

## Supplementary Information

# ***In situ* monitoring of *Lactobacillus parabuchneri* biofilms via real-time infrared spectroscopy**

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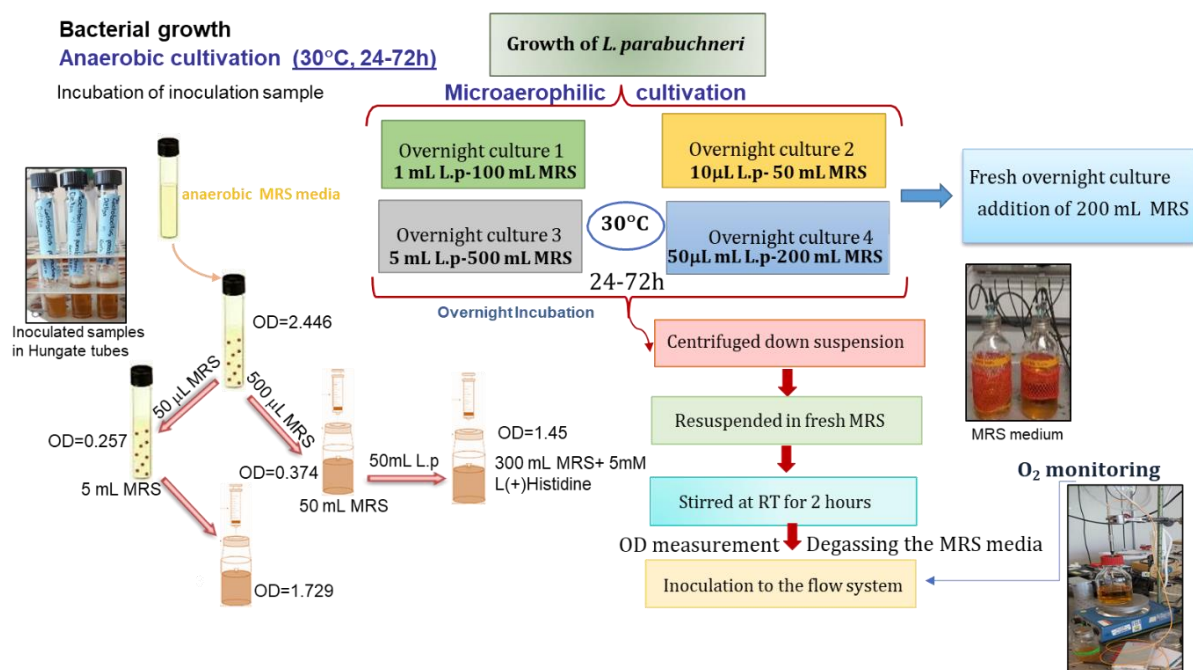
Supplementary Table 1

Supplementary References

## Supplementary note I

### Experimental strategy for *L. parabuchneri* cultivation

Lactic acid bacteria are Gram positive aerotolerant microorganisms that synthesize lactic acid as the main product of sugar fermentation. The culture media and the main fermentation conditions requires a nutritive media rich with carbon source<sup>1</sup>. Mann de Rogosa Sharpe broth has been used as an oxygen free media for cultivation of the *L. parabuchneri* DSM 5987 isolates. To test the capacity of strain for Histamine producing, the cultures have been supplemented with 5mM Histidine<sup>2</sup>. By the scheme you can see the matrix of the overnight inoculated samples incubated on 30°C, 24-72h. After that the supernatant was discarded and resuspended on fresh MRS media, stirred for 2 h and afterwards measuring the OD. Before the inoculation to the flow system, the media was flushed by nitrogen gas (degassing process) and the oxygen level were monitored using an O<sub>2</sub> microsensor.



Supplementary Figure I. Experimental strategy for *L. parabuchneri* cultivation.

