**Table S3.** Differential metabolites between evolved strain and ancestral strain before and after novobiocin treatment and in the presence or absence of novobiocin.

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| --- | --- | --- | --- | --- | --- |
| Strain | Compound Name | FC(Treatment/Control) | Log2FC | Regulation | P\_val |
| ancestral\_NV\_vs\_ancestral\_UT | (S)-2-Acetolactate | 3.33 | 1.74 | up | 0.038325 |
| (S)-3-Methyl-2-oxopentanoic acid | 0.64 | -0.64 | down | 0.014424 |
| 2-Phospho-D-glycerate | 0.23 | -2.12 | down | 0.04866 |
| 3-Hydroxy-3-methyl-2-oxobutanoic acid | 3.33 | 1.74 | up | 0.038325 |
| Acetylornithine | 5.41 | 2.44 | up | 0.006691 |
| Aminocyclopropane carboxylate | 2.33 | 1.22 | up | 0.00573 |
| CAIR | 1.93 | 0.95 | up | 0.027978 |
| CDP | 4.04 | 2.01 | up | 0.000323 |
| cis-2-Methylaconitate | 8.63 | 3.11 | up | 0.003494 |
| Citrulline | 1.78 | 0.83 | up | 0.048602 |
| coenzyme A | 2.88 | 1.53 | up | 0.017958 |
| CTP | 4.78 | 2.26 | up | 0.024561 |
| dCDP | 2.11 | 1.08 | up | 0.007997 |
| Deoxyinosine | 1.52 | 0.6 | up | 0.012378 |
| dephospho-coenzyme A | 0.23 | -2.12 | down | 0.029791 |
| Dimethymalate | 0.36 | -1.47 | down | 0.001429 |
| dTDP | 2.38 | 1.25 | up | 0.006534 |
| dTMP | 5.87 | 2.55 | up | 0.028808 |
| FAICAR | 3.66 | 1.87 | up | 0.006794 |
| GABA | 1.87 | 0.9 | up | 0.005099 |
| Glutamate | 1.93 | 0.95 | up | 0.010408 |
| Glutamine | 1.75 | 0.81 | up | 0.022512 |
| Guanosine | 0.56 | -0.84 | down | 0.00742 |
| Histidine | 2.12 | 1.08 | up | 0.012292 |
| Methylmaleate | 2.48 | 1.31 | up | 0.004505 |
| N6-(1,2-Dicarboxyethyl)-AMP | 1.66 | 0.73 | up | 0.031872 |
| oxo-Proline | 2.37 | 1.24 | up | 0.005184 |
| PEP | 0.23 | -2.12 | down | 0.001575 |
| phosphopantothenate | 2.65 | 1.41 | up | 0.043919 |
| Ribitol | 0.59 | -0.76 | down | 0.049743 |
| Thymine | 2.04 | 1.03 | up | 0.010096 |
| UDP | 3.91 | 1.97 | up | 0.001757 |
| UDP-D-galactose | 4.65 | 2.22 | up | 0.006615 |
| UDP-GlcNAc | 2.9 | 1.54 | up | 0.009762 |
| UDP-glucose | 4.65 | 2.22 | up | 0.006615 |
| Uracil | 1.54 | 0.62 | up | 0.029953 |
| 3-PA | 0.21 | -2.25 | down | 0.048583 |
| N10\_NV\_vs\_ancestral\_NV | (R)-3-Hydroxy-3-methyl-2-oxopentanoate | 2.04 | 1.03 | up | 0.000203 |
| (S)-2-Aceto-2-hydroxybutanoate | 2.04 | 1.03 | up | 0.000203 |
| (S)-2-Acetolactate | 0.31 | -1.69 | down | 0.003165 |
| (S)-3-Methyl-2-oxopentanoic acid | 0.47 | -1.09 | down | 0.035108 |
| 2-dehydropantoate | 2.04 | 1.03 | up | 0.000203 |
| 2-Deoxy-D-ribose 1-phosphate | 0.44 | -1.18 | down | 0.025167 |
| 3-Hydroxy-3-methyl-2-oxobutanoic acid | 0.31 | -1.69 | down | 0.003165 |
| 5,6,7,8-Tetrahydrofolate | 5.17 | 2.37 | up | 0.049394 |
