**Metabolite profiling of the social spider** *Stegodyphus dumicola* **along a climate gradient**

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**Supplementary information**

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**Figure S1** – Heat map of the *S. dumicola* microbiome showing the 15 most abundant ASVs and their taxonomic classification across the three sampled populations. ASVs 3, 5, 6, and 13 could only be classified to family level (Weeksellaceae). Each panel represents a population, and each column shows the microbiome composition of one spider individual representing one nest within the population.

**Table S1** –Metadata from sampling trip February 2019. ID is the name of the sampled nest derived from the name of the sampling site. Latitude, longitude, time, and elevation were recorded with a Garmin eTrex H Handheld GPS Receiver (Garmin, USA). **T** is the ambient temperature at the time of sampling measured with a handheld thermometer next to the nest and **AV T** is the average temperature at the time of sampling for each population. **n** refers to the number of spiders sampled from the individual nest, **AV mind** is the average mass of an individual spider in a nest, and **AV mpop** is the average mass of an individual in a population. The standard error is presented in parentheses. A single factor ANOVA was conducted on average temperatures and average masses. Bold p-value indicate a significant difference between populations (ANOVA; p<0.05).

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Date** (dd-mm-yy) | **Site** | **Latitude** | **Longitude** | **Time** (hh:mm) | **Elevation** (m.a.s.l.) | **T** (°C) | **AV T** (±SE) | **n** | **AV mind** (mg) | **AV mpop**  (±SE) |
| O1 | 23-02-2019 | Otavi | -19.09760 | 17.22477 | 16:14 | 1184 | 43.3 | 43.3 (±0.3) | 26 | 101 | 108  (±26) |
| O2 | 23-02-2019 | Otavi | -19.09691 | 17.22669 | 16:53 | 1182 | 43.8 | 48 | 65 |
| O3 | 23-02-2019 | Otavi | -19.09071 | 17.22721 | 17:25 | 1182 | 42.7 | 12 | 157 |
| W1 | 12-02-2019 | Windhoek | -22.57488 | 17.21947 | 16:23 | 1953 | 37.5 | 37.5 (±0.0) | 55 | 73 | 76  (±6) |
| W2 | 12-02-2019 | Windhoek | -22.57412 | 17.21995 | 16:51 | 1944 | 37.6 | 52 | 87 |
| W3 | 12-02-2019 | Windhoek | -22.57405 | 17.22189 | 17:21 | 1948 | 37.5 | 56 | 66 |
| S1 | 15-02-2019 | Stampriet | -23.74118 | 18.19486 | 16:20 | 1283 | 39.4 | 40.2 (±0.5) | 69 | 57 | 71  (±12) |
| S2 | 15-02-2019 | Stampriet | -23.73767 | 18.19512 | 16:54 | 1287 | 41.4 | 37 | 88 |
| S3 | 15-02-2019 | Stampriet | -23.73767 | 18.19512 | 17:18 | 1287 | 39.2 | 51 | 93 |
| S4 | 15-02-2019 | Stampriet | -23.73119 | 18.19742 | 17:43 | 1285 | 40.8 | 58 | 45 |

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| --- | --- | --- | --- | --- | --- | --- |
| **ANOVA on**  **AV T\*** |  |  |  |  |  |  |
|  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *p-value* | *F crit* |
| Between Groups | 49.40 | 2 | 24.70 | 42.66 | **1.20x10-4** | 4.74 |
| Within Groups | 4.05 | 7 | 0.58 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 53.46 | 9 |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ANOVA on**  **AV mpop\*** |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *p-value* | *F crit* |
| Between Groups | 2572.56 | 2 | 1286.28 | 1.46 | **2.90x10-2** | 4.74 |
| Within Groups | 6159.30 | 7 | 879.90 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 8731.86 | 9 |  |  |  |  |

\*SS: sum of squares, df: degrees of freedom, MS: mean square value, F: F-ratio, F crit: critical value in the F-test.