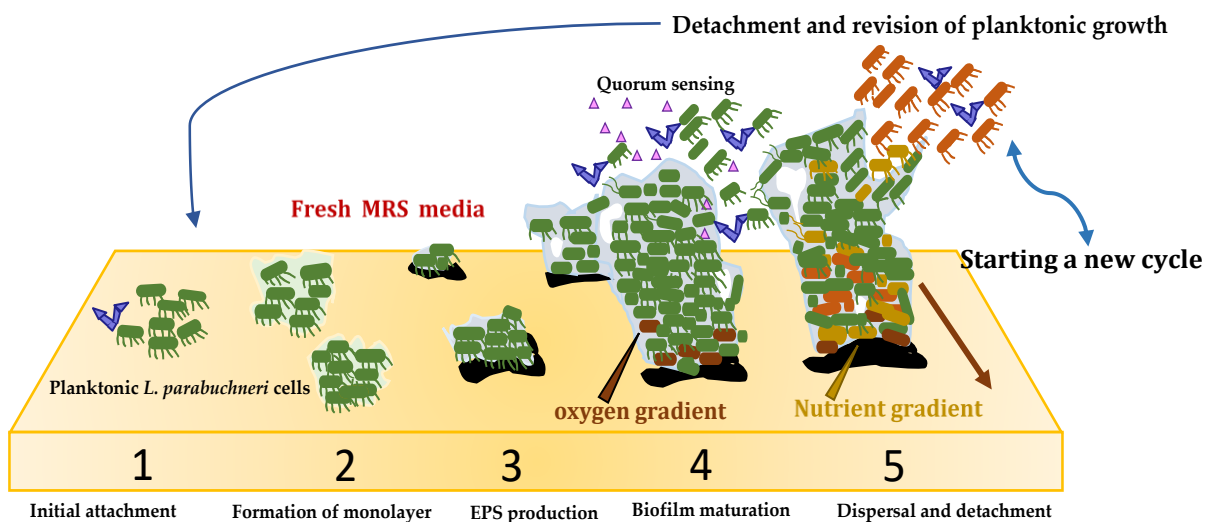
## Supplementary note 2

### The process of *L. parabuchneri* biofilm formation at the ATR waveguide surface with five distinct stages of biofilm evolution

Development of lactobacillus biofilms leads toward series of morphological changes on the substrate<sup>3,4</sup>. The adhesion to surface is dependent physicochemical properties of the abiotic and biotic surfaces like: pH, hydrophobicity, surface texture, temperature, etc<sup>5</sup>. The five proposed stages of biofilm formation are:

- 1) initial attachment of cells at the surface;
- 2) transport and attachment of bacterial cells towards the surface;
- 3) early development of biofilms indicated by EPS production and proliferation into microcolony units;
- 4) maturation of the biofilm architecture, and
- 5) dispersal of mature clusters and cell detachment (schematically illustrated in Figure 2).

Many cells can detach from the surface and come back to the planktonic lifestyle, spreading out to start a new cycle of life. Extracellular polymeric matrix has the key role to adhesion ability and strength the bond between bacteria and the substrate.