| 5-Methylthioadenosine | 0.48 | -1.06 | down | 0.041177 |
| 6-Phospho-D-gluconate | 2.69 | 1.43 | up | 0.007156 |
| Acetylaminobutanal | 1.71 | 0.77 | up | 0.003097 |
| Acetylornithine | 1.51 | 0.59 | up | 0.017853 |
| alpha-D-Glucose | 2.22 | 1.15 | up | 0.014287 |
| alpha-Isopropylmalate | 0.53 | -0.92 | down | 0.017553 |
| Arginine | 9.27 | 3.21 | up | 0.000222 |
| beta-D-Glucose6-phosphate | 2.53 | 1.34 | up | 0.001473 |
| Biotin | 5.08 | 2.34 | up | 0.001075 |
| Carbamoyl-L-aspartate | 0.62 | -0.69 | down | 0.014428 |
| CDP | 0.39 | -1.36 | down | 0.000491 |
| cis-2-Methylaconitate | 0.36 | -1.47 | down | 0.008857 |
| Citrulline | 0.62 | -0.69 | down | 0.001582 |
| CTP | 0.31 | -1.69 | down | 0.04029 |
| Cytosine | 1.66 | 0.73 | up | 0.041731 |
| d-Ala-d-Ala | 1.61 | 0.69 | up | 0.000498 |
| dATP | 2.59 | 1.37 | up | 0.003189 |
| dCDP | 1.88 | 0.91 | up | 0.01751 |
| Deoxyadenosine | 1.78 | 0.83 | up | 0.000438 |
| Deoxyinosine | 3.89 | 1.96 | up | 0.000756 |
| Deoxyribose phosphate | 0.44 | -1.18 | down | 0.025167 |
| DHAP | 4.58 | 2.2 | up | 0.033345 |
| dTDP | 0.61 | -0.71 | down | 0.043251 |
| dTMP | 0.3 | -1.74 | down | 0.048067 |
| dUDP | 3.03 | 1.6 | up | 0.003255 |
| FAICAR | 2.67 | 1.42 | up | 0.012579 |
| Folate | 2.27 | 1.18 | up | 0.030872 |
| G6P | 2.53 | 1.34 | up | 0.001473 |
| GABA | 0.61 | -0.71 | down | 0.011908 |
| Galactitol | 1.82 | 0.86 | up | 0.001839 |
| gamma-L-Glutamyl-L-cysteine | 2.33 | 1.22 | up | 1.48E-05 |
| GlcNAc | 2.71 | 1.44 | up | 8.74E-05 |
| Glucose | 2.22 | 1.15 | up | 0.014287 |
| Glutamate | 0.66 | -0.6 | down | 0.022304 |
| Glutathione | 3.61 | 1.85 | up | 0.003716 |
| Histidine | 1.72 | 0.78 | up | 0.023329 |
| Homocysteine | 3.6 | 1.85 | up | 0.00374 |
| Homoserine | 2.35 | 1.23 | up | 0.007703 |
| IMP | 7.95 | 2.99 | up | 0.001536 |
| Indolepyruvate | 4.52 | 2.18 | up | 0.025879 |
| Isoleucine | 1.6 | 0.68 | up | 0.000884 |
| Leucine | 1.6 | 0.68 | up | 0.000884 |
| Lysine | 1.67 | 0.74 | up | 0.004751 |
| Methionine | 2.13 | 1.09 | up | 0.000136 |
| Methylmaleate | 0.66 | -0.6 | down | 0.042842 |
| N6-(1,2-Dicarboxyethyl)-AMP | 0.62 | -0.69 | down | 0.041538 |
| N-Acetylmuramic acid alpha-1-phosphate | 0.5 | -1 | down | 0.039508 |
| Ornithine | 0.63 | -0.67 | down | 0.001755 |
| oxo-Proline | 0.66 | -0.6 | down | 0.026034 |
| pantetheine | 2.86 | 1.52 | up | 0.018297 |
| pantethine | 3.89 | 1.96 | up | 0.006327 |
| Phe | 1.62 | 0.7 | up | 8.23E-05 |
| phosphopantetheine | 4.23 | 2.08 | up | 3.86E-05 |
| phosphopantothenate | 0.35 | -1.51 | down | 0.036218 |
| Riboflavin | 0.58 | -0.79 | down | 0.008662 |
| S-Acetyldihydrolipoamide-E | 1.87 | 0.9 | up | 0.011389 |
