# SUPPORTING INFORMATION

for

# On-cell MAS NMR: physiological clues from living cells

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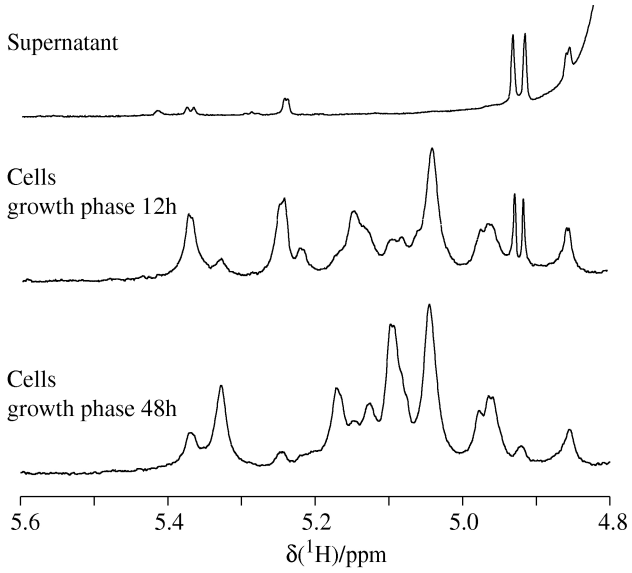


**Figure S1.** $^{1}$H MAS NMR spectra (anomeric region) of living *S.* Typhimurium cells from a bacterial culture in the early (12 h, middle trace) and very late stationary phase (48 h, bottom trace). The spectra correspond to two cell aliquots taken from the same culture at the given time points, centrifuged and then resuspended in buffer/$D_2O$. The MAS rotors were then emptied and the samples were pelleted again. The supernatant solutions added together and measured on a Bruker DMX 400 spectrometer operating at a proton frequency of 400.13 MHz with a 5 mm triple resonance inverse TXI solution probe. The corresponding spectrum is reported on the top of the figure.

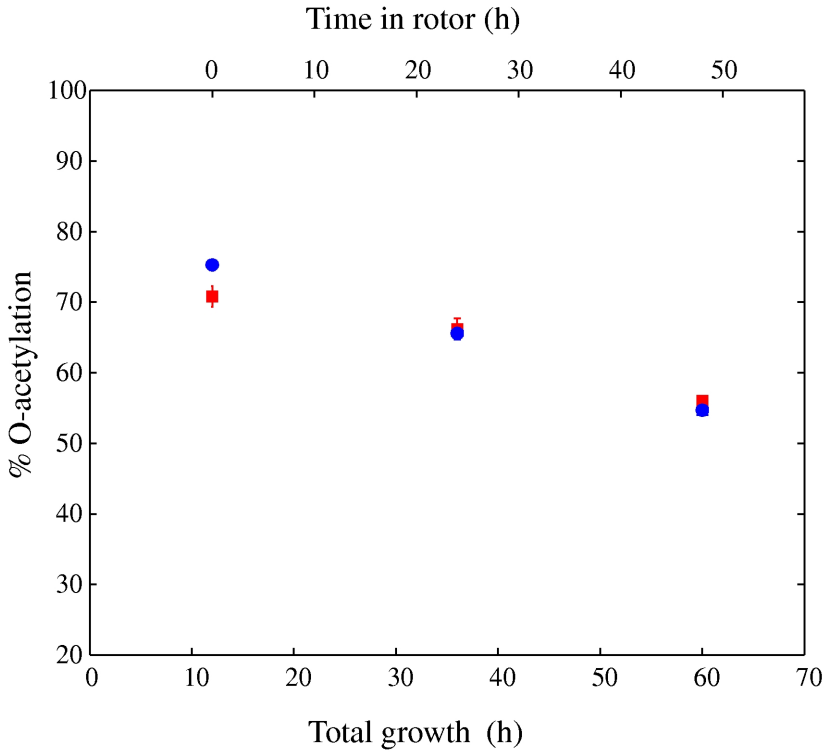


**Figure S2.** *Reproducibility of the experiments in Figure 2A-C.* The O-acetylation degree here reported is calculated from the $^{1}$H on-cell MAS NMR spectra as (area of the Man H1 signal in the O-acetylated O-antigen)/(sum of the areas of Man H1 signals in the O-acetylated and de-O-acetylated O-antigens) for two rotors (indicated by the symbols with the two different colours) containing cells from an early stationary phase (12 h, $OD_{600}$=4.31). The cells were centrifuged and the pellet was resuspended in sterilised spent growth medium and transferred to the NMR rotor. The spectrum taken immediately after the rotor was filled corresponds to the data at time in rotor = 0 h. Afterwards the rotor has been kept open (thus allowing oxygenation) and the sample shaking at 37 °C in order to maintain our system in conditions as close as possible to the ones of the cell batch culture. After 24 h a spectrum was measured (corresponding to the data at 24 h) and after additional 24 h a third spectrum has been taken (data at 48 h). The same culture of bacterial cells has been used to provide the cells filling two rotors which have been treated in the same way generating the two data sets denoted by the red and blue symbols. The data set indicated by the blue filled circles corresponds to the $^{1}$H on-cell MAS NMR spectra in Figure 2A-C.The error-bar indicates the statistical error of the single NMR measurement and it is often smaller than the size of the symbol.