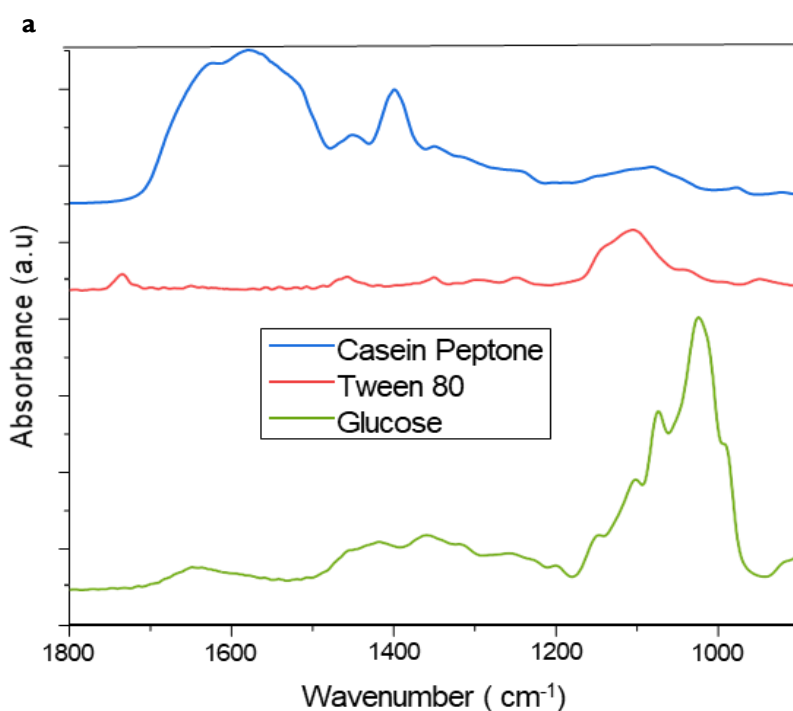


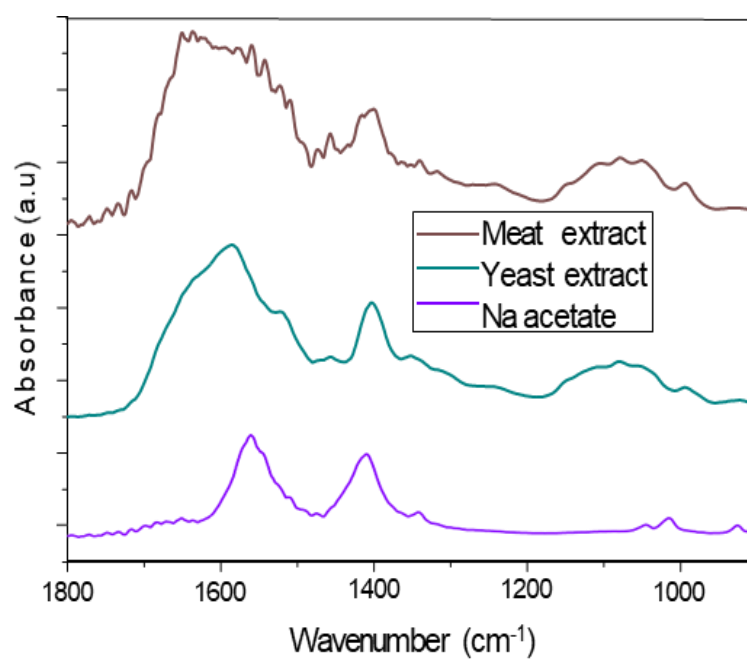
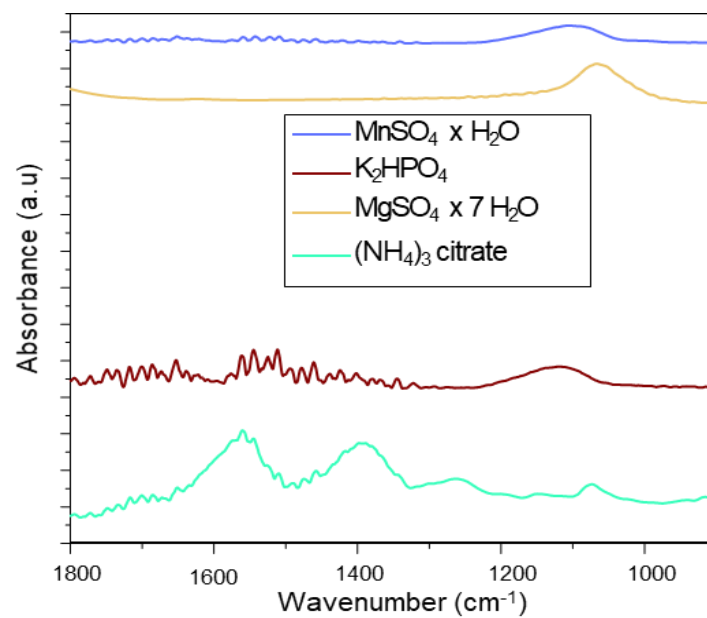
**Supplementary Figure 2. Scheme of the five stages of *L. parabuchneri* biofilm development regulated by quorum sensing.**

