Supplementary Material

Comprehensive analysis of C. glutamicum anaplerotic deletion mutants under defined D-glucose conditions

Jannick Kappelmann¹, Bianca Klein¹, Mathias Papenfuß², Julian Lange³, Bastian Blombach⁴, Ralf Takors⁵, Wolfgang Wiechert¹, Tino Polen¹, Stephan Noack¹,*

Supplementary Table S1. Estimated specific growth rates (μ), D-glucose consumption rates (πGLC), CO₂ formation rates (πCO₂) and instantaneous carbon balance (θ) for anaplerotic deletion mutants during exponential growth.

| Strain        | Replicate | μ [h⁻¹] (BV) | μ [h⁻¹] (CDW) | πGLC [mmol mL⁻¹ h⁻¹] (BV) | πGLC [mmol g CDW⁻¹ h⁻¹] (CDW) | πCO₂ [mmol mL⁻¹ h⁻¹] (BV) | πCO₂ [mmol g CDW⁻¹ h⁻¹] (CDW) | θ               |
|---------------|-----------|--------------|---------------|---------------------------|-------------------------------|----------------------------|-------------------------------|------------------|
| ATCC 13032    | R1        | 0.45 [0.44, 0.46] | 0.44 [0.42, 0.45] | 1.47 [1.45, 1.50] | 4.86 [4.68, 5.05] | 2.20 | 7.16 ± 0.60 | 0.81 ± 0.077 |
| ATCC 13032    | R2        | 0.45 [0.44, 0.46] | 0.45 [0.42, 0.48] | 1.41 [1.36, 1.46] | 4.61 [4.40, 4.85] | 1.92 | 6.41 ± 0.67 | 0.84 ± 0.087 |
| ATCC 13032    | R3        | 0.47 [0.45, 0.48] | 0.46 [0.42, 0.49] | 1.53 [1.48, 1.58] | 4.98 [4.77, 5.23] | 2.23 | 7.24 ± 0.71 | 0.82 ± 0.089 |
| Δpck          | R1        | 0.36 [0.36, 0.37] | 0.37 [0.37, 0.38] | 1.26 [1.25, 1.27] | 4.03 [3.93, 4.14] | 1.63 | 5.32 ± 0.51 | 0.80 ± 0.072 |
| Δpck          | R2        | 0.38 [0.37, 0.38] | 0.39 [0.37, 0.40] | 1.27 [1.25, 1.29] | 4.16 [4.02, 4.29] | 1.75 | 5.69 ± 0.53 | 0.81 ± 0.077 |
| Δppc          | R1        | 0.45 [0.44, 0.47] | 0.46 [0.42, 0.49] | 1.57 [1.53, 1.61] | 5.08 [4.89, 5.32] | 2.43 | 7.60 ± 0.92 | 0.81 ± 0.083 |
| Δppc          | R2        | 0.43 [0.42, 0.44] | 0.43 [0.41, 0.40] | 1.48 [1.43, 1.53] | 5.16 [4.95, 5.38] | 2.36 | 7.68 ± 1.09 | 0.77 ± 0.076 |
| Δpyc          | R1        | 0.38 [0.37, 0.39] | 0.40 [0.38, 0.41] | 1.44 [1.41, 1.46] | 4.60 [4.45, 4.77] | 2.24 | 7.29 ± 0.51 | 0.80 ± 0.072 |
| Δpyc          | R2        | 0.37 [0.36, 0.37] | 0.40 [0.38, 0.41] | 1.55 [1.53, 1.57] | 4.70 [4.57, 4.84] | 2.32 | 7.56 ± 0.76 | 0.79 ± 0.071 |
| ΔmalE         | R1        | 0.48 [0.48, 0.49] | 0.46 [0.45, 0.48] | 1.50 [1.47, 1.52] | 5.04 [4.98, 5.21] | 2.02 | 7.36 ± 0.43 | 0.82 ± 0.075 |
| ΔmalE         | R2        | 0.49 [0.48, 0.50] | 0.46 [0.44, 0.48] | 1.37 [1.36, 1.39] | 4.72 [4.58, 4.87] | 1.82 | 6.64 ± 1.00 | 0.85 ± 0.080 |
| Δpck ΔmalE    | R1        | 0.45 [0.43, 0.47] | 0.45 [0.41, 0.48] | 1.50 [1.45, 1.54] | 4.94 [4.72, 5.18] | 2.30 | 7.45 ± 0.67 | 0.82 ± 0.087 |
| Δpck ΔmalE    | R2        | 0.47 [0.46, 0.48] | 0.47 [0.45, 0.48] | 1.48 [1.44, 1.52] | 4.77 [4.58, 4.99] | 2.26 | 7.37 ± 0.31 | 0.87 ± 0.083 |
| Δpck ΔmalE    | R3        | 0.43 [0.43, 0.44] | 0.45 [0.43, 0.47] | 1.51 [1.49, 1.52] | 4.85 [4.69, 5.02] | 2.15 | 6.98 ± 0.83 | 0.81 ± 0.076 |
| Δpck ΔmalE    | R1        | 0.46 [0.45, 0.47] | 0.43 [0.42, 0.44] | 1.46 [1.43, 1.49] | 5.03 [4.83, 5.25] | 2.14 | 6.96 ± 0.29 | 0.77 ± 0.072 |
| Δpck ΔmalE    | R2        | 0.42 [0.41, 0.42] | 0.42 [0.41, 0.43] | 1.48 [1.45, 1.51] | 4.77 [4.57, 4.96] | 1.89 | 6.16 ± 0.83 | 0.77 ± 0.073 |
| Δpcc Δodx     | R1        | 0.39 [0.38, 0.40] | 0.40 [0.38, 0.42] | 1.40 [1.38, 1.43] | 4.54 [4.37, 4.70] | 2.00 | 6.51 ± 0.96 | 0.78 ± 0.077 |
| Δpcc Δodx     | R2        | 0.38 [0.38, 0.38] | 0.38 [0.37, 0.39] | 1.31 [1.30, 1.32] | 4.15 [4.04, 4.28] | 1.87 | 6.07 ± 0.99 | 0.82 ± 0.074 |
| Δpcc Δodx     | R3        | 0.43 [0.42, 0.44] | 0.42 [0.40, 0.44] | 1.26 [1.22, 1.30] | 4.44 [4.31, 4.60] | 1.78 | 6.36 ± 0.89 | 0.83 ± 0.078 |
| Δpcc Δpyc     | R1        | 0.27 [0.27, 0.28] | 0.27 [0.26, 0.28] | 1.09 [1.06, 1.11] | 3.62 [3.51, 3.77] | 2.30 | 7.52 ± 0.68 | 0.81 ± 0.070 |
| Δpcc Δpyc     | R2        | 0.26 [0.26, 0.28] | 0.26 [0.25, 0.27] | 1.20 [1.18, 1.23] | 3.85 [3.69, 4.01] | 2.23 | 7.27 ± 0.45 | 0.74 ± 0.067 |
**Supplementary Table S2.** Gradient elution for amino acid quantification.