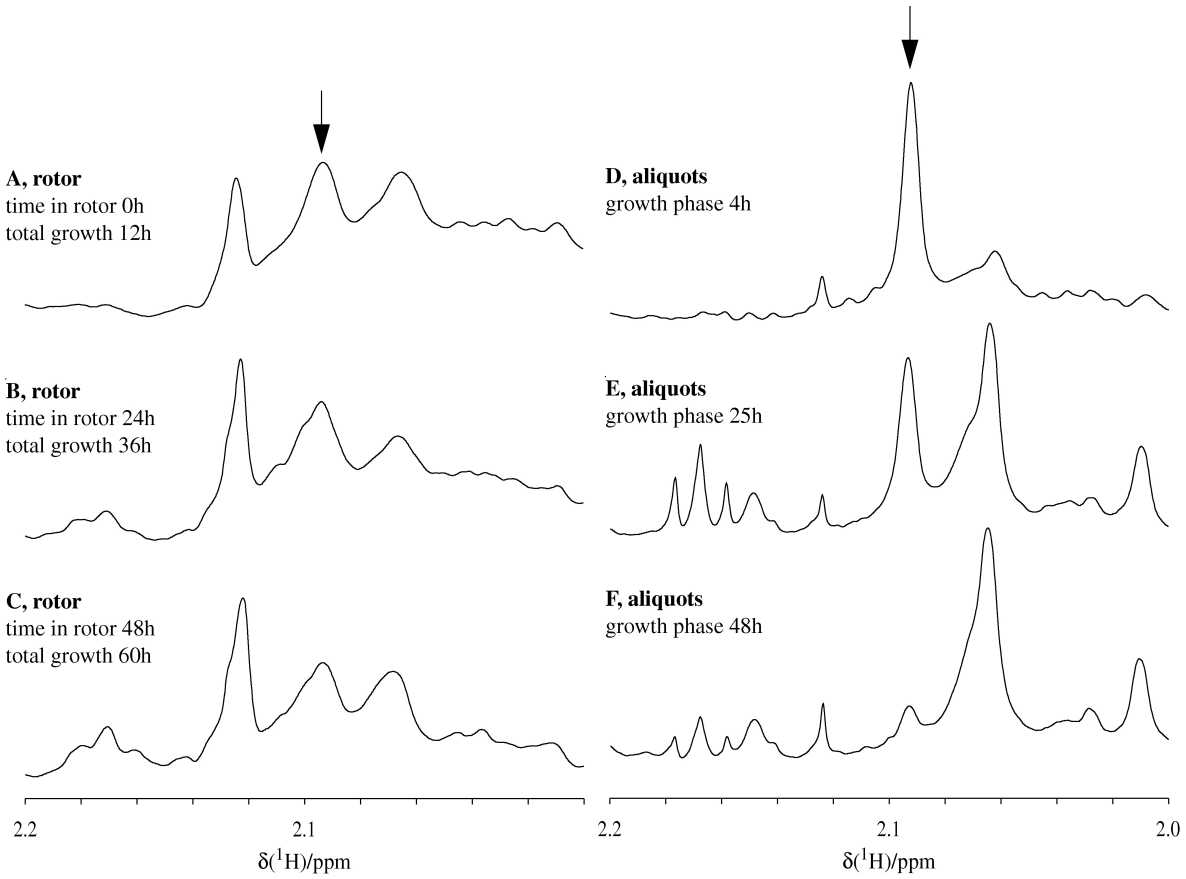


**Figure S3.** $^{1}$H MAS NMR spectra of living *S.* Typhimurium cells. The arrow indicates the –$COCH_3$ peak in the *O*-acetyl group of Abe O-acetylated at C2 (the anomeric region of these spectra is shown in Figure 2 in the manuscript). Left panel corresponds to the spectra in Figure 2A-C: Cells from an early stationary phase culture (**A**: 12 h, $OD_{600}$=4.31) were centrifuged and the pellet was resuspended in the supernatant. The same sample was then measured after further 24 h (**B**) and further 24 h (**C**) in the rotor at 37 °C. "Total growth" indicates the time interval equal to the proper growth phase in LB medium plus the time in the rotor. The corresponding values of the O-acetylation degree are reported in Figure S2 in the Supporting Information. Right panel corresponds to the spectra in Figure 2D-F: Cells from a culture in the exponential phase (**D**: 4 h, $OD_{600}$=1.25), late (**E**: 25 h, $OD_{600}$=3.05) and very late stationary phase (**F**: 48 h, $OD_{600}$=2.74). The spectra correspond to three cell aliquots taken from the same culture at the given time points, centrifuged and then resuspended in buffer. The corresponding values of the O-acetylation degree are reported in Figure S5, together with data from additional experiments.