### Supplementary note 3

#### IR-spectroscopic characterization of the individual components of de Man Rogosa and Sharpe medium (MRS)

IR-ATR spectroscopy with the Alpha I spectrometer of the individual constituents of MRS showed the characteristic spectra in the spectral region from 1800 – 900  $\text{cm}^{-1}$ . The peaks of the individual constituents were integrated and averaged thereafter served as basis for the cumulative impulse fit demonstrated in the manuscript. Spectra were recorded using the same Alpha spectrometer with Platinum Single bounced Diamond Crystal in 2  $\text{cm}^{-1}$  resolution and 100 average scans. The characterization of the MRS growth medium is crucial to chemically analyze *L. parab.* adsorption and biofilm formation in molecular detail and the cumulative impulse fit of the spectra of de Man Rogosa and Sharpe medium (MRS) is fundamental for the analysis of *L. parab.* biofilms in this culture medium.

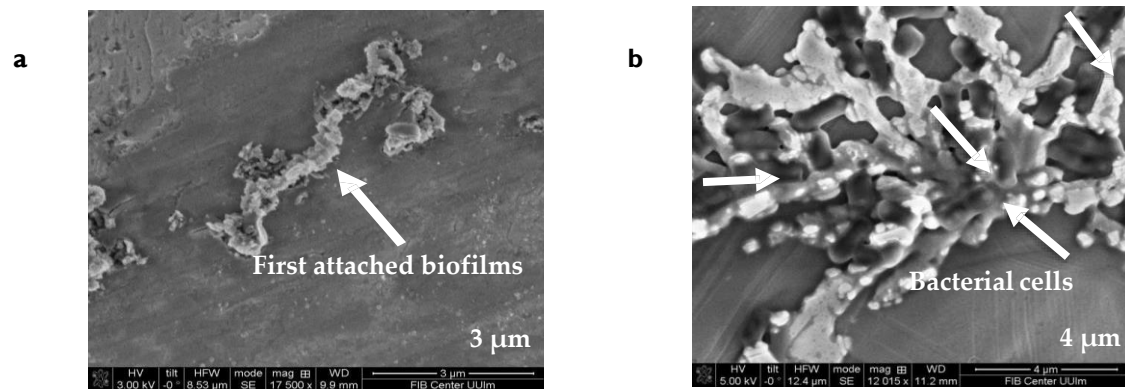


**b****c**

**Supplementary Figure 3. IR-ATR spectra of the individual constituents of MRS in the spectral region from 1800 – 900  $\text{cm}^{-1}$  (see the color label for each component).**

