**Lipopolysaccharides from *Ralstonia solanacearum* Induce a Broad Metabolomic Response in *Solanum lycopersicum***

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**Supplementary data – (Figures S1 – S4 and Tables S1 and S2)**

**Table S1.** 1H, and 13C (*Italic*) chemical shifts of the OPS derived from mild acid hydrolysis of the LPS from *R. solanacearum*. The chemical shifts of the NH of the acetyl group (*N*Ac) were at 1H/13C 1.96/22.3 ppm.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **A** | 1H | 5.07 | 3.99 | 3.85 | 3.40 | 3.66 | 1.25 |  |
| 2-α-L-Rha | 13C | *100.9* | ***77.8*** | *70.1* | *71.2* | *69.3* | *16.7* |  |
| **B’** | 1H | 4.91 | 4.18 | 3.78 | 3.63 | 3.63 | 1.18 |  |
| 3,4-α-L-Rha | 13C | *101.7* | *69.8* | ***74.6*** | ***79.4*** | *69.3* | *16.7* |  |
| **B** | 1H | 4.89 | 4.22 | 3.77 | 3.43 | 3.63 | 1.18 |  |
| 3-α-L-Rha | 13C | *101.7* | *69.7* | ***79.8*** | *71.8* | *69.3* | *16.7* |  |
| **C** | 1H | 4.77 | 3.75 | 3.72 | 3.46 | 3.95 | 1.16 |  |
| 3-α-L-Rha | 13C | *101.1* | *70.7* | ***77.4*** | *71.5* | *69.1* | *16.7* |  |
| **D** | 1H | 4.66 | 3.80 | 3.53 | 3.48 | 3.40 | 3.70/3.85 |  |
| 3-β-D-GlcNAc | 13C | *102.0* | *55.8* | ***81.6*** | *68.3* | *75.8* | *60.6* |  |
| **E** | 1H | 4.34 | 3.41 | 3.33 | 3.50 | 3.90/3.19 |  |  |
| t-β-L-Xyl | 13C | *103.5* | *73.2* | *76.1* | *69.3* | *65.0* |  |  |
| **X** | 1H | 5.28 | 3.95 | 3.93 | 3.61 | n.d. | 4.02 | 3.71 |
| 2,3-α-L,D-Hep | 13C | *100.0* | ***80.0*** | ***72.4*** | *72.0* | *n.d.* | *68.4* | *63.4* |
| **Y** | 1H | 4.75 | 3.96 | n.d. | 3.41 | 3.67 | 1.17 |  |
| *t*-α-L-Rha | 13C | *102.6* | *70.4* | *n.d.* | *71.1* | *69.3* | *16.7* |  |



**Figure S1.** Negative-ion MALDI MS/MS spectrum of precursor ions at *m/z* 1505.6, chosen as a representative ion peak of the cluster ascribed to *mono*-phosphorylated penta-acylated lipid A species from *R. solanacearum* LPS. The assignment of main fragments is reported in the spectrum. The proposed structure for the lipid A species is sketched in the inset.

Chart

Description automatically generated with low confidence

**Figure S2.** Base peak intensity chromatograms of the ESI negative UHPLC-MS analyses of the methanol extracts from the LPS*R.sol*(100 µg/mL) inoculated *S. lycopersicum* leaf tissues. A comparison of the metabolite profiles at the 24 h time interval – **(A)** MgSO4 negative control and **(B)** LPS treatment - revealed concentration-linked variation in relative peak intensities. The *y*-axes of the two chromatograms are linked and represent the relative abundance (%) of the metabolite signatures at their respective retention times (Rt, min). The changes in peak intensities (green) and/or the presence/absence of peaks (purple) could be observed, reflecting the LPS*R.sol*-induced perturbation of leaf metabolism.

Timeline

Description automatically generated

**Figure S3.** Base peak intensity chromatograms of the ESI negative UHPLC-MS analyses of the methanol extracts from the LPS*R.sol*(100 µg/mL) inoculated *S. lycopersicum* leaf tissues. A comparison of the metabolite profiles at the 32 h time interval – **(A)** MgSO4 negative control and **(B)** LPS treatment - revealed concentration-linked variation in relative peak intensities. The *y*-axes of the two chromatograms are linked and represent the relative abundance (%) of the metabolite signatures at their respective retention times (Rt, min). The changes in peak intensities (green) and/or the presence/absence of peaks (purple) could be observed, reflecting the LPS*R.sol* -induced perturbation of leaf metabolism.

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**Figure S4.** Overlaid UHPLC-MS BPI chromatograms (ESI-) of methanolic leaf extracts from the LPS*R. sol* elicitor treated tomato plants after 16 h (black), 24 h (green) and 32 h (blue). The chromatograms highlight time-dependent metabolic variations as a result of LPS treatment. Qualitative differences are reflected by the peak intensities where the *y*-axis represents the relative peak intensity of the metabolites at their respective retention times.

**Table S2.** Statistical validation of the computed OPLS-DA models corresponding to the LPS elicited tomato leaf treatment data matrices. The calculated number of components used in each final model (N), the R2X(cum), the R2Y(cum) and the Q2(cum) values for each of the six OPLS-DA models are presented for both ESI negative and ESI positive modes. The R2 and Q2 values of the permutation analysis (*n =* 200 random permutations) are compared and shown to be significantly lower than the original values. Model values > 0.50 are shaded in green, values < 0.50 but > 0.30 are shaded in orange, and values < 30 are shaded in red. The *p-*value of a 7-fold CV-ANOVA was shown to indicate statistical significance of each investigated model. (AUROC = Area Under the Receiver Operating Characteristics curve; LPS*R. sol* = *R. solanacearum*-derived lipopolysaccharide treatment; all at time intervals of 16 h, 24 h and 32 h post-elicitation; 8 mM MgSO4 controls at the same time points).

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Model** | **N** | **R2X**  **(cum)** | **R2Y**  **(cum)** | **Q2**  **(cum)** | **Permutation** | | **AUROC** | | ***p*-value of CV-ANOVA** |
| **R2** | **Q2** | **Control** | **LPS*R. sol*** |
| ESI (Negative) Supervised Models | | | | | | | | | |
| C\_16 *vs.* LPS16 | 17 | 0.406 | 0.998 | 0.970 | 0.828 | -0.299 | 0.906 | 1.000 | 5.337 x 10-9 |
| C\_24 *vs*. LPS24 | 18 | 0.456 | 0.999 | 0.916 | 0.963 | -0.382 | 0.542 | 0.946 | 2.605 x 10-5 |
| C\_32 *vs*. LPS32 | 16 | 0.363 | 0.997 | 0.945 | 0.904 | -0.320 | 1.000 | 0.690 | 7.348 x 10-7 |
| ESI (Positive) Supervised Models | | | | | | | | | |
| C\_16 *vs.* LPS16 | 18 | 0.582 | 0.999 | 0.989 | 0.806 | -0.344 | 0.882 | 1.000 | 1.033 x 10-12 |
| C\_24 *vs*. LPS24 | 18 | 0.558 | 0.992 | 0.963 | 0.796 | -0.389 | 0.874 | 0.816 | 3.654 x 10-9 |
| C\_32 *vs*. LPS32 | 18 | 0.467 | 0.995 | 0.697 | 0.797 | -0.385 | 0.689 | 0.545 | 1.556 x 10-9 |