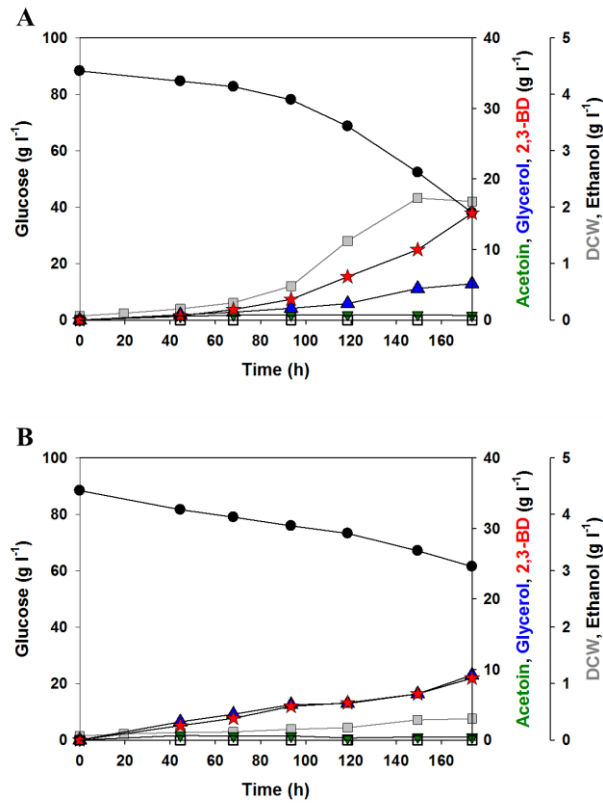


Table S1. Kinetic constants (K_m and V_{max}) of Pdc enzymes.

Strains	CtPDC1	KmPDC1	ScPDC1	ScPDC5	ScPDC6
K_m (mM)	2.7	7.7	4.7	9.9	8.2
V_{max} (mU/mg protein)	107	383	541	437	415

Figure S1. Batch cultivation of the BD4 strain (A and C) and the BD5 strain (B and D) in minimal medium containing 90 g/L glucose without ethanol (A and B) and with 0.5 g/L of ethanol (C and D). Symbols: Glucose (filled circle), dry cell weight (grey square), glycerol (blue triangle), acetoin (green inverted triangle), 2,3-BD (red star), and ethanol (open square).



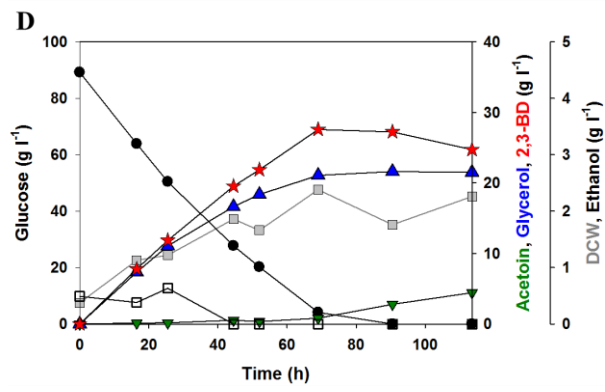
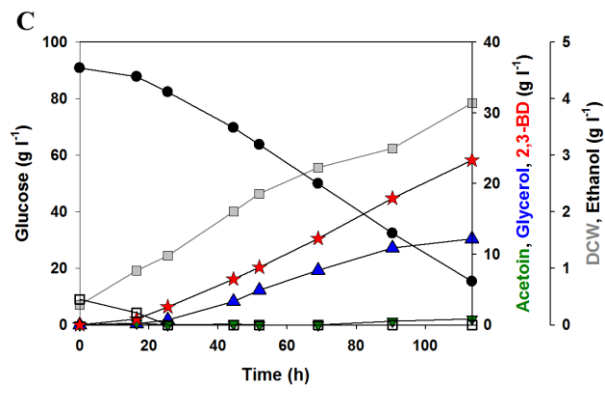


Figure S2. Amino acid sequence of the *C. tropicalis* pyruvate decarboxylase I. Amino acids of Mg²⁺ and ThDP binding loop (bold and underlined) and substrate activation (bold) that are conserved throughout Pdc enzymes.

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1                               40
MSEITLGRFFFERLHQLQVDTVFG LPGDFNLALLDKIYEV
41                               77
DGMRWAGNANELNAGYAADGYARVNPNGLAALVSTFG
78                               117
VGELSLTNAIAGSYSEHVGIIINLVGVPSSSAQAKQLLLHH
118                               158
TLGNGDFTVFHRMFKNISQTSAFISDPNTAAASEIDRCIRDA
159                               198
YVYQRPVYIGLPSNLVDVKVPKSLLDKKIDLSLHPNEPES
199                               239
QAEVVETVEKFISEASNPVILVDACAIRHNCLKEVAELIAE
240                               278
TQFPVFTTPMGKSSVDESNPRFGGVYVGSLSPPDVKEAV
279                               316
ESADLVLSVGAMLSDFNTGAFSYNYKTRNVVEFHSDYT
317                               355
KIRQATFPGVQMKEALQVLLKTVKKSVNPKYVPAPVPAT
356                               395
KAITTPGNNDPVSQEYLWRKVSDWFQEGDVISETGTSAF
396                               435
GIVQSKFPKNAIGISQVLWGSIGYATGATCGAAMAAQEID
436                               474
PKKRVLFTGDGSLQLTVQEISTMCKWDCYNTYLYVLNN
475                               513
DGYTIERLIHGEKAQYNDIQPWNNLQLLPLFNAKKYETK
514                               553
RISTVGELNDLFTNKEFAVPDRIRMVEIMLPVMDAPANLV
554
AQAKQSAATNAAQE

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Figure S3. *In vitro* Pdc activities in the control and four engineered *S. cerevisiae* strains expressing *C. tropicalis* pyruvate decarboxylase gene (*CtPDC1*) differentially. Cells were grown to mid-exponential phase in YNB medium containing 80 g/L glucose and 0.5 g/L ethanol and analyzed.