## Supplementary note 4

### Scanning electron microscopy of *L. parabuchneri* biofilms



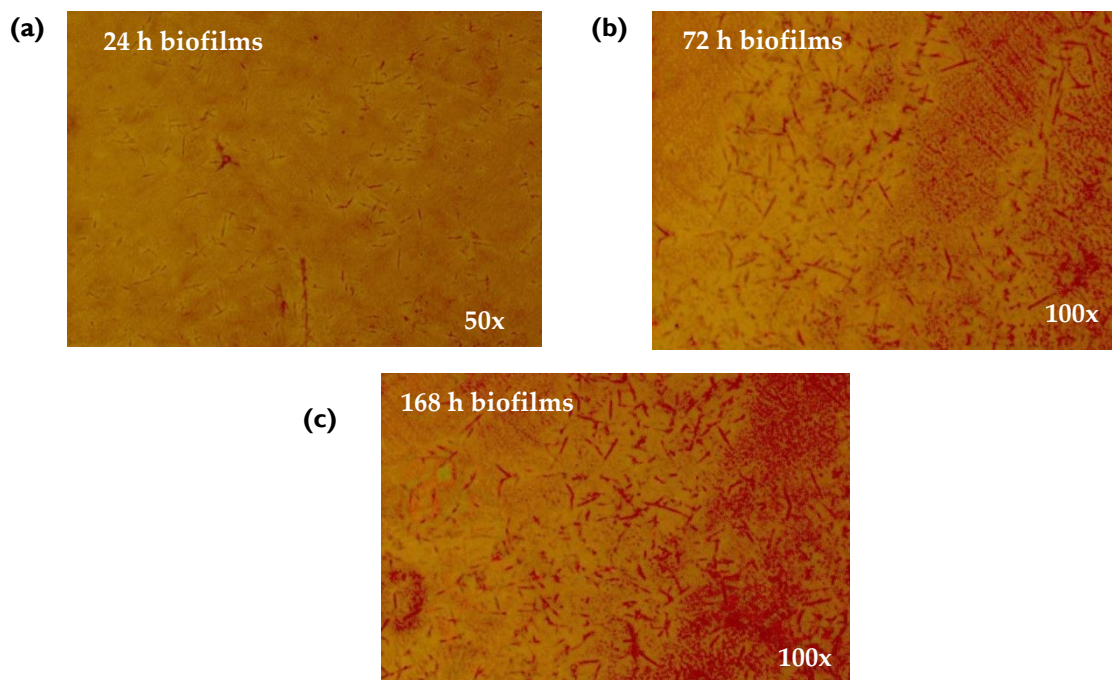
**Supplementary Figure 4. Scanning electron photomicrographs of *Lactobacillus parabuchneri* biofilms adhered to stainless steel coupons within a) the first attached microcolonies formed on surface and b) bacterial cells well adaption.**

## Supplementary discussion

### Optical microscopy of *L. parabuchneri* biofilms

The characterization of the chemistry and architecture of biofilms can be evaluated by the combination of IR-ATR spectroscopy and light microscopy and therefore achieve the information on the region which is restricted by evanescent wave depth penetration<sup>6</sup>. For 7 days inoculation on the flow ATR system, the ZnSe surface has become highly colonized by *L. parabuchneri* bacteria creating high density biofilms with different heterogeneity types. These images show uniform coverage of ZnSe surface by *L. parabuchneri* biofilms. Figure 5 a show the biofilms visualized after 24 h (1 day), figure 5 b shows the biofilm attached to waveguide surface after 72 h (3 days) and figure 5 c shows the mature biofilm after 168 h (7 days).

For 7 days of inoculation within the IR-ATR assembly, the ZnSe surface has become highly colonized by *L. parabuchneri* bacteria creating high-density biofilms with spatially different heterogeneities. Optical microscopy is a basic technique to track the structural heterogeneity of biofilms. The optical micrographs prove the coverage with biofilms at the waveguide surface. These images reveal biofilm structures adherent to the ZnSe surface in small colonies of cells after 24 h (Fig 5 a), whereby the number of *L. parabuchneri* cells that were near the attached 'pioneering cells' was higher than those observed close to the initially uncovered zones of the ZnSe substrate. A uniform coverage of the ZnSe surface by *L. parabuchneri* biofilms was observed after 3 days (Fig 5.b) of real-time monitoring with the addition of fresh nutritive media every 16 h. The aged biofilms after 7 days (Fig 5.c) reveal an increased thickness of the biofilm enriched with nutrients for a week. Microscopic images were captured with Zeiss Microscope (Zeiss, Germany) and defined by the image analysis micro software.



**Supplementary Figure 5. Optical micrograms of *L. parabuchneri* bacteria that have attached the surface of ZnSe crystal during 7 days of flow-through IR measurements whereby changes in biofilm parameters that are resolved and quantified by light microscopy and computer-controlled image analysis can be related to surface area-averaged chemical changes at the biofilm-substrate interface.**