| S-Adenosylmethionine | 1.64 | 0.71 | up | 0.019958 |
| Serine | 2.56 | 1.36 | up | 0.010045 |
| Succinyl diaminopimelate | 2.22 | 1.15 | up | 0.005378 |
| Thiamine | 2.95 | 1.56 | up | 0.000141 |
| Threonine | 2.35 | 1.23 | up | 0.007703 |
| Trehalose | 1.91 | 0.93 | up | 0.016641 |
| Trp | 1.56 | 0.64 | up | 0.012331 |
| Tyr | 1.79 | 0.84 | up | 0.000269 |
| UDP | 0.6 | -0.74 | down | 0.010709 |
| UDP-D-galactose | 0.39 | -1.36 | down | 0.023315 |
| UDP-GlcNAc | 0.47 | -1.09 | down | 0.014339 |
| UDP-glucose | 0.39 | -1.36 | down | 0.023315 |
| Uridine | 1.7 | 0.77 | up | 0.01258 |
| N10\_NV\_vs\_N10\_UT | (S)-3-Methyl-2-oxopentanoic acid | 3.77 | 1.91 | up | 0.028131 |
| 2-Phospho-D-glycerate | 0.55 | -0.86 | down | 0.047695 |
| 3-Methyl-2-oxobutanoic acid | 0.62 | -0.69 | down | 0.044677 |
| 3-Oxopropanoate | 0.48 | -1.06 | down | 0.000569 |
| 3-PA | 0.55 | -0.86 | down | 0.018969 |
| CAIR | 1.67 | 0.74 | up | 0.018589 |
| CDP | 1.59 | 0.67 | up | 0.027869 |
| cis-2-Methylaconitate | 4.19 | 2.07 | up | 0.000709 |
| Citrulline | 1.7 | 0.77 | up | 0.005865 |
| coenzyme A | 1.69 | 0.76 | up | 0.000487 |
| CTP | 7.95 | 2.99 | up | 0.034152 |
| Cysteine | 0.65 | -0.62 | down | 0.018969 |
| d-Ala-d-Ala | 1.64 | 0.71 | up | 0.009857 |
| dAMP | 3.52 | 1.82 | up | 0.007697 |
| Deoxyadenosine | 1.81 | 0.86 | up | 0.016368 |
| Deoxyribose phosphate | 1.54 | 0.62 | up | 0.000561 |
| dephospho-coenzyme A | 0.39 | -1.36 | down | 0.000554 |
| Dimethymalate | 0.49 | -1.03 | down | 0.031529 |
| dTMP | 2.26 | 1.18 | up | 0.012381 |
| FBP | 2.81 | 1.49 | up | 0.046166 |
| FGAM | 1.77 | 0.82 | up | 0.021049 |
| GABA | 0.44 | -1.18 | down | 0.000356 |
| GTP | 4.26 | 2.09 | up | 0.046685 |
| meso-Diaminopimelate | 0.62 | -0.69 | down | 0.010461 |
| NADH | 1.53 | 0.61 | up | 0.004425 |
| NADP+ | 1.95 | 0.96 | up | 0.010527 |
| OXG | 0.22 | -2.18 | down | 0.038658 |
| PEP | 0.6 | -0.74 | down | 0.001158 |
| Riboflavin | 0.63 | -0.67 | down | 0.019487 |
| Sedoheptulose 7-phosphate | 1.5 | 0.58 | up | 0.000561 |
| UDP | 2.9 | 1.54 | up | 0.004012 |
| UDP-GlcNAc | 1.73 | 0.79 | up | 0.000861 |
| UMP | 2.92 | 1.55 | up | 0.000296 |
| Uracil | 0.6 | -0.74 | down | 0.001119 |
| N10\_UT\_vs\_ancestral\_UT | (R)-3-Hydroxy-3-methyl-2-oxopentanoate | 2.15 | 1.1 | up | 0.002264 |
| (S)-2-Aceto-2-hydroxybutanoate | 2.15 | 1.1 | up | 0.002264 |
| 10-Formyl-THF | 2.88 | 1.53 | up | 0.018751 |
| 2-dehydropantoate | 2.15 | 1.1 | up | 0.002264 |
| 2-Deoxy-D-ribose 1-phosphate | 0.24 | -2.06 | down | 0.009877 |
| 3-Oxopropanoate | 2.13 | 1.09 | up | 0.019084 |
| 5,6,7,8-Tetrahydrofolate | 3.16 | 1.66 | up | 0.030902 |
| 5-Methylthioadenosine | 0.6 | -0.74 | down | 0.020444 |
