Supplementary

Utilizing the food-pathogen metabolome to putatively identify biomarkers for the detection of Shiga toxin-producing *E. coli* (STEC) from spinach.

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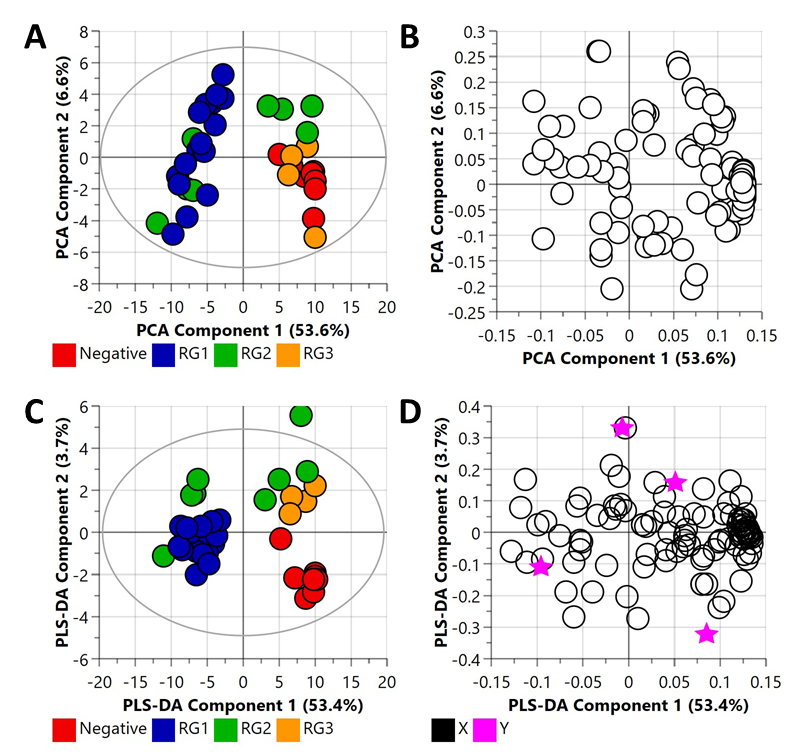
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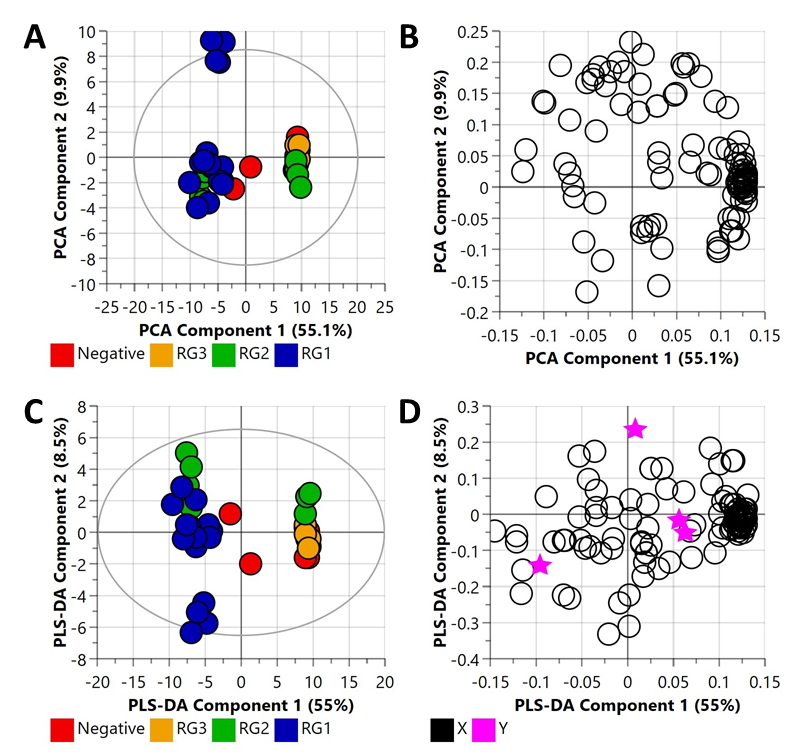
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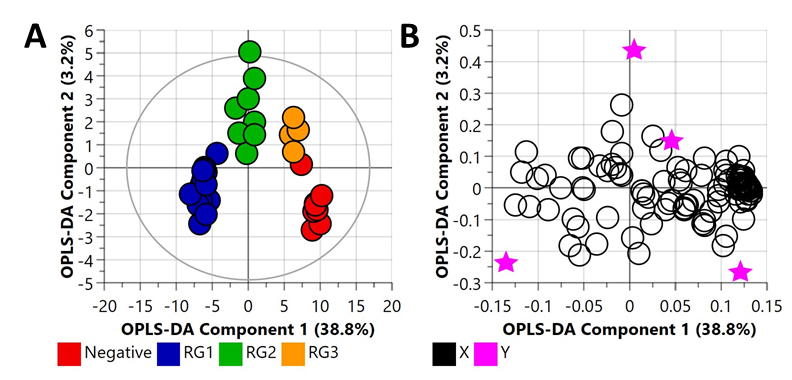
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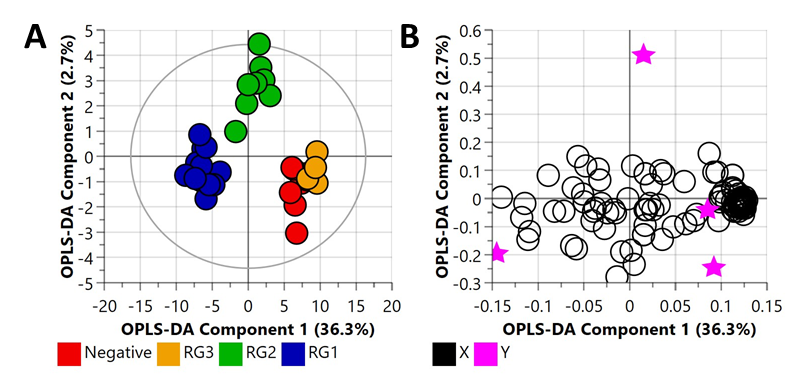


**Figure S1.** Principal component analysis (PCA) (R2X (cum) = 0.653, Q2 = 0.521) and partial least square-discriminant analysis (PLS-DA) (R2X (cum) = 0.571, R2Y (cum) = 0.494, Q2 = 0.312) of bacterial pellet samples collected from buffered peptone water (BPW) cultures*. Note, the eclipse presented in Figure S1A and S1C represents the Hotelling's confidence limit (95%). Note: The colored circles in panel “A” and “C” represent each analysed samples, while the purple-colored stars in panel “B” and “D” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*



**Figure S2**. Principal component analysis (PCA) (R2X (cum) = 0.649, Q2 = 0.581) and partial least square-discriminant analysis (PLS-DA) (R2X (cum) = 0.696, R2Y (cum) = 0.697, Q2 = 0.347) of bacterial supernatant samples collected from buffered peptone water (BPW) cultures. *Note, the eclipse presented in Figure S2A and S2C represents the Hotelling's confidence limit (95%). The colored circles in panel “A” and “C” represent each analysed samples, while the purple-colored stars in panel “B” and “D” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*

