; \*\*δN–H coupled with νC–N; \*\*\*νC–N coupled with δN–H, with weak contributions from δC=O.

**Reference**

Cassanas G, Morssli M, Fabrègue E, Bardet L. 1991. Vibrational spectra of lactic acid and lactates. J Raman Spectrosc. 22(7):409–413.