**Table S2** – Metabolites identified by GC-MS. Fold change of metabolites is calculated from difference in relative abundance (based on nest averages) between the sampled populations: Otavi (O), Stampriet (S), and Windhoek (W). Color-coding indicates the direction of the difference (blue, lower relative abundance; red, higher relative abundance). Metabolites are sorted according to their retention time in minutes (**RT). RI** is the retention index calculated from the RT. **m/z value** is mass to charge value referring to the mass of the identified metabolite. **Match score** is the similarity (in %) of the measured mass spectrum with the mass spectrum of the library. The **p-value** and **FDR q-value** (the false discovery rate to correct for multiple comparisons) were calculated on average relative abundance, e.g. average of metabolite x in nest y, in the *post hoc* ANOVA. **Significant metabolites are shown in bold** (ANOVA; p<0.05). **VIP score** is thevariable importance parameter based on the PLS-DA modelindicating an individual metabolite’s weight in separating the populations (data not shown).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolite** | **Molecular formula** | **m/z value** | **RT (min)** | **RI** | **Match score (%)** | **Fold change** | | | **p-value** | **FDR q-value** | **VIP score** | **ID level\*** |
| **O vs S** | **O vs W** | **S vs W** |
| Lactate | C3H5O3- | 89.0700 | 8.60 | 851 | 91.54 | 1.61 | 1.51 | 0.94 | 5.95x10-2 | 2.61x10-1 | 2.21 | 1 |
| Alanine | C3H7NO2 | 89.0900 | 9.95 | 983 | 89.56 | 1.00 | 0.85 | 0.85 | 7.72x10-1 | 8.13x10-1 | 0.32 | 1 |
| Valine | C5H11NO2 | 117.1500 | 13.36 | 1318 | 87.28 | 0.81 | 0.96 | 1.18 | 4.93 x10-1 | 6.13x10-1 | 0.72 | 1 |
| Ethanolamine | C2H7NO | 61.0800 | 14.83 | 1462 | 84.72 | 1.28 | 1.30 | 1.02 | 1.25 x10-1 | 3.50x10-1 | 0.73 | 2 |
| Glycerol | C3H8O3 | 92.0900 | 15.13 | 1492 | 78.99 | 1.02 | 0.68 | 0.66 | 8.46x10-2 | 3.00x10-1 | 0.61 | 2 |
| Isoleucine | C6H13NO2 | 131.1700 | 15.60 | 1538 | 80.67 | 0.69 | 0.85 | 1.24 | 1.42x10-1 | 3.88x10-1 | 1.11 | 2 |
| Proline | C5H9NO2 | 115.1300 | 15.65 | 1543 | 78.07 | > 1c | 12.68 | < 1b | 1.31x10-2 | 1.19x10-1 | 1.23 | 1 |
| Glycine | C2H5NO2 | 75.0700 | 15.92 | 1569 | 89.82 | 1.14 | 1.14 | 1.01 | 6.72x10-1 | 7.24x10-1 | 0.93 | 1 |
| Uracil | C4H4N2O2 | 112.0868 | 16.78 | 1653 | 82.88 | 1.09 | > 1b | > 1b | 3.42x10-2 | 1.92x10-1 | 0.10 | 2 |
| Serine | C3H7NO3 | 105.0900 | 17.49 | 1723 | 89.66 | 0.75 | 0.79 | 1.05 | 3.62x10-1 | 5.71x10-1 | 1.54 | 2 |