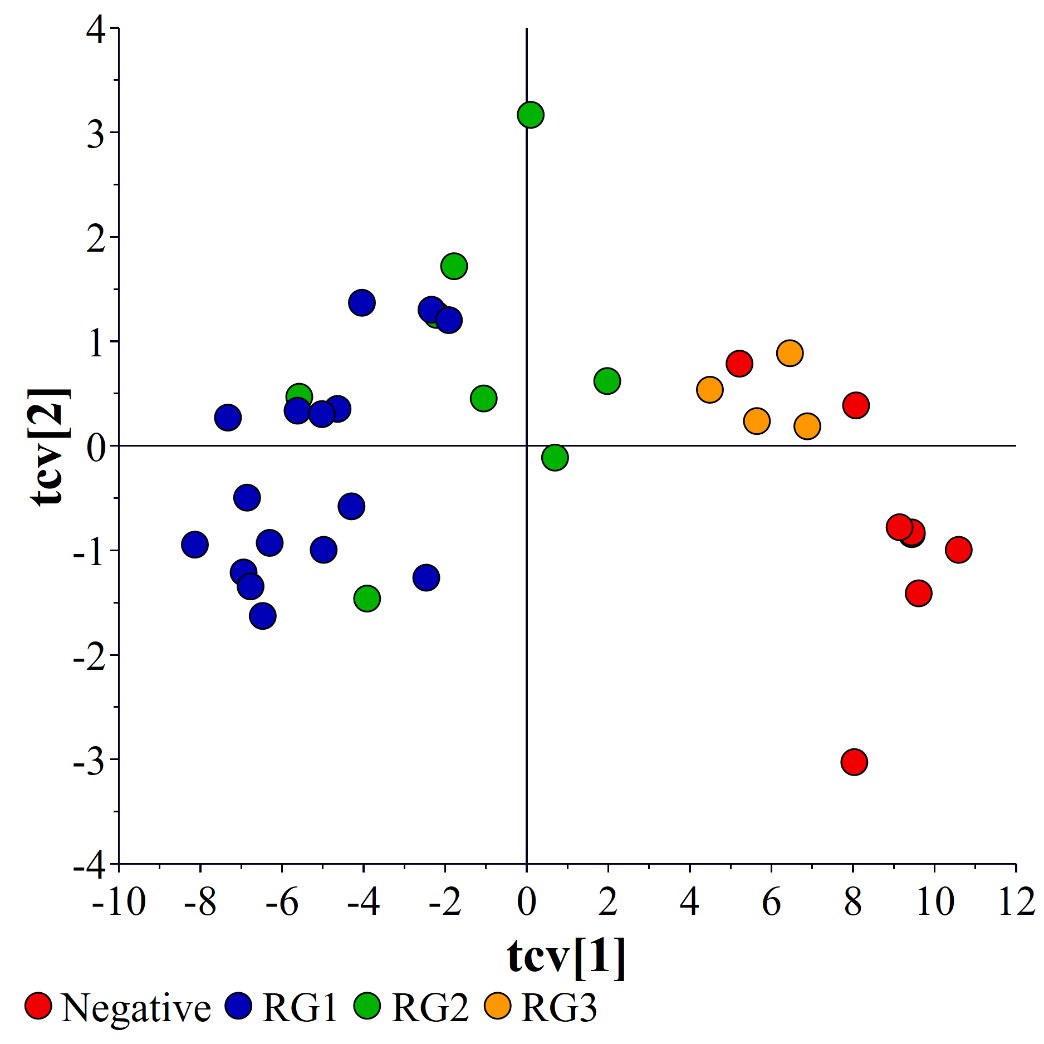
**Figure S3**. Orthogonal Partial Least Square-Discriminant Analysis (A) scatter plot and (B) loading plot of bacterial pellet samples collected from BPW cultures (n=38; note, the negative group includes *Salmonella*). R2X (cum) = 0.697, R2Y (cum) = 0.637, Q2 = 0.345. Note, the eclipse presented in Figure 2A represents the Hotelling's confidence limit (95%). ***Note:*** *The colored circles in panel “A” represent each analysed samples, while the purple-colored stars in panel “B” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*

**Figure S4**. Orthogonal Partial Least Square-Discriminant Analysis (A) scatter plot and (B) loading plot of bacterial supernatant samples collected from BPW cultures (n=38; note, the negative group includes *Salmonella*). R2X (cum) = 0.765, R2Y (cum) = 0.845, Q2 = 0.421. Note, the eclipse presented in Figure 3A represents the Hotelling's confidence limit (95%). ***Note:*** *The colored circles in panel “A” represent each analysed samples, while the purple-colored stars in panel “B” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*

**Table S1: Cross validation (CV)-ANOVA of the OPLS-DA bacterial pellet model (Figure S3).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **OPLS-DA (Figure S3)** | **SS** | **DF** | **MS** | **F-test** | **P value** | **SD** |
| **Total corr.** | 105 | 105 | 1 |  |  | 1 |
| **Regression** | 46.6142 | 30 | 1.55381 | 1.99595 | 0.00844 | 1.24652 |
| **Residual** | 58.3858 | 75 | 0.778478 |  |  | 0.882314 |

*In this table, SS: sum-of-squares, DF: degrees of freedom, MS: mean squares, SD: standard deviation*

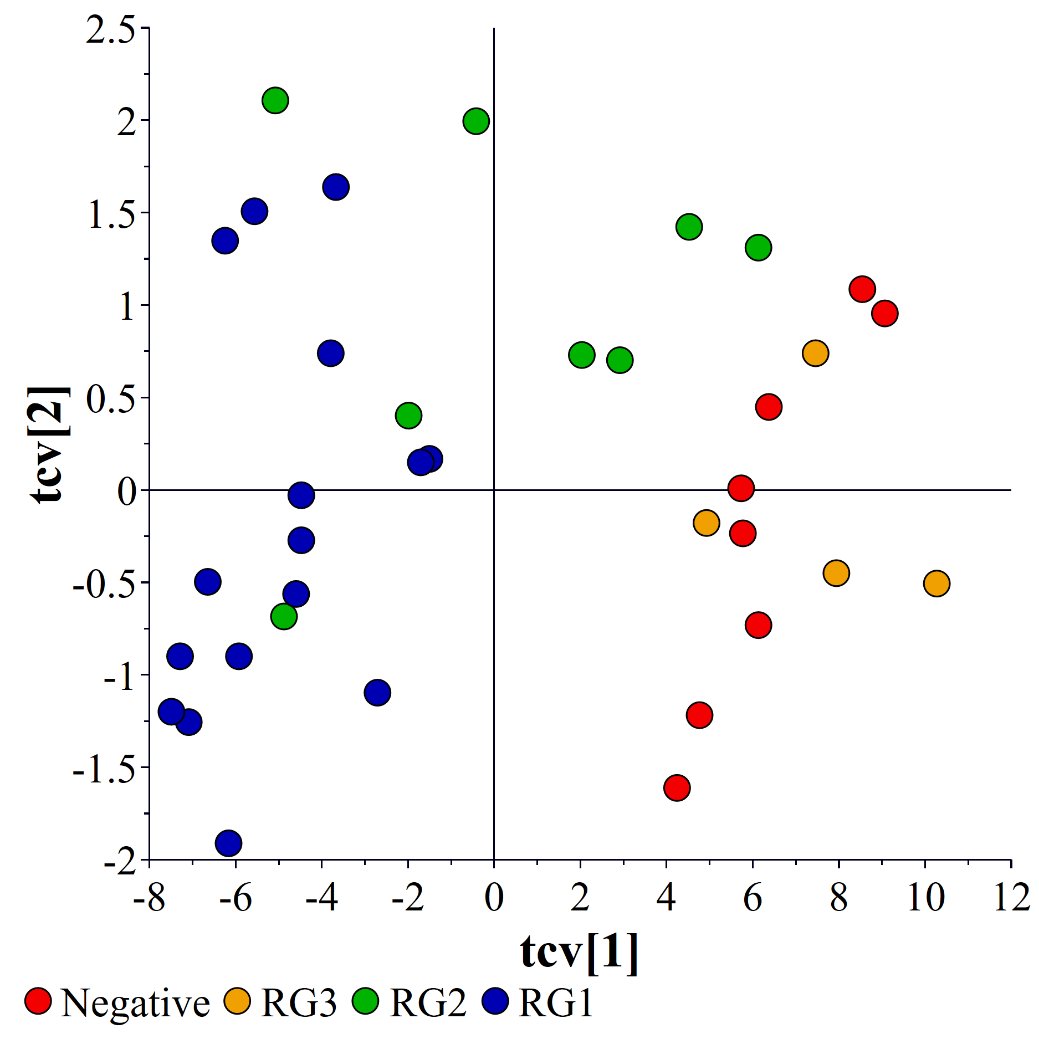
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**Figure S5**: Cross Validation (CV) Scores plots of the OPLS-DA bacterial pellet model. Note, the scatter plot of the cross-validated score vectors is analogous to the scatter plot of regular score vectors (Figure 1) but illustrates the sample stability of each point in relation to the other groups.