| Time [min] | Flow rate [µL min⁻¹] | % B  |
|------------|---------------------|------|
| 0          | 400                 | 15   |
| 10         | 400                 | 15   |
| 16         | 400                 | 100  |
| 28         | 400                 | 100  |
| 30         | 400                 | 15   |
| 35         | 400                 | 15   |

**Supplementary Table S3.** Gradient elution for sugar and nucleoside phosphate quantification.

| Time [min] | Flow rate [µL min⁻¹] | % B  |
|------------|---------------------|------|
| 0          | 450                 | 0    |
| 2          | 450                 | 0    |
| 5          | 450                 | 20   |
| 8          | 450                 | 20   |
| 10         | 450                 | 35   |
| 14         | 450                 | 100  |
| 15         | 450                 | 100  |
| 15.5       | 450                 | 0    |
| 17         | 450                 | 0    |

**Supplementary Table S4.** Gradient elution for SWATH acquisition.

| Time [min] | Flow rate [µL min⁻¹] | % B  |
|------------|---------------------|------|
| -12        | 200                 | 3    |
| 0          | 200                 | 3    |
| 70         | 200                 | 40   |
| 78         | 200                 | 40   |
| 79         | 200                 | 60   |
| 89         | 200                 | 60   |
| 90         | 200                 | 3    |
Supplementary Table S5. SWATH Q1 windows monitored during elution on the TripleTOF 6600. The SWATH windows were not scheduled. CES, collision energy spread was always fixed to 5.000.