| Threonine | C4H9NO3 | 119.1200 | 18.16 | 1789 | 89.20 | 0.60 | 0.84 | 1.40 | 4.17x10-2 | 2.11x10-1 | 1.77 | 2 |
| Malate | C4H4O5-2 | 132.0700 | 20.82 | 2049 | 89.79 | 1.45 | 1.14 | 0.79 | 4.16x10-2 | 2.11x10-1 | 0.98 | 1 |
| Methionine | C5H11NO2S | 149.2100 | 21.47 | 2113 | 69.56 | 0.59 | 0.58 | 0.98 | 3.61x10-3 | 6.32x10-2 | 1.46 | 1 |
| Aspartic acid | C4H7NO4 | 133.1100 | 21.58 | 2124 | 88.08 | 0.90 | 1.21 | 1.35 | 9.82x10-1 | 9.92x10-1 | 0.20 | 2 |
| 4-Aminobutyric acid (GABA) | C4H9NO2 | 103.1198 | 21.76 | 2142 | 81.49 | 0.35 | > 1b | > 1b | 8.25x10-1 | 8.51x10-1 | 0.28 | 2 |
| Glutamic acid | C5H9NO4 | 147.1293 | 21.85 | 2150 | 77.02 | < 1a | 0.00 | > 1b | 3.67x10-3 | 6.32x10-2 | 0.90 | 2 |
| Pipecolic acid | C6H11NO2 | 129.1570 | 23.36 | 2298 | 72.38 | 0.45 | 0.61 | 1.36 | 1.62x10-1 | 4.29x10-1 | 1.49 | 2 |
| 5-Hydroxypipecolic acid | C6H11NO3 | 145.1600 | 23.51 | 2313 | 71.45 | 0.00 | 0.00 | 0.30 | 2.60x10-1 | 5.24x10-1 | 0.47 | 2 |
| Glutamate | C5H9NO4 | 147.1293 | 23.97 | 2358 | 75.90 | 1.32 | 1.30 | 0.98 | 1.01x10-2 | 1.02x10-1 | 2.83 | 1 |
| Taurine | C2H7NO3S | 125.1469 | 24.88 | 2447 | 84.89 | 1.01 | 1.32 | 1.30 | 3.86x10-1 | 5.99x10-1 | 0.84 | 2 |
| Asparagine | C4H8N2O3 | 132.1179 | 25.03 | 2462 | 65.15 | 0.33 | 0.23 | 0.69 | 1.20x10-1 | 3.50x10-1 | 1.15 | 1 |
| Ribose | C5H10O5 | 150.1299 | 25.28 | 2487 | 77.30 | 0.36 | > 1b | > 1b | 1.75x10-1 | 4.32x10-1 | 0.63 | 2 |
| Sorbitol | C6H14O6 | 182.1700 | 25.70 | 2528 | 74.56 | 0.00 | 0.00 | 0.12 | 5.81x10-1 | 6.45x10-1 | 0.13 | 2 |
| Putrescine | C4H12N2 | 88.1515 | 26.49 | 2605 | 78.91 | 1.13 | 0.40 | 0.36 | 5.12x10-1 | 6.13x10-1 | 0.09 | 1 |
| Ciliatine | C2H8NO3P | 125.0636 | 26.57 | 2613 | 79.34 | 0.00 | < 1a | < 1c | 5.28x10-1 | 6.13x10-1 | 0.03 | 2 |
| Glycerol-1-phosphate | C3H9O6P | 172.0737 | 27.11 | 2666 | 84.13 | 0.75 | 0.49 | 0.65 | 2.98x10-2 | 1.88x10-1 | 0.43 | 1 |
| **3,4-Dihydroxyphenylglycol** | **C8H10O4** | **170.1626** | **27.32** | **2687** | **76.01** | **0.39** | **0.38** | **0.99** | **5.52x10-5** | **5.58x10-3** | **2.26** | **2** |
| Phosphorylethanolamine | C2H8NO4P | 141.0630 | 27.47 | 2701 | 78.37 | 0.70 | 0.84 | 1.19 | 9.06x10-2 | 3.05x10-1 | 0.91 | 1 |
| Hypoxanthine | C5H4N4O | 136.1115 | 27.89 | 2743 | 83.46 | 2.48 | 2.81 | 1.13 | 1.41x10-2 | 1.19x10-1 | 1.56 | 1 |
| Ornithine | C5H12N2O2 | 132.1610 | 28.24 | 2777 | 82.94 | 0.00 | < 1a | < 1c | 5.28x10-1 | 6.13x10-1 | 0.04 | 2 |
| Citric acid | C6H8O7 | 192.1235 | 28.30 | 2783 | 85.04 | 2.18 | 0.42 | 0.19 | 4.09x10-1 | 6.13x10-1 | 0.83 | 1 |
| D-Pinitol | C7H14O6 | 194.1825 | 28.50 | 2802 | 80.67 | < 1a | 0.00 | > 1b | 6.75x10-2 | 2.73x10-1 | 1.45 | 2 |