**Supplementary Table 1 . Fundamental infrared vibrational band assignments of the 3000 – 500 cm<sup>-1</sup> spectral region of the IR-ATR spectrum of *L. parabuchneri* bacterial cells and biofilm (ν: stretching, δ: bending, τ: twist, a: antisymmetric, s: symmetric, **ARN**: Ribonucleic acid; **AND**: Deoxyribonucleic acid and **LPS**: lipopolysaccharides)<sup>7-9</sup>.**

| Wavenumber (cm <sup>-1</sup> ) | Assignment                                                             | Principal constituents                                    | Cellular components                              |
|--------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------|--------------------------------------------------|
| 2961                           | ν <sub>a</sub> CH <sub>3</sub>                                         | Fatty chains                                              | Cell membrane                                    |
| 2925                           | ν <sub>a</sub> CH <sub>2</sub>                                         | Fatty acids                                               | Cell membrane                                    |
| 2897                           | νCH tertiary                                                           | Fatty acids                                               | Cell membrane                                    |
| 2874                           | ν <sub>s</sub> CH <sub>3</sub>                                         | Fatty acids                                               |                                                  |
| 2854                           | ν <sub>s</sub> CH <sub>2</sub>                                         |                                                           |                                                  |
| 1736                           | νC=O                                                                   | Esters from lipids                                        | Cell membrane                                    |
| 1713                           | νC=O                                                                   | Esters, carboxylic acids                                  | Nucleoid, ribosomes                              |
| 1700–1580                      | C=O C=N C=C NH                                                         | ADN/ARN bases                                             | Nucleoid, ribosomes                              |
| 1693–1627                      | Amide I (ν <u>C=O</u> coupled with N-H δH <sub>2</sub> O)              | Proteins, water (1640 cm <sup>-1</sup> )                  | Membranes, cytoplasm, flagella, pili, ribosomes  |
| 1568–1531                      | Amide II (δ <u>N-H</u> coupled with C-N)                               | Proteins                                                  | Membranes, cytoplasm, flagella, pili, ribosomes  |
| 1468–1455                      | δCH <sub>2</sub> , δ <sub>a</sub> CH <sub>3</sub>                      | Lipids                                                    | Membranes                                        |
| 1400                           | ν <sub>s</sub> COO <sup>-</sup>                                        | Amino acids, fatty acid chains                            | Capsules, peptidoglycan                          |
| 1350; 1281                     | τCH <sub>2</sub> , ρCH <sub>2</sub> Amide III (νC-N coupled with δN-H) | Fatty acid chains, proteins                               | Membranes, nucleoid, ribosomes                   |
| 1238                           | PO <sub>2</sub> <sup>-</sup>                                           | Phosphodiester, phospholipids, LPS, nucleic acids, ribose |                                                  |
| 1220                           | νC-O-C                                                                 | Polysaccharides                                           |                                                  |
| 1200-900                       | νC-O, νC-C, δC-O-C                                                     | Polysaccharides                                           | Capsule, storage inclusions                      |
| 1172; 1153                     | ν <sub>s</sub> C-OH, νC-O                                              | Proteins, carbohydrates, esters                           |                                                  |
| 1118                           | ν <sub>s</sub> CC                                                      | ARN, ADN                                                  | Nucleoid, ribosomes                              |
| 1086                           | ν <sub>s</sub> PO <sub>2</sub> <sup>-</sup>                            | Phosphodiester, phospholipids, LPS, nucleic acids         | Membranes, nucleoid, ribosomes                   |
| 1058                           | ν <sub>s</sub> C-O-C, ν <sub>s</sub> P-O-C                             | Polysaccharides                                           | Capsule, peptidoglycan                           |
| 1041                           | <u>νO-H</u> coupled with δC-O                                          | Polysaccharides                                           | Capsule, peptidoglycan, cell wall                |
| 1026                           | CH <sub>2</sub> OH                                                     | Carbohydrates                                             | Storage inclusion                                |
| 993                            |                                                                        | Ribose skelet (ARN)                                       | Ribosomes                                        |
| 970                            | νC- C, νP-O-P                                                          | RNA backbone                                              | Ribosomes                                        |
| 900-500                        | –                                                                      | “true” fingerprint region                                 | Bands not assigned to specific functional groups |

## Supplementary References

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