| SWATH Exp Index | StartMass [Da] | StopMass [Da] |
|-----------------|----------------|--------------|
| SWATH Exp 1     | 199.50         | 222.10       |
| SWATH Exp 2     | 221.10         | 242.80       |
| SWATH Exp 3     | 241.80         | 264.40       |
| SWATH Exp 4     | 263.40         | 280.60       |
| SWATH Exp 5     | 279.60         | 295.00       |
| SWATH Exp 6     | 294.00         | 307.60       |
| SWATH Exp 7     | 306.60         | 319.30       |
| SWATH Exp 8     | 318.30         | 330.10       |
| SWATH Exp 9     | 329.10         | 340.00       |
| SWATH Exp 10    | 339.00         | 349.90       |
| SWATH Exp 11    | 348.90         | 358.90       |
| SWATH Exp 12    | 357.90         | 367.90       |
| SWATH Exp 13    | 366.90         | 376.00       |
| SWATH Exp 14    | 375.00         | 385.00       |
| SWATH Exp 15    | 384.00         | 393.10       |
| SWATH Exp 16    | 392.10         | 401.20       |
| SWATH Exp 17    | 400.20         | 410.20       |
| SWATH Exp 18    | 409.20         | 419.20       |
| SWATH Exp 19    | 418.20         | 428.20       |
| SWATH Exp 20    | 427.20         | 438.10       |
| SWATH Exp 21    | 437.10         | 448.90       |
| SWATH Exp 22    | 447.90         | 459.70       |
| SWATH Exp 23    | 458.70         | 472.30       |
| SWATH Exp 24    | 471.30         | 485.80       |
| SWATH Exp 25    | 484.80         | 500.20       |
| SWATH Exp 26    | 499.20         | 517.30       |
| SWATH Exp 27    | 516.30         | 537.10       |
| SWATH Exp 28    | 536.10         | 559.60       |
| SWATH Exp 29    | 558.60         | 584.80       |
| SWATH Exp 30    | 583.80         | 613.60       |
| SWATH Exp 31    | 612.60         | 645.10       |
| SWATH Exp 32    | 644.10         | 679.30       |
| SWATH Exp 33    | 678.30         | 718.90       |
| SWATH Exp 34    | 717.90         | 766.60       |
| SWATH Exp 35    | 765.60         | 826.00       |
| SWATH Exp 36    | 825.00         | 903.40       |
| SWATH Exp 37    | 902.40         | 1013.20      |
| SWATH Exp 38    | 1012.20        | 1171.60      |
| SWATH Exp 39    | 1170.60        | 1419.10      |
| SWATH Exp 40    | 1418.10        | 1998.70      |
**Supplementary Table S6.** SNPs or affected amino acids in intergenic regions or protein-coding genes identified in the *C. glutamicum Δppc Δpyc* mutant adapted to D-glucose as sole carbon source in comparison to *C. glutamicum* WT as reference. The column ‘Reads of position’ refers to the number of sequencing reads supporting the alteration. The relative frequency refers to the number of reads supporting the alteration relative to the total number of reads of the position.

| Affected region                                                                 | Reads of position | Rel. frequency |
|---------------------------------------------------------------------------------|-------------------|----------------|
| nt 3,163,180; SNV C to T, intergenic region of cg3314 (conserved hypothetical protein) and cg3315 (malR, transcriptional regulator MalR) | 181               | 1              |
| nt 2,030,620; SNV C to A, intergenic region of cg2136 (gluA, glutamate uptake system) and cg2137 (gluB, secreted glutamate-binding protein) | 125               | 1              |
| SNV C to T, T306T in cg1574, pheS, phenylalanyl-tRNA synthetase α subunit         | 116               | 1              |
| SNV T to C, exchange L328S in cg1245, putative membrane protein                  | 102               | 1              |
| SNV T to C, A22A in cg3237, sodA (sod), manganese superoxide dismutase           | 125               | 1              |
| SNV A to G, exchange Y158H in cg1676, lip3, putative lipase                      | 106               | 0.99           |
| SNV A to C, exchange E324A in cg1451, serA, phosphoglycerate dehydrogenase       | 75                | 0.35           |
| SNV C to T, exchange G577S in cg0766, icd, isocitrate dehydrogenase             | 148               | 0.33           |
| SNV G to A, exchange P583S in cg0766, icd, isocitrate dehydrogenase             | 149               | 0.14           |
| SNV A to T, Stop L508* in cg2267, putative membrane protein                     | 89                | 0.11           |
| SNV A to T, exchange V117E in cg0237, putative short chain dehydrogenase         | 76                | 0.11           |
Supplementary Figure S1. Evolution of the *C. glutamicum* Δppc Δpyc mutant on 1 % d-glucose CGXII medium. A) Time course of biomass growth where each line represents one well of a certain inoculation density. The inoculation density is indicated on the left hand side of each plot, decreasing from top to bottom. B) Histograms of the lag-times in each well of a certain inoculation density. Lag-time is defined as the time after inoculation needed to reach the Backscatter value of 50. In each plot the number of wells is given that were inoculated to the OD_{init, 600} indicated under A) alongside the number of wells that reached the Backscatter value of 50.
Supplementary Figure S2. Time course of biovolume (BV) and substrate concentration as well as CO₂ exhaust gas volume fraction from a cultivation of the A) Δpck mutant, and B) evolved Δppc Δpyc mutant on defined CGXII medium with 1% D-glucose. Dashed lines show the computed time course of BV and glucose concentrations for the best fit of Eqs. (2) and (3) in the main text to the shown experimental data. Green filled circles denote the time-dependent volume fraction of CO₂ in the exhaust gas stream.
Supplementary Figure S3. Correlation between BV and CDW for *C. glutamicum*. Data were derived by growing the Δpyc Δodx, Δppe ΔmalE and wild-type strain in defined CGXII medium with D-glucose as sole carbon and energy source. The fitted coefficients for the function $f(CDW) = p_1 \cdot BV + p_2$ are as follows (coefficient estimate followed by 95% confidence interval): slope $p_1 = 0.3098$ (0.3045, 0.3151) and intercept $p_2 = -0.008692$ ($-0.02189, 0.004509$). The goodness of fit is SSE: 0.2256, R-square: 0.994, Adjusted R-square: 0.9939, RMSE: 0.05278.