| Cadaverine | C5H14N2 | 102.1781 | 28.59 | 2811 | 65.08 | 0.00 | 0.00 | 1.69 | 3.44x10-1 | 5.61x10-1 | 0.78 | 1 |
| Quinic acid | C7H12O6 | 192.1666 | 29.11 | 2862 | 69.36 | 1.07 | > 1b | > 1b | 4.40x10-1 | 6.13x10-1 | 0.21 | 2 |
| Sedoheptulose | C7H14O7 | 210.1819 | 29.68 | 2918 | 81.83 | 1.28 | 1.26 | 0.98 | 9.00x10-1 | 9.18x10-1 | 0.10 | 2 |
| Mannose | C6H12O6 | 180.1600 | 29.80 | 2930 | 90.41 | 2.12 | 2.85 | 1.34 | 3.92x10-1 | 5.99x10-1 | 0.42 | 2 |
| Glucose | C6H12O6 | 180.1559 | 29.89 | 2939 | 81.19 | 0.98 | 1.13 | 1.16 | 6.79x10-1 | 7.24x10-1 | 0.61 | 1 |
| Lysine | C6H14N2O2 | 146.1876 | 30.39 | 2988 | 81.90 | 0.78 | 1.04 | 1.34 | 4.25x10-1 | 6.13x10-1 | 1.09 | 1 |
| Tyrosine | C9H11NO3 | 181.1885 | 30.68 | 3016 | 80.11 | 0.59 | 0.84 | 1.42 | 9.87x10-3 | 1.02x10-1 | 3.18 | 1 |
| Allo inositol | C6H12O6 | 612.3000 | 31.02 | 3049 | 83.03 | 0.00 | 0.00 | 5.45 | 6.92x10-3 | 8.74x10-2 | 1.96 | 2 |
| Galactopyranose | C6H12O6 | 180.1559 | 31.57 | 3103 | 85.63 | 1.71 | 2.18 | 1.28 | 2.18x10-1 | 4.89x10-1 | 0.43 | 2 |
| Pyridoxine | C8H11NO3 | 169.1778 | 31.79 | 3125 | 70.04 | < 1a | 0.00 | > 1b | 3.32x10-2 | 1.92x10-1 | 1.01 | 2 |
| Gluconic acid | C6H12O7 | 196.1553 | 31.87 | 3133 | 90.57 | 0.25 | 1.48 | 5.93 | 4.56x10-1 | 6.13x10-1 | 1.17 | 1 |
| Scyllo-inositol | C6H12O6 | 180.1559 | 32.43 | 3188 | 84.25 | 0.78 | 1.25 | 1.61 | 2.65x10-2 | 1.78x10-1 | 1.09 | 2 |
| Palmitic acid | C16H32O2 | 256.4241 | 32.80 | 3224 | 82.40 | 1.22 | 0.93 | 0.76 | 8.16x10-2 | 3.00x10-1 | 1.04 | 2 |
| Myo-inositol | C6H12O6 | 180.1559 | 33.57 | 3299 | 88.09 | 3.69 | 3.05 | 0.83 | 3.33x10-1 | 5.61x10-1 | 2.51 | 2 |
| Uric acid | C5H4N4O3 | 168.1103 | 33.77 | 3319 | 79.98 | 0.49 | 0.04 | 0.09 | 1.23x10-1 | 3.50x10-1 | 0.30 | 1 |
| Tryptophan | C11H12N2O2 | 204.2252 | 35.27 | 3466 | 70.56 | 1.04 | > 1b | > 1b | 5.01x10-2 | 2.30x10-1 | 0.28 | 2 |
| Linoleic acid | C18H32O2 | 280.4455 | 35.29 | 3468 | 61.21 | 0.49 | 0.05 | 0.09 | 3.76x10-3 | 6.32x10-2 | 0.18 | 1 |
| Oleic acid | C18H34O2 | 282.4614 | 35.36 | 3475 | 74.83 | 1.05 | 0.87 | 0.83 | 7.02x10-2 | 2.73x10-1 | 1.08 | 2 |
| Stearic acid | C18H36O2 | 284.4772 | 35.64 | 3502 | 68.44 | 0.86 | 0.68 | 0.79 | 3.68x10-3 | 6.32x10-2 | 0.12 | 2 |
| Spermidine | C7H19N3 | 145.2459 | 35.70 | 3508 | 77.11 | 1.23 | 2.11 | 1.72 | 8.63x10-2 | 3.00x10-1 | 0.72 | 2 |
| Fructose-6-phosphat | C6H13O9P | 260.1358 | 36.17 | 3554 | 71.34 | 0.00 | < 1a | < 1c | 3.20x10-1 | 5.61x10-1 | 0.07 | 2 |