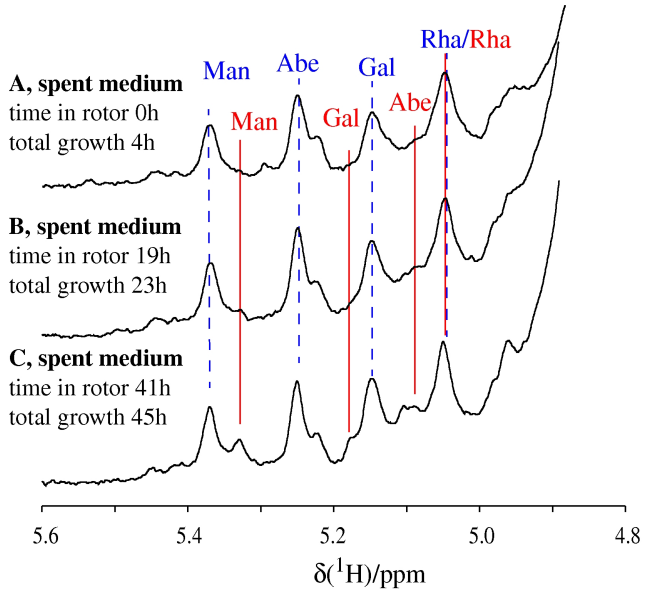


**Figure S4.** $^{1}$H MAS NMR spectra (anomeric region) of living *S.* Typhimurium cells from a bacterial culture in the exponential phase (**A**: 4 h, $OD_{600}$=3.01). Cells were centrifuged and the pellet was resuspended in the supernatant. The same sample was then measured after further 19 h (**B**) and further 22 h (**C**) in the rotor at 37 °C. "Total growth" indicates the time interval equal to the proper growth phase in LB medium plus the time in the rotor. The experiment is similar to the one in Figure 2 A-C in the main text. The assignment of the anomeric protons of the O-antigen is reported, the blue broken line and the red line correspond to the O-antigen which is O-acetylated and de-O-acetylated at Abe C2, respectively.

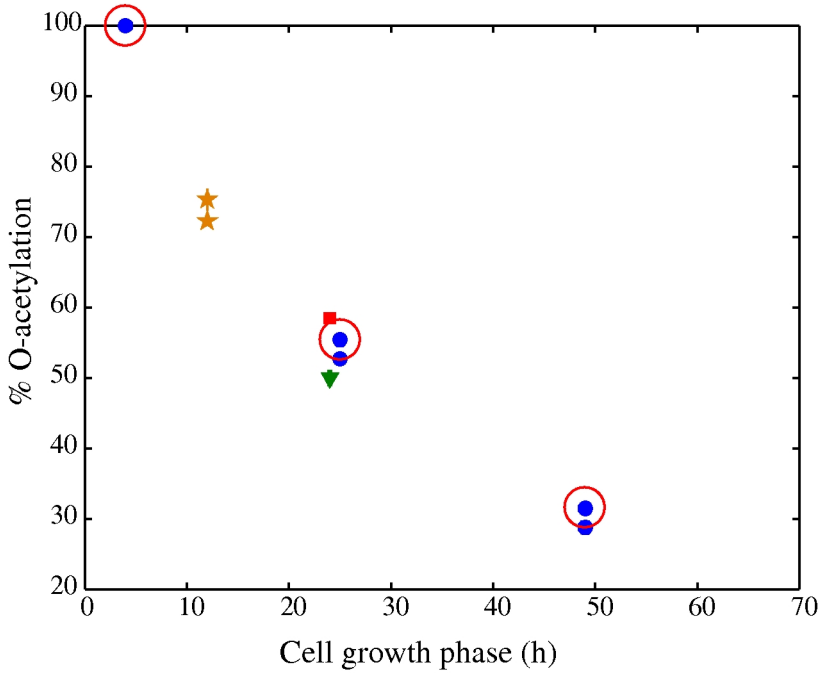


**Figure S5.** *Reproducibility of the experiments in Figure 2D-F.* The O-acetylation degree here reported is calculated from the $^{1}$H on-cell MAS NMR spectra as reported for Figure S2 for 12 cell aliquots from 4 cultures (distinguished by symbols with different shape) at different growth phases. Four data points were collected at 4 h and superimpose in the plot. The spectra in Figure 2D-F correspond to the symbols marked by a red circle. Identical symbols appearing twice at one cell growth phase refer to independent measurements (i.e. different NMR experiments on different rotors filled with cells from the same culture with the same growth phase). The error-bar indicates the statistical error of the single NMR measurement and it is often smaller than the size of the symbol.