| Acetylaminobutanal | 1.86 | 0.9 | up | 0.00019 |
| Acetylornithine | 1.66 | 0.73 | up | 0.04354 |
| Alanine | 1.59 | 0.67 | up | 0.016921 |
| alpha-Isopropylmalate | 0.51 | -0.97 | down | 0.002996 |
| Arginine | 2.73 | 1.45 | up | 0.019858 |
| Asparagine | 1.63 | 0.7 | up | 0.024535 |
| Biotin | 6.52 | 2.7 | up | 8.11E-05 |
| dADP | 0.34 | -1.56 | down | 0.019322 |
| dATP | 2.19 | 1.13 | up | 0.021281 |
| Deoxyadenosine | 1.73 | 0.79 | up | 0.003243 |
| Deoxyinosine | 2.74 | 1.45 | up | 0.020132 |
| Deoxyribose phosphate | 0.24 | -2.06 | down | 0.009877 |
| dephospho-coenzyme A | 2.02 | 1.01 | up | 0.01177 |
| Dimethymalate | 1.99 | 0.99 | up | 0.001522 |
| dTDP | 0.54 | -0.89 | down | 0.01294 |
| dUDP | 2.79 | 1.48 | up | 0.006921 |
| dUMP | 0.33 | -1.6 | down | 0.03537 |
| F6P | 2.82 | 1.5 | up | 0.012545 |
| FAICAR | 7.82 | 2.97 | up | 0.01074 |
| Folate | 3.29 | 1.72 | up | 0.000143 |
| G1P | 2.82 | 1.5 | up | 0.012545 |
| Galactitol | 2.3 | 1.2 | up | 0.00141 |
| Galactono-1,4-lactone | 1.65 | 0.72 | up | 0.002939 |
| gamma-L-Glutamyl-L-cysteine | 2.78 | 1.48 | up | 0.00019 |
| GlcNAc | 2.77 | 1.47 | up | 0.000536 |
| Glutathione | 3.73 | 1.9 | up | 0.001669 |
| Glycine | 1.73 | 0.79 | up | 0.006083 |
| Histidine | 2.39 | 1.26 | up | 0.033192 |
| Homocysteine | 3.91 | 1.97 | up | 0.001594 |
| Homoserine | 2.94 | 1.56 | up | 0.000758 |
| IMP | 7.7 | 2.94 | up | 0.000122 |
| Indolepyruvate | 4.26 | 2.09 | up | 0.003945 |
| Isoleucine | 1.85 | 0.89 | up | 0.001269 |
| Leucine | 1.85 | 0.89 | up | 0.001269 |
| Lysine | 1.85 | 0.89 | up | 0.000192 |
| meso-Diaminopimelate | 2.05 | 1.04 | up | 0.013544 |
| Methionine | 2.25 | 1.17 | up | 0.001697 |
| Methylglyoxal | 1.64 | 0.71 | up | 0.009683 |
| Oxaloacetate | 1.7 | 0.77 | up | 0.030752 |
| OXG | 3.95 | 1.98 | up | 0.042886 |
| oxo-Proline | 1.51 | 0.59 | up | 0.009119 |
| pantethine | 3.56 | 1.83 | up | 0.000415 |
| PEP | 0.4 | -1.32 | down | 0.009472 |
| Phe | 1.59 | 0.67 | up | 0.000872 |
| phosphopantetheine | 4.89 | 2.29 | up | 0.000522 |
| PYR | 2.13 | 1.09 | up | 0.019084 |
| Riboflavin | 0.59 | -0.76 | down | 0.041888 |
| Saccharopine | 1.63 | 0.7 | up | 0.033471 |
| S-Adenosylmethionine | 1.85 | 0.89 | up | 0.031507 |
| Sedoheptulose 7-phosphate | 0.65 | -0.62 | down | 0.010979 |
| Serine | 2.94 | 1.56 | up | 0.004172 |
| Succinyl diaminopimelate | 2.13 | 1.09 | up | 0.007137 |
| Thiamine | 3.19 | 1.67 | up | 0.003433 |
| Threonine | 2.94 | 1.56 | up | 0.000758 |
| Trp | 1.98 | 0.99 | up | 0.002336 |
| Tyr | 1.95 | 0.96 | up | 0.000193 |
| Uridine | 2.37 | 1.24 | up | 0.001401 |
| Xanthine | 1.62 | 0.7 | up | 0.023044 |
|  |  |  |  |  |  |