Structure of the O-Antigen and the Lipid A from the Lipopolysaccharide of *Fusobacterium nucleatum* ATCC 51191

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Figure S1. Electrophoresis analysis of bacterial LPS. A. *E. coli* O111:B4 LPS (8 µg). B. *F. nucleatum* ATCC 51191 LPS (8 µg) C. BlueEye protein standard. The samples were run on a 12% SDS-PAGE and visualized by silver staining.

Figure S2. GC-MS profile of *F. nucleatum* ATCC 51191 LPS A. acetylated methyl glycosides. B. lipid compositional analysis. i: impurities; 16:1 and 18:0 are impurities or cell derived fatty acids.
Figure S3: Chromatogram profile of *F. nucleatum* ATCC 51191 O-antigen purification on a Sephacryl S200 column. The O-antigen corresponds to the second (highest) peak of the chromatogram.

Figure S4: $^1$H NMR spectrum of the O-antigen of *F. nucleatum* ATCC 51191 showing the lack of signals around 8 ppm and therefore the absence of a formyl group (600 MHz, 25 °C, 550 μL of D$_2$O, neutral pH).
Figure S5. Negative-ion MALDI MS/MS spectrum of precursor ion at m/z 1801.1 of the lipid A of *F. nucleatum* ATCC 51191. This is a representative ion peak of the cluster ascribed to hexa-acylated lipid A species decorated by two phosphates. The main fragments’ assignment is indicated in the spectrum. The proposed structure is reported in the inset. The loss of C\(_{12}\)H\(_{24}\)O (184 mass units) and C\(_{14}\)H\(_{28}\)O (212 mass units) is also indicated and was due to a rearrangement typically occurring on primary acyl chains only when their 3-OH group is free, thus contributing to the establishment of the location of the secondary acyl substitution.

Table S1. The main MALDI-TOF MS ion peaks *F. nucleatum* ATCC 51191 lipid A. The table reports the predicted mass and the proposed interpretation of the substituting fatty acids and phosphates on the *F. nucleatum* ATCC 51191 lipid A backbone. See Figure 6 for full spectrum.

| Predicted mass (Da) | Observed ion peaks (m/z) | Acyl substitution | Proposed fatty acid/phosphate composition |
|---------------------|--------------------------|-------------------|------------------------------------------|
| 1364.96             | 1364.68                  | Tetra-acyl        | HexN\(\text{P}^\bullet\)\{14:0(3-OH)\}\{16:0(3-OH)\}; (14:0) |
| 1444.92             | 1444.69                  | Tetra-acyl        | HexN\(\text{P}^\bullet\)\{14:0(3-OH)\}\{16:0(3-OH)\}; (14:0) |
| 1581.15             | 1580.86                  | Penta-acyl        | HexN\(\text{P}^\bullet\)\{14:0(3-OH)\}\{16:0(3-OH)\}; (14:0) |
| 1671.11             | 1670.87                  | Penta-acyl        | HexN\(\text{P}^\bullet\)\{14:0(3-OH)\}\{16:0(3-OH)\}; (14:0) |
| 1801.35             | 1801.15                  | Hexa-acyl         | HexN\(\text{P}^\bullet\)\{14:0(3-OH)\}\{16:0(3-OH)\}; (14:0) |
| 1881.31             | 1881.15                  | Hexa-acyl         | HexN\(\text{P}^\bullet\)\{14:0(3-OH)\}\{16:0(3-OH)\}; (14:0) |