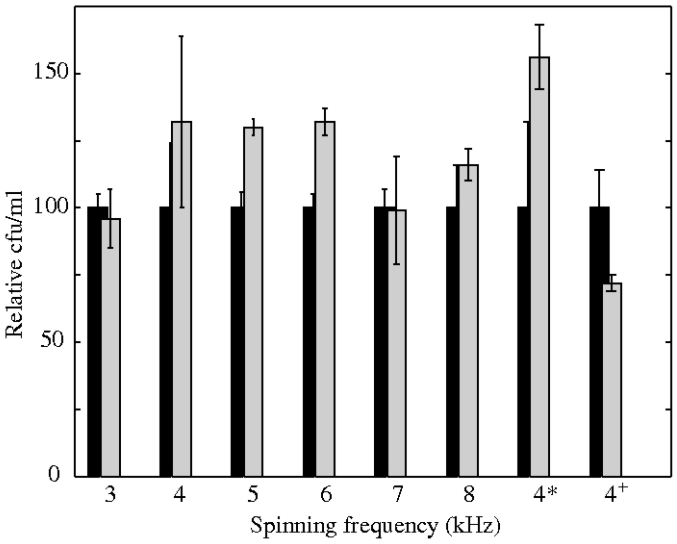


**Figure S6.** Average number of *S.* Typhimurium cells in the late stationary phase (24 h) before (black bars) and after (grey bars) the NMR experiments where the rotor was spun at the indicated MAS spinning frequency for about 1 h. Data denoted with an asterisk (*) refer to a sample where a 2D NOESY was recorded and the sample was therefore spun about 19 h long. Data denoted with a plus (+) refer to the sample which was kept in the NMR rotor 44 hours long and subjected three times to 1 h MAS spinning at 4 kHz. In fact, immediately after rotor filling, after 20 h, and finally after further 24 h the rotor was submitted to the 1D NMR experiments in Figure S8. The error bars refer to the maximum deviation over three measures of the number of cells. The cfu (colony-forming units) are normalized to the average values before the NMR experiment (=100). *S.* Typhimurium cells before and after the NMR experiments were diluted $1x10^8$ fold and spread on LB agar plates containing streptomycin. The number of cfu was determined by counting colonies after overnight incubation of the plates at 37 ˚C.

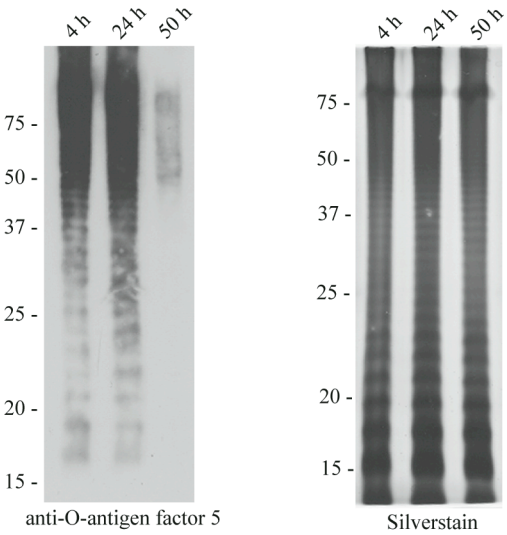


**Figure S7.** Exemplary immunoblot (left panel) and silverstain (right panel) of proteinase K treated whole *S.* Typhimurium cells at given time points show decrease in *O*-acetylation state of abequose. The immunoblot was developed with anti-O-antigen factor 5 antiserum (BD Diagnostics, catalogue number 226601) which recognizes the *O*-acetyl group at abequose. The decrease of O-acetylated Abe at longer growth phases is evident.

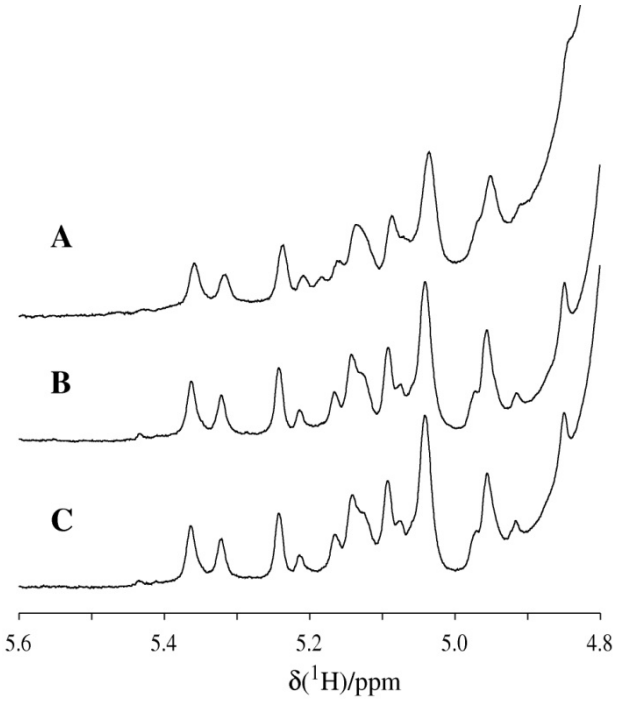


**Figure S8**. $^{1}$H MAS NMR spectra (anomeric region) of living cells of *S.* Typhimurium in the late stationary phase (24 h) measured on the same rotor after having been pelleted and then resuspended into buffer/$D_2O$. After spectrum **A** has been measured on a fresh sample, the rotor was kept sealed 20 h at 37 ˚C under shaking and then measured again to give **B**. After B has been measured, the rotor was kept in the same conditions as above for additional 24 h and then measured again to give **C**. After 2 days the pH of the sample was still neutral. The cfu corresponding to spectra A and C are reported in Figure S6, data denoted with (+). The experiment proves that the cells survive at least three days in the NMR rotor and that there is no significant modification in the O-antigen composition when the rotor is kept sealed and cells are in a buffer.