| Glucose-6-phosphat | C6H13O9P | 260.1358 | 36.26 | 3563 | 83.23 | > 1c | 0.71 | < 1b | 1.02x10-1 | 3.11x10-1 | 0.84 | 2 |
| Myo-Inositol phosphate | C6H13O9P | 260.1358 | 36.94 | 3630 | 64.22 | 1.18 | 0.86 | 0.73 | 4.71x10-3 | 6.79x10-2 | 1.40 | 2 |
| Uridine | C9H12N2O6 | 244.2014 | 37.14 | 3649 | 60.14 | 0.96 | 0.83 | 0.86 | 1.69x10-1 | 4.29x10-1 | 0.24 | 2 |
| Inosine | C10H12N4O5 | 268.2261 | 37.85 | 3719 | 86.13 | 0.97 | 0.99 | 1.02 | 9.94x10-1 | 9.94x10-1 | 0.35 | 2 |
| Melezitose | C18H32O16 | 504.4371 | 38.64 | 3796 | 75.74 | 0.80 | 1.86 | 2.33 | 4.48x10-1 | 6.13x10-1 | 0.73 | 1 |
| Trehalose | C12H22O11 | 342.2965 | 38.74 | 3806 | 63.93 | 0.94 | 1.56 | 1.67 | 2.35x10-1 | 4.98x10-1 | 0.32 | 2 |

\* According to Sumner et al. (2007): 1 = verified by authentic standard, 2 = putatively annotated compounds verified by MS spectral libraries. **a**Not identified in Otavi, **b**not identified in Windhoek or **c**not identified in Stampriet and hence not possible to calculate a fold difference.

**Table S3** – Metabolites identified by LC-MS (both positive and negative mode). Fold change of metabolites is calculated from difference in relative abundance (based on nest averages) between the sampled populations: Otavi (O), Stampriet (S), and Windhoek (W). Color-coding indicates the direction of the difference (blue, lower relative abundance; red, higher relative abundance). Metabolites are sorted alphabetically. **Mode** refers to the operating mode of the mass spectrometer. **m/z value** is mass to charge value referring to the mass of the identified metabolite. **RT** is the retention time in seconds. The **p-value** and **FDR q-value** (the false discovery rate to correct for multiple comparisons) were calculated on average relative abundance, e.g. average of metabolite x in nest y, in the *post hoc* ANOVA. **Significant metabolites are shown in bold** (ANOVA; p<0.05). **VIP score** is thevariable importance parameter based on the PLS-DA modelindicating an individual metabolite’s weight in separating the populations (data not shown).

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolite** | **Mode** | **Molecular formula** | **m/z value** | **RT (s)** | **Fold change** | | | **p-value** | **FDR q-value** | **VIP score** | **ID level\*** |
| **O vs S** | **O vs W** | **S vs W** |
| 2'-Deoxycytidine 5'-monophosphate (dCMP) | Negative | C9H14N3O7P | 306.0499 | 59 | 2.13 | 1.94 | 0.91 | 3.26x10-3 | 4.79x10-2 | 0.70 | 1 |
| 6-Phosphogluconic acid | Negative | C6H13O10P | 275.0173 | 37 | 0.51 | 0.41 | 0.80 | 9.66x10-3 | 7.57x10-2 | 0.94 | 1 |