**Table S2:** Cross validation (CV)-ANOVA of the OPLS-DA bacterial supernatant model (Figure S4).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **OPLS-DA (Figure S4)** | **SS** | **DF** | **MS** | **F-test** | **P value** | **SD** |
| **Total corr.** | 105 | 105 | 1 |  |  | 1 |
| **Regression** | 36.9623 | 39 | 0.947751 | 0.919366 | 0.605361 | 0.973525 |
| **Residual** | 68.0377 | 66 | 1.03087 |  |  | 1.01532 |

*In this table, SS: sum-of-squares, DF: degrees of freedom, MS: mean squares, SD: standard deviation*

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**Figure S6:** Cross Validation (CV) Scores plots of the OPLS-DA bacterial supernatant model. Note, the scatter plot of the cross-validated score vectors is analogous to the scatter plot of regular score vectors (Figure 1) but illustrates the sample stability of each point in relation to the other groups.

**Table S3: Significant metabolites in bacterial pellet samples collected from BPW cultures** (n=38; note, the negative group includes *Salmonella*).

| **Metabolites** | **Fold change** | **Adjusted P value** | **Regulation** |
| --- | --- | --- | --- |
| Compound\_18 | 36.416 | 0.000146 | up |
| Compound\_14 | 35.204 | 8.28E-06 | up |
| Compound\_12 | 33.318 | 0.001508 | up |
| 2-amino-2-methyl-1,3-propanediol | 31.664 | 9.19E-05 | up |
| D-sphingosine | 25.018 | 1.85E-13 | up |
| Compound\_73 | 24.241 | 7.12E-10 | up |
| Compound\_107 | 16.051 | 0.001213 | up |
| Compound\_87 | 15.753 | 0.000257 | up |
| Compound\_21 | 12.48 | 5.78E-07 | up |
| Compound\_104 | 10.114 | 7.33E-10 | up |
| Behenic acid | 9.6772 | 4.92E-05 | up |
| 2,3-dihydroxybiphenyl | 9.5005 | 0.000127 | up |
| Compound\_2 | 8.2141 | 0.004631 | up |
| Compound\_96 | 8.2125 | 0.001037 | up |
| Compound\_44 | 8.1728 | 3.47E-05 | up |
| Compound\_50 | 8.1701 | 7.38E-05 | up |
| Compound\_61 | 8.0776 | 0.000731 | up |
| Compound\_11 | 7.9889 | 4.92E-05 | up |
| Compound\_24 | 6.6523 | 0.000146 | up |
| Compound\_38 | 6.5173 | 0.000712 | up |
| Acetohydroxamic acid | 6.2431 | 0.000146 | up |
| Compound\_27 | 6.2068 | 0.004362 | up |
| 3-hydroxyanthranilic acid | 5.8699 | 0.000731 | up |
| Compound\_51 | 5.8517 | 0.002949 | up |
| Compound\_103 | 5.5829 | 1.30E-05 | up |
| Pelargonic acid | 5.4489 | 4.92E-05 | up |
| Compound\_97 | 5.2038 | 0.007991 | up |
| Compound\_9 | 5.1091 | 0.000808 | up |
| Compound\_5 | 4.5896 | 0.004927 | up |
| Compound\_99 | 4.5847 | 0.000158 | up |
| 4-aminophenol | 4.5141 | 0.000143 | up |
| Compound\_48 | 4.4353 | 0.006312 | up |
| Compound\_106 | 4.3723 | 0.000245 | up |
| Compound\_49 | 4.2725 | 0.013932 | up |
| DL-2-amino-3-phosphonopropionic acid | 4.1294 | 0.000466 | up |
| Compound\_16 | 3.9466 | 0.000811 | up |
| Glycolic acid | 3.9064 | 0.000182 | up |
| Compound\_19 | 3.8993 | 0.001678 | up |
| Halostachine | 3.8843 | 0.002475 | up |
| Compound\_26 | 3.8763 | 0.000781 | up |
| Compound\_82 | 3.7364 | 0.003348 | up |
| Compound\_68 | 3.5628 | 0.00204 | up |
| Lauric acid | 2.2008 | 0.026559 | up |
| 2,6-Dihydroxy-4-Methoxytoluene | 2.0793 | 0.002067 | up |
| 1-hexadecanol | 2.0793 | 0.002067 | up |
| Compound\_105 | 2.0371 | 0.017356 | up |
| Pipecolic acid | 0.48959 | 2.58E-06 | down |
| Trimethyllysine | 0.41389 | 1.40E-05 | down |
| Compound\_70 | 0.37818 | 3.95E-07 | down |
| L-methionine | 0.37376 | 3.95E-07 | down |
| Compound\_79 | 0.34472 | 4.07E-07 | down |
| Cytidine | 0.29306 | 3.72E-06 | down |
| Compound\_111 | 0.25147 | 8.28E-06 | down |
| Compound\_76 | 0.24706 | 1.61E-05 | down |
| Compound\_77 | 0.23977 | 7.69E-05 | down |
| N-acetyl-ornithine | 0.23468 | 2.48E-05 | down |
| Compound\_83 | 0.21965 | 1.56E-06 | down |
| Compound\_109 | 0.21267 | 1.61E-05 | down |

**Table S4: Significant metabolites in bacterial supernatant samples collected from BPW cultures** (n=38; note, the negative group includes *Salmonella*).