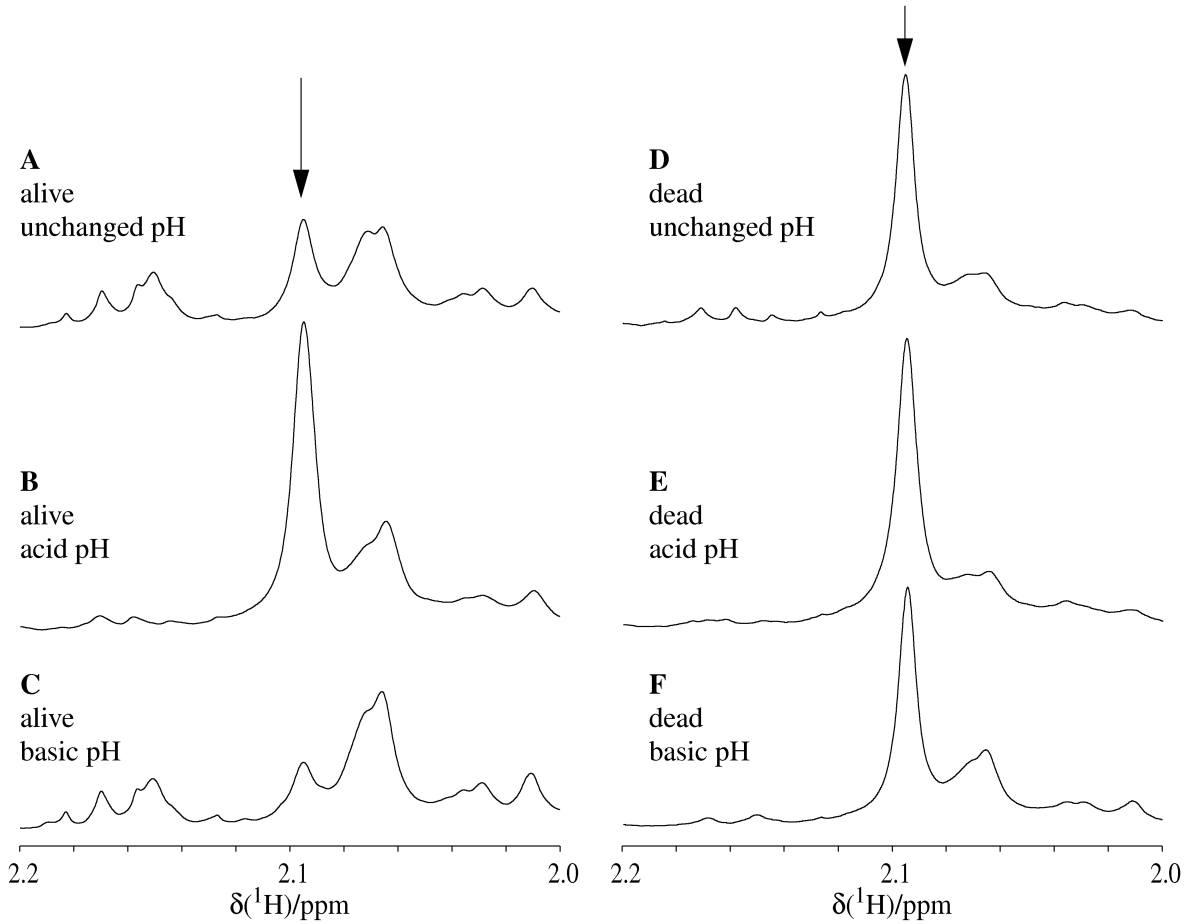


**Figure S9**. $^{1}$H MAS NMR spectra of living *S*. Typhimurium cells (the corresponding anomeric region is shown in Figure 4 in the manuscript). The arrow indicates the –$COCH_3$ peak in the Acetyl group of Abe O-acetylated at C2. See caption of Figure 4 for the description of the samples.