| Acetyl carnitine | Positive | C9H17NO4 | 204.1230 | 69 | 0.87 | 1.73 | 1.99 | 3.12x10-1 | 4.62x10-1 | 2.44 | 1 |
| Adenine | Positive | C5H5N5 | 136.0610 | 59 | 0.55 | 1.15 | 2.07 | 2.82x10-1 | 4.39x10-1 | 0.60 | 1 |
| Adenosine | Negative | C10H13N5O4 | 266.0898 | 213 | 2.81 | 1.84 | 0.65 | 1.55x10-2 | 9.40x10-2 | 0.46 | 1 |
| Alanine | Positive | C3H7NO2 | 90.0545 | 35 | 0.98 | 0.82 | 0.84 | 4.16x10-1 | 5.37x10-1 | 1.50 | 1 |
| Asparagine | Negative | C4H8N2O3 | 133.0599 | 35 | 0.57 | 0.40 | 0.70 | 8.34x10-3 | 7.12x10-2 | 1.32 | 1 |
| Positive | C4H8N2O3 | 131.0456 | 34 | 0.50 | 0.44 | 0.87 | 4.86x10-2 | 1.70x10-1 | 1.31 | 1 |
| Butanoyl carnitine | Positive | C11H21NO4 | 232.1533 | 244 | 1.58 | 2.98 | 1.88 | 8.11x10-1 | 8.56x10-1 | 0.21 | 1 |
| Carnitine | Positive | C7H15NO3 | 162.1121 | 37 | 1.39 | 2.07 | 1.49 | 1.28x10-1 | 2.82x10-1 | 4.24 | 1 |
| Choline | Positive | C5H14NO+ | 104.1069 | 35 | 0.87 | 0.76 | 0.88 | 4.09x10-2 | 1.57x10-1 | 4.62 | 1 |
| Citric acid | Negative | C6H8O7 | 191.0199 | 74 | 1.47 | 0.65 | 0.44 | 1.99x10-1 | 3.45x10-1 | 3.37 | 1 |
| Flavin mononucleotide (FMN) | Negative | C17H21N4O9P | 455.0978 | 301 | 0.43 | 0.90 | 2.09 | 5.72x10-2 | 1.74x10-1 | 0.01 | 1 |
| Fructose/Glucose† | Positive | C6H12O6 | 203.0519 | 37 | 1.73 | 1.80 | 1.04 | 3.39x10-1 | 4.80x10-1 | 1.52 | 2 |
| Fumarate | Negative | C4H4O4 | 115.0033 | 86 | 1.43 | 1.22 | 0.85 | 4.17x10-1 | 5.37x10-1 | 0.49 | 1 |
| **Glutamate** | **Positive** | **C5H9NO4** | 148.0598 | **36** | **1.42** | **1.59** | **1.12** | **1.82x10-3** | **3.63x10-2** | **2.83** | **1** |
| **Glutamine** | **Negative** | **C5H10N2O3** | 147.0757 | **36** | **0.44** | **0.25** | **0.58** | **1.17x10-5** | **9.61x10-3** | **2.43** | **1** |
| **Positive** | **C5H10N2O3** | 145.0616 | **35** | **0.43** | **0.35** | **0.81** | **3.42x10-6** | **1.22x10-3** | **3.00** | **1** |
| Glutathione, oxidized | Positive | C20H32N6O12S2 | 613.1598 | 139 | 0.19 | 0.27 | 1.38 | 1.96x10-2 | 1.05x10-1 | 1.28 | 1 |
| Glutathione, reduced | Negative | C10H17N3O6S | 308.0908 | 73 | 0.52 | 0.49 | 0.94 | 9.60x10-2 | 2.27x10-1 | 1.07 | 1 |
| Glutathione, reduced | Positive | C10H17N3O6S | 306.0769 | 72 | 0.48 | 0.44 | 0.92 | 1.04x10-1 | 2.49x10-1 | 1.28 | 1 |
| Glycerate | Negative | C3H6O4 | 105.0187 | 42 | 1.44 | 0.52 | 0.36 | 5.76x10-2 | 1.74x10-1 | 1.19 | 1 |
| Glycerol 3-phosphate | Negative | C3H9O6P | 171.0064 | 36 | 2.05 | 2.71 | 1.32 | 6.53x10-2 | 1.87x10-1 | 1.68 | 1 |
| Glycerophosphorylcholine | Positive | C8H20NO6P | 258.1105 | 36 | 1.69 | 1.11 | 0.66 | 6.70x10-2 | 1.99x10-1 | 1.46 | 1 |