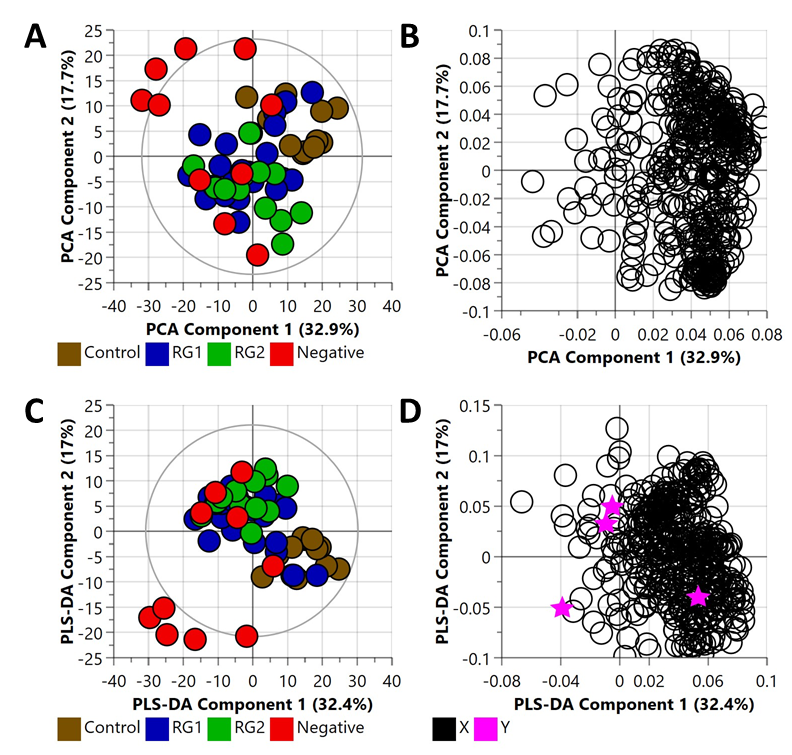
| **Metabolites** | **Fold change** | **Adjusted P value** | **Regulation** |
| --- | --- | --- | --- |
| Compound\_103 | 38.929 | 6.63E-05 | up |
| Compound\_21 | 34.426 | 3.12E-07 | up |
| Compound\_104 | 22.265 | 6.98E-07 | up |
| Compound\_107 | 19.281 | 6.74E-07 | up |
| 2-amino-2-methyl-1,3-propanediol | 18.976 | 0.002331 | up |
| Compound\_87 | 17.041 | 0.000872 | up |
| 2,3-dihydroxybiphenyl | 10.598 | 0.000988 | up |
| Compound\_105 | 6.9911 | 0.000729 | up |
| Compound\_38 | 6.8391 | 0.013821 | up |
| Compound\_44 | 6.6591 | 0.004764 | up |
| Compound\_102 | 6.6434 | 0.020342 | up |
| Compound\_96 | 6.4177 | 0.00816 | up |
| Compound\_48 | 6.2733 | 0.002057 | up |
| behenic acid | 5.4042 | 0.001537 | up |
| Compound\_18 | 5.3986 | 0.000729 | up |
| 2,3-butanediol | 5.0483 | 0.001857 | up |
| Compound\_4 | 5.0482 | 0.001857 | up |
| Compound\_14 | 4.9289 | 0.002907 | up |
| Compound\_51 | 4.6055 | 0.002113 | up |
| Compound\_49 | 4.5877 | 0.025265 | up |
| pelargonic acid | 3.8394 | 0.018413 | up |
| Compound\_11 | 3.7539 | 0.002784 | up |
| Compound\_61 | 3.6594 | 0.012926 | up |
| Compound\_24 | 3.4676 | 0.003368 | up |
| Compound\_12 | 3.4617 | 0.034869 | up |
| 4-aminophenol | 3.3531 | 0.000729 | up |
| acetohydroxamic acid | 3.3156 | 0.003907 | up |
| Compound\_5 | 3.1738 | 0.012242 | up |
| Compound\_9 | 3.073 | 0.005124 | up |
| Compound\_99 | 2.865 | 0.001857 | up |
| Compound\_106 | 2.813 | 0.00348 | up |
| Compound\_26 | 2.7512 | 0.013821 | up |
| glycolic acid | 2.6654 | 0.002113 | up |
| 3-hydroxyanthranilic acid | 2.5867 | 0.031998 | up |
| Compound\_27 | 2.5864 | 0.031998 | up |
| halostachine | 2.5024 | 0.025265 | up |
| Compound\_19 | 2.5024 | 0.025265 | up |
| Compound\_22 | 2.4491 | 0.00816 | up |
| Compound\_16 | 2.4334 | 0.031998 | up |
| DL-2-amino-3-phosphonopropionic acid | 2.4136 | 0.019143 | up |
| D-sphingosine | 2.3341 | 0.001613 | up |
| Compound\_68 | 2.2822 | 0.049952 | up |
| Compound\_73 | 2.2638 | 0.003307 | up |
| Compound\_76 | 0.48847 | 0.007747 | down |
| Compound\_77 | 0.48806 | 0.018413 | down |
| Compound\_111 | 0.46467 | 0.004764 | down |
| epsilon-caprolactam | 0.31819 | 0.003841 | down |
| Compound\_109 | 0.30493 | 0.001613 | down |
| N-acetyl-ornithine | 0.25191 | 0.000804 | down |

**Table S5:** ANOVA analysis of bacterial pellet samples collected from BPW cultures.

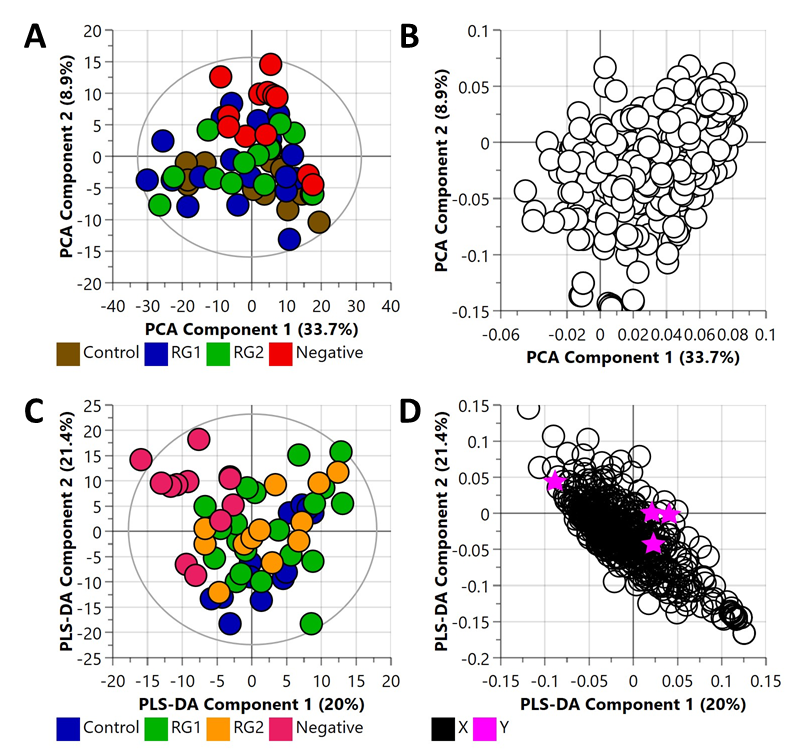
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Metabolites** | **Pubchem ID** | **KEGG ID** | **F-statistic** | **P value** | **FDR** | **Fisher's LSD** |
| D-sphingosine | 5280335 | C00319 | 55.978 | 1.05E-15 | 1.12E-13 | RG1 > Negative; RG2 > Negative; RG1 > RG2; RG1 > RG3; RG2 > RG3 |
| L-methionine | 6137 | C00073 | 15.094 | 4.74E-07 | 8.38E-06 | Negative > RG1; Negative > RG2; RG3 > RG1; RG3 > RG2 |
| Pipecolic acid | 439227 | C00408 | 12.302 | 4.31E-06 | 4.24E-05 | Negative > RG1; Negative > RG2; RG3 > RG1; RG3 > RG2 |
| Cytidine | 596 | NA | 12.163 | 4.84E-06 | 4.27E-05 | Negative > RG1; Negative > RG2; RG3 > RG1; RG3 > RG2 |
| N-acetyl-ornithine | 439232 | C00437 | 10.016 | 3.08E-05 | 2.17E-04 | Negative > RG1; Negative > RG2; RG3 > RG1; RG3 > RG2 |
| Trimethyllysine | 159660 | NA | 9.5429 | 4.72E-05 | 2.94E-04 | Negative > RG1; Negative > RG2; RG3 > RG1; RG3 > RG2 |
| Behenic acid | 8215 | C08281 | 8.806 | 9.31E-05 | 4.93E-04 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| Conduritol epoxide | 119054 | NA | 8.3638 | 1.41E-04 | 6.80E-04 | Negative > RG1; Negative > RG2; RG3 > RG1 |
| Pelargonic acid | 8158 | C01601 | 8.0606 | 1.89E-04 | 8.42E-04 | RG1 > Negative; RG2 > Negative; RG1 > RG3 |
| 2-amino-2-methyl-1,3-propanediol | 1531 | C11260 | 7.4813 | 3.31E-04 | 1.35E-03 | RG1 > Negative; RG2 > Negative; RG1 > RG3 |
| 2,6-Dihydroxy-4-Methoxytoluene | 238769 | NA | 7.2436 | 4.19E-04 | 1.48E-03 | RG1 > Negative; Negative > RG3; RG1 > RG3; RG2 > RG3 |
| 1-hexadecanol | 2682 | C00823 | 7.2436 | 4.19E-04 | 1.48E-03 | RG1 > Negative; Negative > RG3; RG1 > RG3; RG2 > RG3 |
| 2,3-dihydroxybiphenyl | 254 | C02526 | 6.817 | 6.41E-04 | 2.12E-03 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| 4-aminophenol | 403 | C02372 | 6.5666 | 8.25E-04 | 2.65E-03 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| Acetohydroxamic acid | 1990 | C06808 | 6.4834 | 8.98E-04 | 2.72E-03 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| Glycolic acid | 757 | C00160 | 6.2912 | 1.09E-03 | 3.13E-03 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| DL-2-amino-3-phosphonopropionic acid | 3857 | C05672 | 5.3854 | 2.82E-03 | 7.11E-03 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| Glycine | 750 | C00037 | 5.2306 | 3.32E-03 | 8.19E-03 | Negative > RG1; Negative > RG2; RG3 > RG1 |
| 3-hydroxyanthranilic acid | 86 | C00632 | 5.0685 | 3.95E-03 | 9.31E-03 | RG1 > Negative; RG2 > Negative; RG1 > RG3 |
| Acetol | 8299 | C05235 | 5.0174 | 4.18E-03 | 9.62E-03 | Negative > RG1; RG3 > RG1 |
| DL-3-aminoisobutyric acid | 64956 | C05145 | 3.9524 | 1.34E-02 | 2.59E-02 | RG1 > Negative; RG1 > RG3 |
| Lauric acid | 3893 | C02679 | 3.7978 | 1.60E-02 | 2.95E-02 | RG1 > RG2; RG1 > RG3 |
| Halostachine | 913 | C03711 | 3.7885 | 1.62E-02 | 2.95E-02 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| L-proline | 145742 | C00148 | 3.4894 | 2.26E-02 | 3.69E-02 | Negative > RG1 |
| Acetoacetate | 96 | C00164 | 3.2799 | 2.87E-02 | 4.51E-02 | RG1 > RG2; RG1 > RG3 |