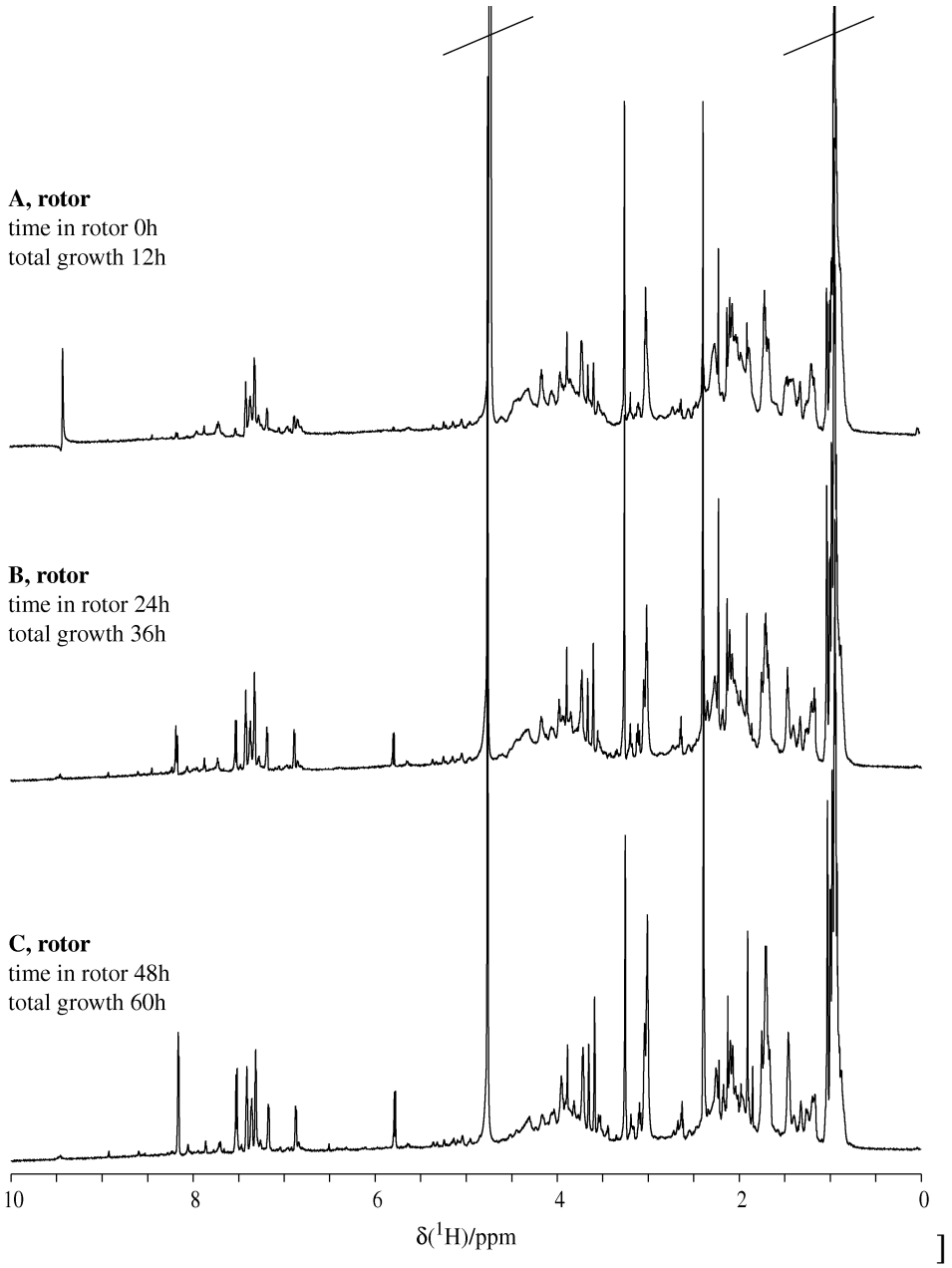


**Figure S10**. Full spectra whose anomeric region is shown in Figure 2A-C. $^{1}$H MAS NMR spectra of living S. Typhimurium cells. Cells from an early stationary phase culture (**A**: 12 h, $OD_{600}$=4.31) were centrifuged and the pellet was resuspended in the supernatant. The same sample was then measured after further 24 h (**B**) and further 24 h (**C**) in the rotor at 37 °C. "Total growth" indicates the time interval equal to the proper growth phase in LB medium plus the time in the rotor. The intense peaks which have been cut correspond to HDO and acetone.