| Glycine | Positive | C2H5NO2 | 76.0385 | 34 | 0.98 | 0.84 | 0.86 | 3.26x10-1 | 4.69x10-1 | 0.56 | 1 |
| Guanine | Negative | C5H5N5O | 152.0566 | 61 | 1.22 | 1.32 | 1.08 | 4.91x10-2 | 1.63x10-1 | 2.67 | 1 |
| Positive | C5H5N5O | 150.0422 | 60 | 1.31 | 1.53 | 1.17 | 6.40x10-2 | 1.95x10-1 | 6.45 | 1 |
| Guanosine-5'-monophosphate (GMP) | Positive | C10H14N5O8P | 364.0647 | 72 | 1.24 | 3.53 | 2.84 | 1.45x10-1 | 3.05x10-1 | 1.00 | 1 |
| Histidine | Positive | C6H9N3O2 | 156.0759 | 33 | 0.31 | 0.68 | 2.18 | 5.47x10-2 | 1.81x10-1 | 0.65 | 1 |
| **Hypoxanthine** | **Positive** | **C5H4N4O** | 137.0454 | **81** | **2.09** | **2.23** | **1.06** | **2.84x10-4** | **1.97x10-2** | **6.36** | **1** |
| Lactate | Negative | C3H6O3 | 89.0240 | 65 | 1.47 | 1.35 | 0.92 | 1.21x10-1 | 2.61x10-1 | 3.31 | 1 |
| Leucine | Positive | C6H13NO2 | 132.1015 | 126 | 0.56 | 0.70 | 1.25 | 3.39x10-2 | 1.43x10-1 | 2.52 | 2 |
| Negative | C6H13NO2 | 130.0875 | 125 | 0.61 | 0.81 | 1.32 | 8.21x10-2 | 2.10x10-1 | 0.52 | 1 |
| Lysophosphatidylcholine; 16:0 | Positive | C24H50NO7P | 496.3407 | 662 | 1.10 | 0.43 | 0.39 | 1.60x10-1 | 3.25x10-1 | 3.29 | 1 |
| Lysophosphatidylcholine; 18:0 | Positive | C26H54NO7P | 524.3708 | 716 | 1.16 | 0.44 | 0.38 | 6.94x10-2 | 2.01x10-1 | 3.57 | 1 |
| Malate | Negative | C4H6O5 | 133.0139 | 49 | 1.24 | 1.07 | 0.86 | 3.21x10-1 | 4.59x10-1 | 0.44 | 1 |
| Malonic Acid | Negative | C3H4O4 | 103.0031 | 54 | 1.75 | 0.86 | 0.49 | 1.53x10-1 | 2.99x10-1 | 0.34 | 1 |
| Maltose | Negative | C12H22O11 | 341.1094 | 42 | 1.39 | 1.76 | 1.26 | 2.23x10-1 | 3.71x10-1 | 1.81 | 1 |
| Methionine | Positive | C5H11NO2S | 150.0578 | 68 | 0.72 | 0.80 | 1.11 | 7.60x10-2 | 2.12x10-1 | 1.31 | 1 |
| Methylthioadenosine (MTA) | Positive | C11H15N5O3S | 298.0959 | 270 | 0.51 | 0.58 | 1.13 | 2.89x10-2 | 1.32x10-1 | 0.42 | 1 |
| N-acetyl-DL-methionine | Negative | C7H13NO3S | 190.0547 | 277 | 0.68 | 0.81 | 1.20 | 2.62x10-2 | 1.17x10-1 | 0.47 | 1 |
| Pantothenic acid | Positive | C9H17NO5 | 220.1173 | 247 | 0.93 | 1.22 | 1.32 | 3.73x10-1 | 5.05x10-1 | 0.32 | 1 |
| Phenylalanine | Positive | C9H11NO2 | 166.0859 | 224 | 0.62 | 0.79 | 1.28 | 6.50x10-3 | 5.90x10-2 | 1.96 | 1 |
| Phosphorylcholine | Positive | C5H14NO4P | 184.0725 | 35 | 1.45 | 1.86 | 1.28 | 1.40x10-2 | 9.09x10-2 | 2.06 | 2 |
| Proline | Positive | C5H9NO2 | 116.0701 | 42 | 1.32 | 0.90 | 0.68 | 4.84x10-1 | 5.87x10-1 | 0.79 | 1 |
| Proline-betaine | Positive | C7H13NO2 | 144.1015 | 45 | 0.06 | 1.51 | 27.02 | 2.57x10-2 | 1.22x10-1 | 1.40 | 1 |