**Table S6:** ANOVA analysis of bacterial supernatant samples collected from BPW cultures.

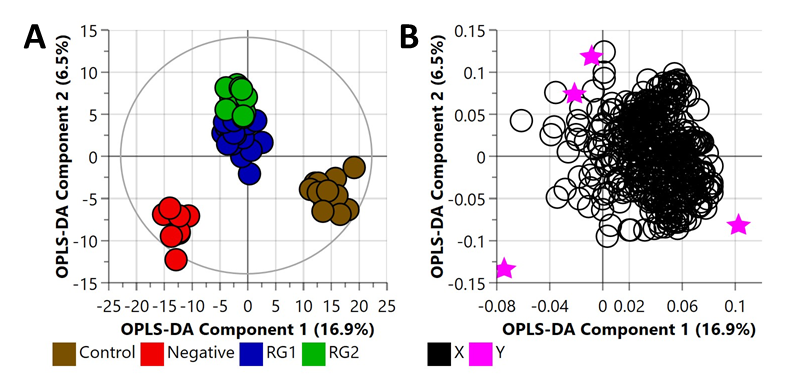
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Metabolites** | **Pubchem ID** | **KEGG ID** | **F statistic** | **P value** | **FDR** | **Fisher's LSD** |
| D-sphingosine | 5280335 | C00319 | 26.235 | 3.42E-10 | 1.81E-08 | RG1 > Negative; Negative > RG3; RG1 > RG2; RG1 > RG3; RG2 > RG3 |
| pelargonic acid | 8158 | C01601 | 9.2378 | 6.24E-05 | 8.26E-04 | RG1 > Negative; RG1 > RG2; RG1 > RG3 |
| 2,3-dihydroxybiphenyl | 254 | C02526 | 8.6094 | 1.12E-04 | 1.32E-03 | RG1 > Negative; RG1 > RG2; RG1 > RG3 |
| DL-3-aminoisobutyric acid | 64956 | C05145 | 8.2684 | 1.55E-04 | 1.64E-03 | RG1 > Negative; RG1 > RG2; RG1 > RG3 |
| 4-aminophenol | 403 | C02372 | 7.4483 | 3.42E-04 | 3.02E-03 | RG1 > Negative; RG1 > RG2; RG1 > RG3 |
| behenic acid | 8215 | C08281 | 6.8098 | 6.45E-04 | 4.56E-03 | RG1 > Negative; RG1 > RG2; RG1 > RG3 |
| N-acetyl-ornithine | 439232 | C00437 | 6.0757 | 1.37E-03 | 8.85E-03 | Negative > RG1; Negative > RG2; RG3 > RG1; RG3 > RG2 |
| acetoacetate | 96 | C00164 | 6.0289 | 1.43E-03 | 8.85E-03 | RG3 > Negative; RG3 > RG1; RG3 > RG2 |
| 2,3-butanediol | 262 | NA | 5.5697 | 2.32E-03 | 1.12E-02 | RG1 > Negative; RG2 > Negative; RG1 > RG3; RG2 > RG3 |
| L-alanine | 5950 | C00041 | 5.5275 | 2.42E-03 | 1.12E-02 | RG1 > Negative; RG1 > RG3 |
| glycolic acid | 757 | C00160 | 5.1918 | 3.46E-03 | 1.47E-02 | RG1 > Negative; RG1 > RG3; RG2 > RG3 |
| 2-amino-2-methyl-1,3-propanediol | 1531 | C11260 | 4.9224 | 4.63E-03 | 1.75E-02 | RG1 > Negative; RG1 > RG3 |
| acetohydroxamic acid | 1990 | C06808 | 4.377 | 8.39E-03 | 2.74E-02 | RG1 > Negative; RG1 > RG3; RG2 > RG3 |
| epsilon-caprolactam | 7768 | C06593 | 4.2255 | 9.92E-03 | 3.09E-02 | Negative > RG1; RG3 > RG1; RG3 > RG2 |

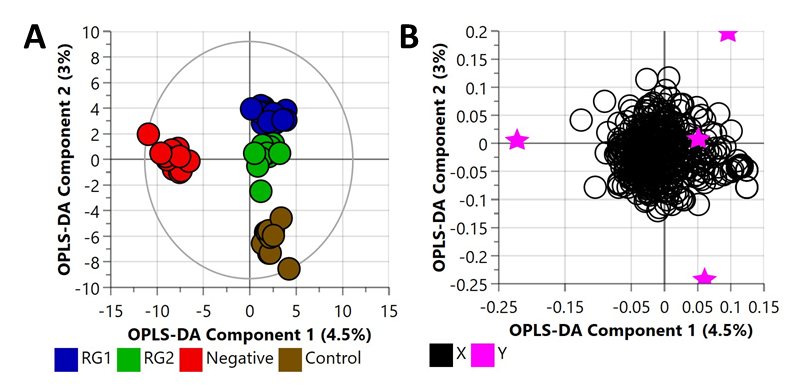


**Figure S7.** Principal component analysis (PCA) (R2X (cum) = 0.714, Q2 = 0.572) and partial least square-discriminant analysis (PLS-DA) (R2X (cum) = 0.671, R2Y (cum) = 0.757, Q2 = 0.431) of bacterial pellet samples collected from inoculated spinach samples. *Note: the eclipse presented in Figure S4A and S4C represents the Hotelling's confidence limit (95%). The colored circles in panel “A” and “C” represent each analyzed samples, while the purple-colored stars in panel “B” and “D” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*



**Figure S8.** Principal component analysis (PCA) (R2X (cum) = 0.712, Q2 = 0.464) and partial least square-discriminant analysis (PLS-DA) (R2X (cum) = 0.52, R2Y (cum) = 0.746, Q2 = 0.329) of bacterial supernatant samples collected from inoculated spinach samples. *Note: the eclipse presented in Figure S5A and S5C represents the Hotelling's confidence limit (95%). The colored circles in panel “A” and “C” represent each analysed samples, while the purple-colored stars in panel “B” and “D” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*

**Figure S9**. Orthogonal Partial Least Square-Discriminant Analysis (A) scatter plot and (B) loading plot of bacterial pellet samples collected from inoculated spinach samples (n=58). R2X (cum) = 0.763, R2Y (cum) = 0.920, Q2 = 0.314. Note, the eclipse presented in Figure 4A represents the Hotelling's confidence limit (95%). ***Note:*** *The colored circles in panel “A” represent each analysed samples, while the purple-colored stars in panel “B” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*