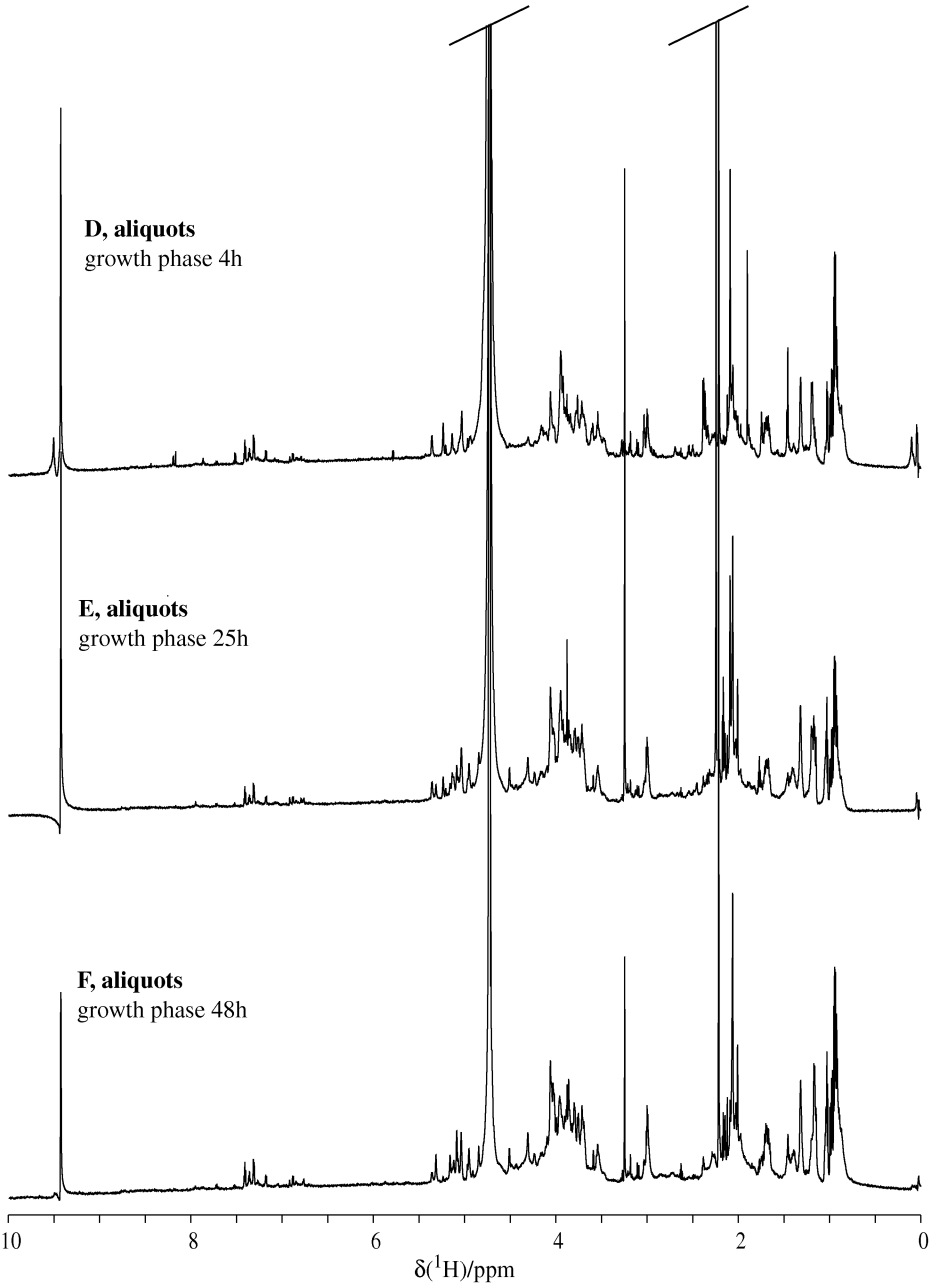


**Figure S11**. Full spectra whose anomeric region is shown in Figure 2D-F. $^{1}$H MAS NMR spectra of living S. Typhimurium cells. Cells from a culture in the exponential phase (**D**: 4 h, $OD_{600}$=1.25), late (**E**: 25 h, $OD_{600}$=3.05) and very late stationary phase (**F**: 48 h, $OD_{600}$=2.74). The spectra correspond to three cell aliquots taken from the same culture at the given time points, centrifuged and then resuspended in buffer. The intense peaks which have been cut correspond to HDO and acetone.