| **Propionyl carnitine** | **Positive** | **C10H19NO4** | 218.1381 | **160** | **0.77** | **1.91** | **2.48** | **9.49x10-5** | **1.03x10-2** | **0.76** | **1** |
| **Riboflavin** | **Positive** | **C17H20N4O6** | 377.1455 | **321** | **0.83** | **1.06** | **1.28** | **1.69x10-6** | **8.40x10-4** | **0.97** | **1** |
| Serine | Positive | C3H7NO3 | 106.0492 | 34 | 0.72 | 0.73 | 1.01 | 4.08x10-2 | 1.57x10-1 | 1.36 | 1 |
| Succinate | Negative | C4H6O4 | 117.0189 | 98 | 1.70 | 1.24 | 0.73 | 3.68x10-2 | 1.39x10-1 | 0.88 | 1 |
| Succinyladenosine | Negative | C14H17N5O8 | 384.1148 | 248 | 2.34 | 1.32 | 0.56 | 3.98x10-2 | 1.46x10-1 | 0.75 | 1 |
| Positive | C14H17N5O8 | 382.1010 | 247 | 2.20 | 1.27 | 0.57 | 2.37x10-1 | 4.06x10-1 | 0.05 | 1 |
| Taurine | Positive | C2H7NO3S | 126.0214 | 34 | 1.09 | 1.24 | 1.13 | 8.34x10-2 | 2.19x10-1 | 2.00 | 1 |
| Threonine | Positive | C4H9NO3 | 120.0647 | 36 | 0.62 | 0.71 | 1.14 | 1.91x10-2 | 1.04x10-1 | 1.22 | 1 |
| Tryptophan | Positive | C11H12N2O2 | 205.0967 | 261 | 1.12 | 2.11 | 1.88 | 7.94x10-2 | 2.15x10-1 | 2.91 | 1 |
| Tyrosine | Positive | C9H11NO3 | 182.0803 | 120 | 0.56 | 0.96 | 1.72 | 7.60x10-3 | 6.25x10-2 | 0.30 | 1 |
| Uric acid | Positive | C5H4N4O3 | 169.0348 | 74 | 1.33 | 0.25 | 0.19 | 8.41x10-2 | 2.20x10-1 | 2.17 | 1 |
| Uridine | Negative | C9H12N2O6 | 245.0761 | 126 | 0.73 | 0.69 | 0.96 | 6.68x10-3 | 6.57x10-2 | 1.85 | 1 |
| Positive | C9H12N2O6 | 243.0627 | 116 | 0.74 | 0.69 | 0.94 | 6.40x10-2 | 1.95x10-1 | 0.80 | 1 |
| Uridine 5′-monophosphate (UMP) | Negative | C9H13N2O9P | 323.0289 | 54 | 0.65 | 0.56 | 0.85 | 2.79x10-2 | 1.21x10-1 | 1.47 | 1 |
| Valine | Positive | C5H11NO2 | 118.0856 | 56 | 0.94 | 0.75 | 0.80 | 6.83x10-2 | 2.00x10-1 | 3.65 | 1 |
| Xanthine | Negative | C5H4N4O2 | 151.0262 | 111 | 5.12 | 3.07 | 0.60 | 9.27x10-2 | 2.24x10-1 | 0.83 | 1 |
| Positive | C5H4N4O2 | 153.0398 | 97 | 3.80 | 2.21 | 0.58 | 4.27x10-2 | 1.61x10-1 | 0.98 | 1 |
| **Xanthosine** | **Negative** | **C10H12N4O6** | 283.0692 | **220** | **3.97** | **1.95** | **0.49** | **3.18x10-4** | **1.77x10-2** | **1.10** | **1** |
| Xanthurenic acid | Negative | C10H7NO4 | 204.0305 | 269 | 0.76 | 0.99 | 1.29 | 3.68x10-1 | 4.99x10-1 | 0.19 | 1 |
| Positive | C10H7NO4 | 206.0439 | 269 | 0.69 | 0.93 | 1.34 | 3.17x10-1 | 4.65x10-1 | 0.23 | 1 |

\* According to Sumner et al. (2007): 1 = verified by authentic standard, 2 = putatively annotated compounds verified by MS spectral libraries. † Fructose/Glucose cannot be separated as fructose and glucose have the same retention time (RT) and fragments used for identification.