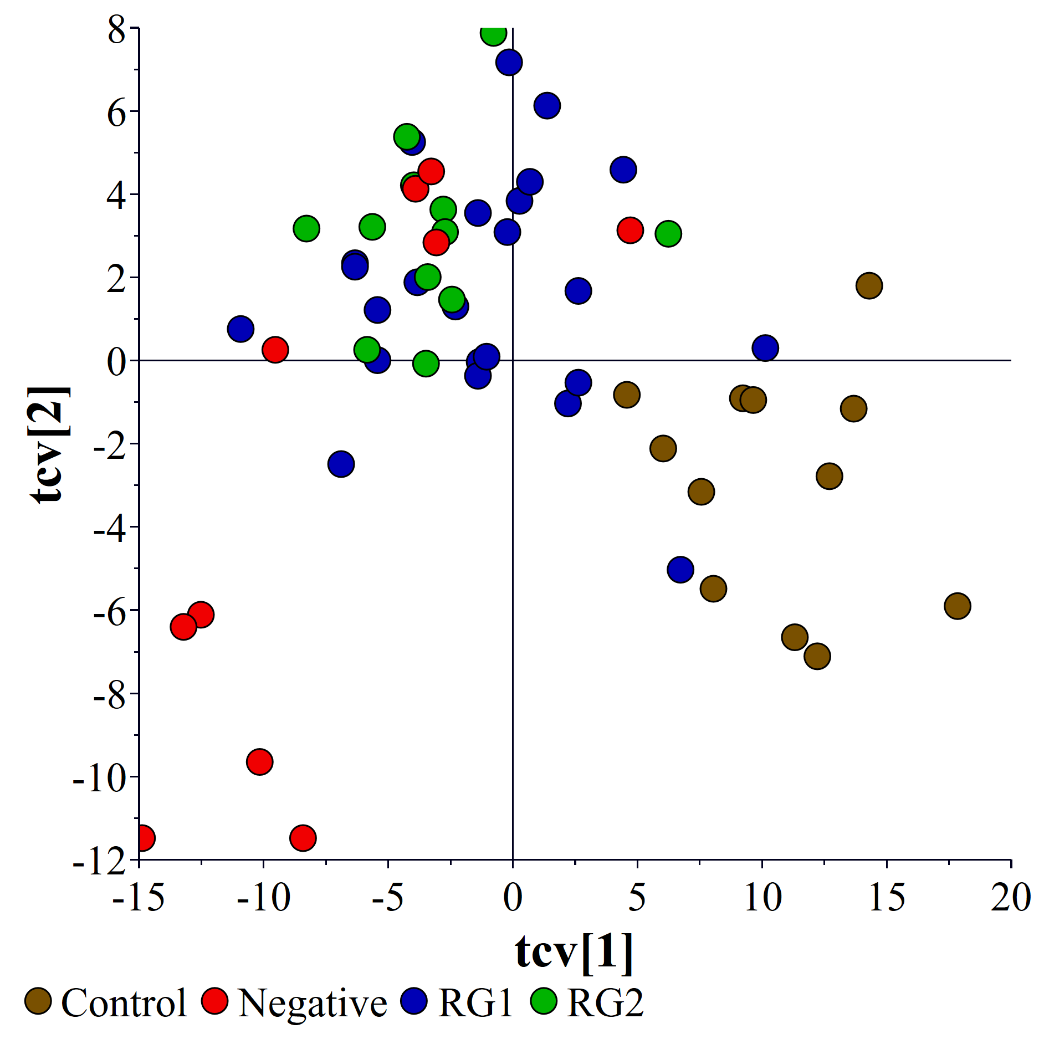


**Figure S10**. Orthogonal Partial Least Square-Discriminant Analysis (A) scatter plot and (B) loading plot of bacterial supernatant samples collected from inoculated spinach samples (n=58). R2X (cum) = 0.635, R2Y (cum) = 0.945, Q2 = 0.429. Note, the eclipse presented in Figure 5A represents the Hotelling's confidence limit (95%). ***Note:*** *The colored circles in panel “A” represent each analysed samples, while the purple-colored stars in panel “B” indicate the average group position for each sample cluster, with the white circles representing the distribution of metabolite features between these groups.*

**Table S7:** Cross validation (CV)-ANOVA of the OPLS-DA inoculated spinach pellet model.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **OPLS-DA (Inoculated spinach pellet model)** | **SS** | **DF** | **MS** | **F-test** | **P value** | **SD** |
| **Total corr.** | 171 | 171 | 1 |  |  | 1 |
| **Regression** | 79.5159 | 54 | 1.47252 | 1.88322 | 0.00234138 | 1.21347 |
| **Residual** | 91.4841 | 117 | 0.781916 |  |  | 0.88426 |

*In this table, SS: sum-of-squares, DF: degrees of freedom, MS: mean squares, SD: standard deviation*

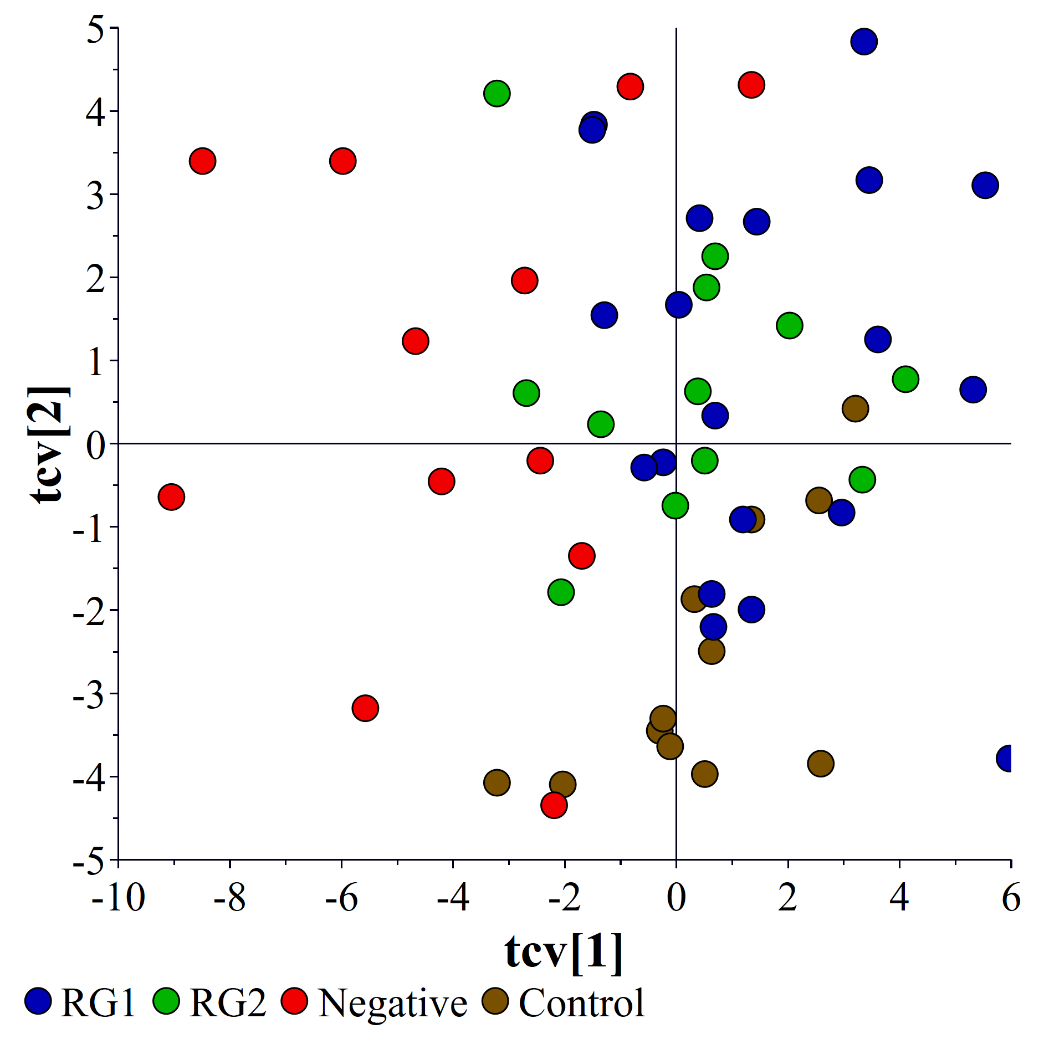
**

**Figure S11:** Cross Validation (CV) Scores plots of the OPLS-DA bacterial supernatant model. Note, the scatter plot of the cross-validated score vectors is analogous to the scatter plot of regular score vectors (Figure 1) but illustrates the sample stability of each point in relation to the other groups.