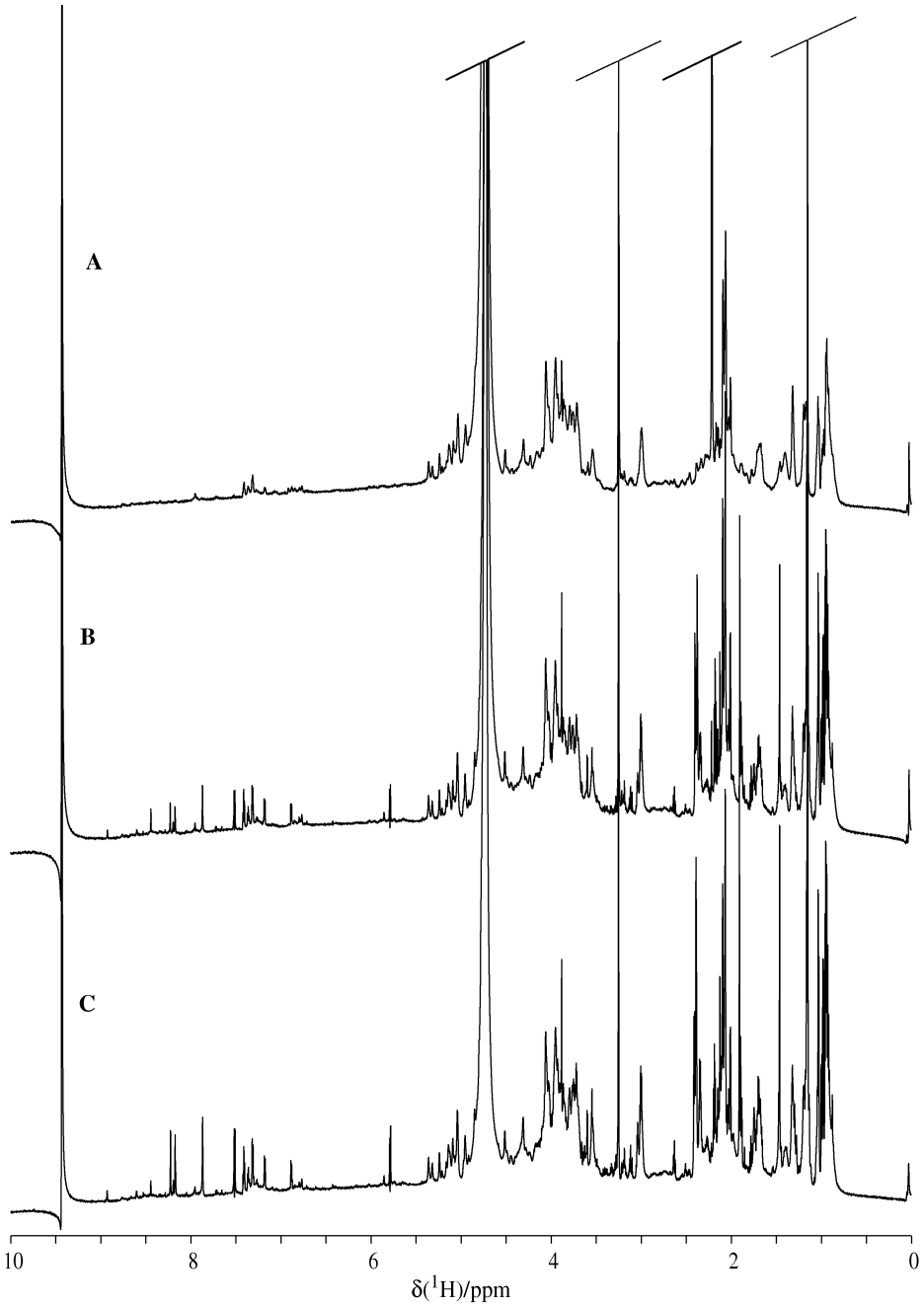


**Figure S12**. Full spectra whose anomeric region is shown in Figure S8 in the Supporting Information. $^{1}$H MAS NMR spectra of living S. Typhimurium cells in the late stationary phase (24 h) measured in the same rotor after having been pelleted and then resuspended into buffer/$D_2O$. After spectrum **A** has been measured on a fresh sample, the rotor was kept sealed 20 h at 37 ˚C under shaking and then measured again to give **B**. After B had been measured, the rotor was kept in the same conditions as above for additional 24 h and then measured again to give **C**. After 2 days the pH of the sample was still neutral. The cfu corresponding to spectra A and C are reported in Figure S6, data denoted with (+). The experiment proves that the cells survive at least three days in the NMR rotor and that there is no significant modification in the O-antigen composition when the rotor is kept sealed and cells are in a buffer. The intense peaks which have been cut correspond to HDO and acetone.

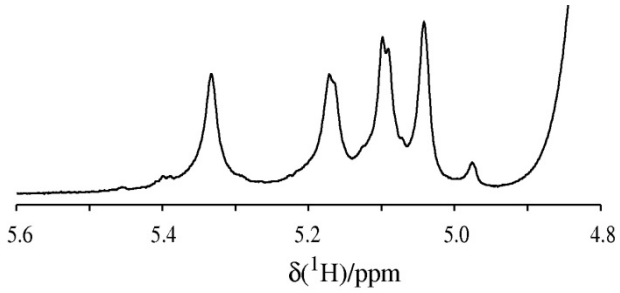


**Figure S13.** $^{1}$H NMR spectrum (anomeric region) of isolated de-O-acylated LPS from *S*. Typhimurium cells in the late exponential phase. A mild hydrazine treatment was employed to isolate the LPS by cleaving off the *O*-linked acyl-chains from lipid A.[1,2] This process causes de-O-acylation at Abe C2. The spectrum has been measured on a Bruker DMX 400 spectrometer operating at a proton frequency of 400.13 MHz with a 5 mm triple resonance inverse TXI solution probe. The temperature was 23 ˚C.

**Table S1**

**Table 1. $^1$H chemical shifts (ppm) of the *S.* Typhimurium O-antigen and relative integrals for the H1 peaks**

| Residue | H1 | H2 | H3 | H4 | H5 | H6 | $CH_3$-CO | Relative Area H1 peak |
|---|---|---|---|---|---|---|---|---|
| O-antigen with O-acetylated Abe | | | | | | | | |
| Man | 5.37 | 3.96 | 3.95 | n.d. | n.d. | n.d. | - | 1.0 |
| Ac-2-Abe | 5.25 | 5.06 | 2.06 | 3.93 | 4.15 | 1.20 | 2.09 | 1.2 |
| Gal | 5.15 | 3.91 | n.d. | 4.06 | 4.12 | 3.72 | - | 1.3 |
| Rha | 5.04 | 4.07 | 3.97 | 3.55 | 3.92 | 1.33 | - | n.d[a] |
| O-antigen with de-O-acetylated Abe[b] | | | | | | | | |
| Man | 5.33 | 4.00 | n.d. | n.d. | 3.98 | 3.81 | - | 1.0[c] |
| Abe | 5.09 | 4.03 | 1.98 | 3.87 | 4.11 | 1.18 | - | 1.5 |
| Gal | 5.17 | 3.91 | 3.94 | 4.07 | 4.09 | 3.71 | - | 1.3 |
| Rha | 5.05 | 4.07 | 3.97 | 3.55 | 3.93 | 1.33 | - | 1.1 |

[a] The area of Rha H1 in the spectrum of the O-antigen O-acetylated at Abe C2 is difficult to determine because Rha H1 and Abe H2 signals are superimposed. [b] The assignment is supported by the comparison with the spectra of fully de-O-acetylated isolated LPS (Figure S13). [c] Due to the signals crowding the relative areas of the H1 peaks of de-O-acetylated O-antigen could not be measured from the on-cell spectra. The values here reported are from the fully de-O-acetylated isolated LPS (Figure S13).

**References:**

(1) Gupta, R. K.; Szu, S. C.; Finkelstein, R. A.; Robbins, J. B. *Infect. Immun*. **1992**, *60*, 3201-8.

(2) Haishima, Y. ; Holst, O.; Brade, H. *Eur. J. Biochem*. **1992**, *203*, 127-134.