**Table S8:** Cross validation (CV)-ANOVA of the OPLS-DA inoculated spinach supernatant model.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **OPLS-DA (Inoculated spinach supernatant model)** | **SS** | **DF** | **MS** | **F-test** | **P value** | **SD** |
| **Total corr.** | 165 | 165 | 1 |  |  | 1 |
| **Regression** | 71.263 | 54 | 1.31969 | 1.56272 | 0.0246481 | 1.14878 |
| **Residual** | 93.737 | 111 | 0.844478 |  |  | 0.918955 |

*In this table, SS: sum-of-squares, DF: degrees of freedom, MS: mean squares, SD: standard deviation*

**

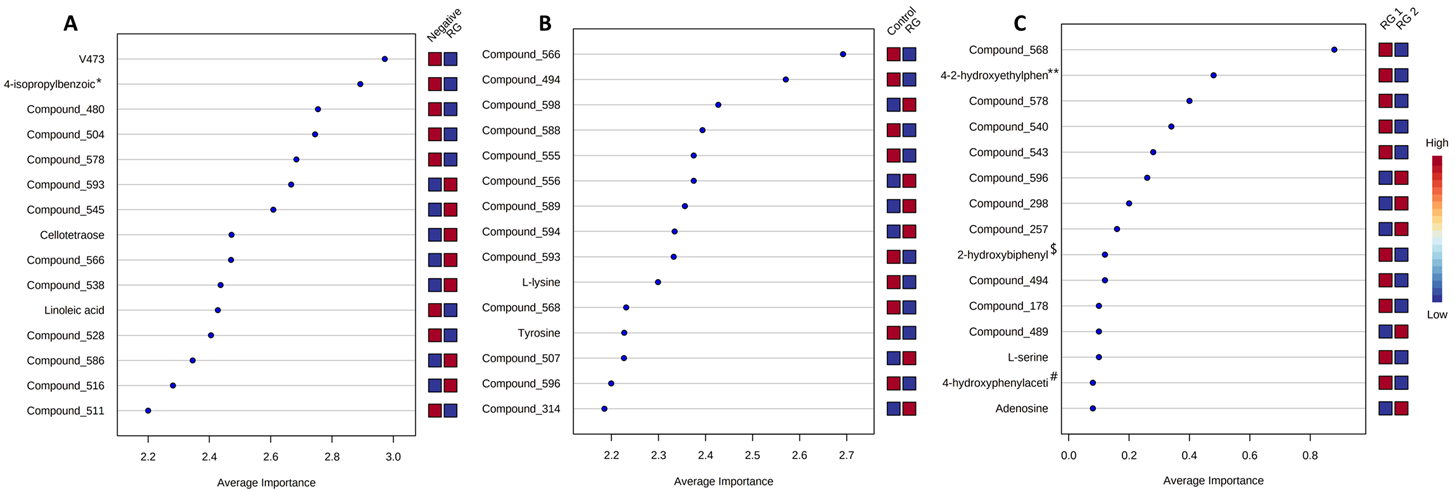
**Figure S12:** Cross Validation (CV) Scores plots of the OPLS-DA inoculated spinach supernatant model. Note, the scatter plot of the cross-validated score vectors is analogous to the scatter plot of regular score vectors (Figure 1) but illustrates the sample stability of each point in relation to the other groups.

**Table S9:** Significant metabolites identified in bacterial pellet from RG1-inoculated spinach samples.

| **Metabolites** | **Human Metabolome Database** | **KEGG** | **P value** | **Fold change** | **Regulation** |
| --- | --- | --- | --- | --- | --- |
| L-methionine | HMDB0000696 | C00073 | 0.0045 | 2.36097 | Up |
| 4-hydroxycinnamic acid | HMDB0002035 | C00811 | 0.01295 | 2.09518 | Up |
| Putrescine | HMDB0001414 | C00134 | 0.005317 | 0.490402 | Down |
| Citric acid | HMDB0000094 | C00158 | 0.012117 | 0.487528 | Down |
| L-threonine | HMDB0000167 | C00188 | 0.001047 | 0.483517 | Down |
| Inosine | HMDB0000195 | C00294 | 0.024845 | 0.46021 | Down |
| O-phosphocolamine | HMDB0000224 | C00346 | 0.049315 | 0.452313 | Down |
| Phosphoric acid | HMDB0002142 | C00009 | 0.011027 | 0.451882 | Down |
| Stearic acid | HMDB0000827 | C01530 | 0.000723 | 0.451536 | Down |
| L-glutamic acid | HMDB0000148 | C00025 | 0.000483 | 0.447883 | Down |
| Methyl Caprate | HMDB0033848 | No result | 0.002917 | 0.446061 | Down |
| Myristic acid | HMDB0000806 | C06424 | 0.000247 | 0.4452 | Down |
| Linoleic acid | HMDB0000673 | C01595 | 0.001348 | 0.439841 | Down |
| Methyl-beta-D-galactopyranoside | No result | C03619 | 0.000918 | 0.432168 | Down |
| Behenic acid | HMDB0000944 | C08281 | 0.004614 | 0.409336 | Down |
| Spermidine | HMDB0001257 | C00315 | 0.001029 | 0.409256 | Down |
| Acetol | HMDB0006961 | C05235 | 0.037705 | 0.402971 | Down |
| Glycerol 1-phosphate | No result | C03189 | 0.000989 | 0.390908 | Down |
| Methyl linolenate | No result | No result | 0.000984 | 0.388655 | Down |
| 3-phosphoglyceric acid | HMDB0060180 | C00197 | 0.049638 | 0.386142 | Down |
| L-proline | HMDB0000162 | C00148 | 0.001112 | 0.339837 | Down |
| Guanosine | HMDB0000133 | C00387 | 0.018204 | 0.307033 | Down |
| Hypoxanthine | HMDB0000157 | C00262 | 0.000188 | 0.303996 | Down |
| Adenosine | HMDB0000050 | C00212 | 0.004923 | 0.29823 | Down |
| Eicosane | HMDB0059909 | No result | 0.005486 | 0.280899 | Down |
| Methyl Palmitate | HMDB0061859 | C16995 | 0.000534 | 0.271417 | Down |
| 3,7-Dihydroxyflavone | HMDB0134547 | No result | 0.000689 | 0.266666 | Down |
| Aspartic acid | HMDB0000191 | C00049 | 0.000139 | 0.262345 | Down |
| Nitrazepam | HMDB0015534 | C07487 | 0.000594 | 0.254608 | Down |
| Phytol | No result | No result | 0.000145 | 0.254329 | Down |
| Adenine | HMDB0000034 | C00147 | 4.50E-05 | 0.252255 | Down |
| Cytosine | HMDB0000630 | C00380 | 0.009951 | 0.247596 | Down |
| Citraconic acid | HMDB0000749 | C01732 | 9.96E-06 | 0.243214 | Down |
| 5,6-dihydro-5-methyluracil | HMDB0000079 | C00906 | 0.000315 | 0.24311 | Down |
| N-methylalanine | HMDB0094692 | C02721 | 8.90E-06 | 0.237281 | Down |
| Epsilon-caprolactam | HMDB0062769 | C06593 | 1.54E-06 | 0.231002 | Down |
| 1-hexadecanol | HMDB0003424 | C00823 | 0.000528 | 0.230051 | Down |
| L-ornithine | HMDB0000214 | C00077 | 0.000239 | 0.230008 | Down |
| Lauric acid | HMDB0000638 | C02679 | 0.000154 | 0.226546 | Down |
| L-valine | HMDB0000883 | C00183 | 8.20E-08 | 0.226404 | Down |
| Cellotetraose | No result | C02013 | 1.23E-06 | 0.213537 | Down |
| L-lysine | HMDB0000182 | C00047 | 6.19E-06 | 0.213351 | Down |
| Methyl palmitoleate | No result | No result | 0.024343 | 0.20616 | Down |
| L-norleucine | HMDB0001645 | C01933 | 6.13E-08 | 0.171248 | Down |
| Tyrosine | HMDB0000158 | C00082 | 6.40E-08 | 0.161078 | Down |
| Xanthine | HMDB0000292 | C00385 | 4.85E-07 | 0.152953 | Down |
| L-tryptophan | HMDB0000929 | C00078 | 4.51E-07 | 0.090689 | Down |

**Table S10:** Significant metabolites identified in bacterial pellet from RG2-inoculated spinach samples.

| **Metabolites** | **Human Metabolome Database** | **KEGG** | **P value** | **Fold change** | **Regulation** |
| --- | --- | --- | --- | --- | --- |
| 4-hydroxyphenylacetic acid | HMDB0000020 | C00642 | 0.00289 | 0.250798 | Down |
| 5-aminovaleric acid | HMDB0003355 | C00431 | 5.33E-05 | 0.326284 | Down |
| Adenine | HMDB0000034 | C00147 | 1.49E-06 | 0.141199 | Down |
| Citric acid | HMDB0000094 | C00158 | 0.000386 | 0.269741 | Down |
| 1,3-diaminopropane | HMDB0000002 | C00986 | 0.000867 | 0.352664 | Down |
| Cytosine | HMDB0000630 | C00380 | 0.01771 | 0.122146 | Down |
| D-Ala-D-Ala2 | No result | No result | 2.89E-05 | 0.396973 | Down |
| Glycerol 1-phosphate | No result | C03189 | 3.64E-05 | 0.279513 | Down |
| Hypoxanthine | HMDB0000157 | C00262 | 0.007248 | 0.340268 | Down |
| Phenethylamine | HMDB0012275 | C05332 | 0.018288 | 0.483771 | Down |
| Phosphoric acid | HMDB0002142 | C00009 | 0.009625 | 0.411665 | Down |
| Putrescine | HMDB0001414 | C00134 | 1.24E-05 | 0.347272 | Down |
| Spermidine | HMDB0001257 | C00315 | 0.004649 | 0.418087 | Down |
| Xanthine | HMDB0000292 | C00385 | 1.43E-06 | 0.093152 | Down |
| 1-hexadecanol | HMDB0003424 | C00823 | 0.002572 | 0.157649 | Down |
| Lauric acid | HMDB0000638 | C02679 | 0.000927 | 0.122289 | Down |
| Thymidine | HMDB0000273 | C00214 | 0.033617 | 0.168474 | Down |
| Trans-4-hydroxy-L-proline | HMDB0000725 | C01157 | 0.041933 | 0.231242 | Down |
| L-serine | HMDB0000187 | C00065 | 0.003752 | 0.423077 | Down |
| Aspartic acid | HMDB0000191 | C00049 | 2.58E-05 | 0.140077 | Down |
| L-lysine | HMDB0000182 | C00047 | 3.27E-06 | 0.139814 | Down |
| Inosine | HMDB0000195 | C00294 | 0.001703 | 0.155599 | Down |
| Tyrosine | HMDB0000158 | C00082 | 2.29E-07 | 0.127933 | Down |
| L-methionine | HMDB0000696 | C00073 | 0.000106 | 3.08852 | Up |
| L-ornithine | HMDB0000214 | C00077 | 0.001884 | 0.117089 | Down |
| L-valine | HMDB0000883 | C00183 | 0.00029 | 0.34979 | Down |
| L-threonine | HMDB0000167 | C00188 | 3.12E-03 | 0.372341 | Down |
| L-tryptophan | HMDB0000929 | C00078 | 4.92E-05 | 0.062864 | Down |
| Guanosine | HMDB0000133 | C00387 | 0.000264 | 0.061949 | Down |
| 2-hydroxybiphenyl | HMDB0032582 | C02499 | 0.001607 | 0.193868 | Down |
| Epsilon-caprolactam | HMDB0062769 | C06593 | 2.43E-06 | 0.176559 | Down |
| Methyl Palmitate | HMDB0061859 | C16995 | 0.000227 | 0.130236 | Down |
| Behenic acid | HMDB0000944 | C08281 | 0.000872 | 0.258145 | Down |
| Eicosane | HMDB0059909 | No result | 0.0025 | 0.046264 | Down |
| Acetol | HMDB0006961 | C05235 | 0.02656 | 0.194565 | Down |
| Dioctyl phthalate | No result | C03690 | 3.28E-05 | 0.434437 | Down |
| Hexanoic acid | HMDB0000535 | C01585 | 0.002573 | 0.288155 | Down |
| Methyl linolenate | No result | No result | 0.000702 | 0.289092 | Down |
| 4-(2-hydroxyethyl)phenol | HMDB0004284 | C06044 | 6.63E-14 | 0.13254 | Down |
| Myristic acid | HMDB0000806 | C06424 | 0.000162 | 0.313572 | Down |
| Phendimetrazine | HMDB0015519 | No result | 0.000406 | 0.238234 | Down |
| L-norleucine | HMDB0001645 | C01933 | 2.32E-07 | 0.124477 | Down |
| L-glutamic acid | HMDB0000148 | C00025 | 4.98E-06 | 0.331559 | Down |
| Adenosine | HMDB0000050 | C00212 | 0.003319 | 0.14406 | Down |
| 5,6-dihydro-5-methyluracil | HMDB0000079 | C00906 | 0.000585 | 0.206062 | Down |
| Methyl-beta-D-galactopyranoside | No result | C03619 | 0.003075 | 0.342085 | Down |
| 3-Hydroxy-3',4'-Dimethoxyflavone | No result | No result | 0.028674 | 7.18358 | Up |
| L-proline | HMDB0000162 | C00148 | 4.40E-05 | 0.17678 | Down |
| 3-phosphoglyceric acid | HMDB0060180 | C00197 | 0.016312 | 0.262672 | Down |
| Cellotetraose | No result | C02013 | 3.89E-05 | 0.130264 | Down |
| 4-hydroxycinnamic acid | HMDB0002035 | C00811 | 0.000889 | 2.54912 | Up |
| Citraconic acid | HMDB0000749 | C01732 | 4.81E-06 | 0.212431 | Down |
| D-sphingosine | No result | C00319 | 0.002491 | 0.340146 | Down |
| Squalene | No result | No result | 0.016476 | 0.413256 | Down |
| Linoleic acid | HMDB0000673 | C01595 | 0.002483 | 0.36246 | Down |
| N-methylalanine | HMDB0094692 | C02721 | 0.000153 | 0.190999 | Down |
| 3,7-Dihydroxyflavone | HMDB0134547 | No result | 5.52E-05 | 0.032102 | Down |
| Phytol | No result | No result | 0.000137 | 0.111386 | Down |



**Figure S13**. The plots indicate the top 15 validated biomarkers, as analysed by PLS-DA classification and feature ranking through a Monte-Carlo cross-validation (MCVV) method. The comparisons consisted of (A) Negative vs RG and (B) Control vs RG and, (C) RG 1 vs RG 2. ***Note****: The symbols denote \*4-isopropyl benzoic acid, \*\*4-(2-hydroxyethyl)phenol, $2-hydroxybiphenyl and #4-hydroxyphenylacetic acid (A).* ***Note****: metabolites annotated “Compound\_###” are indicative of unknown compounds.*