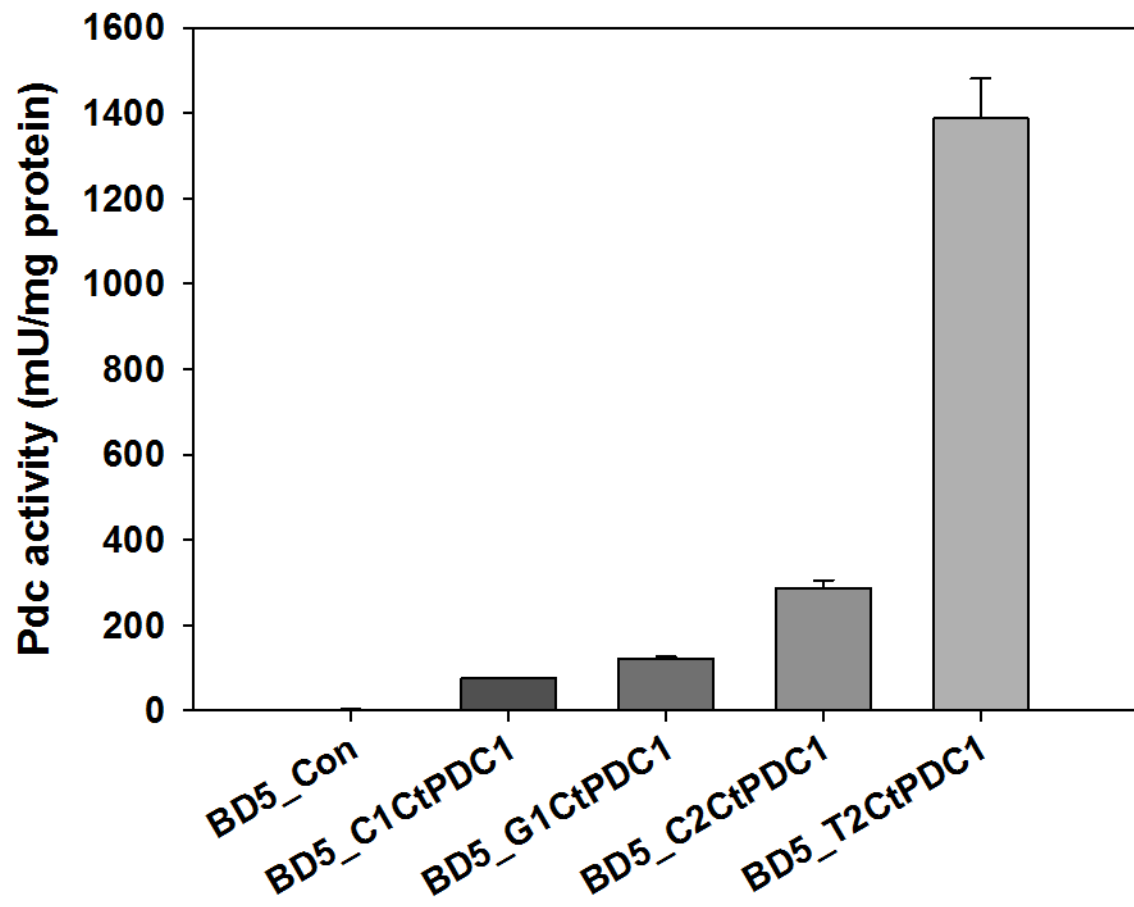
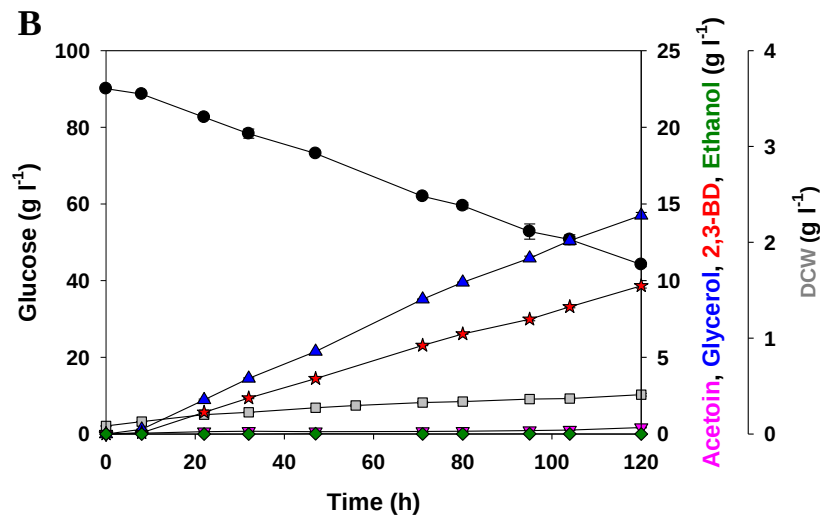
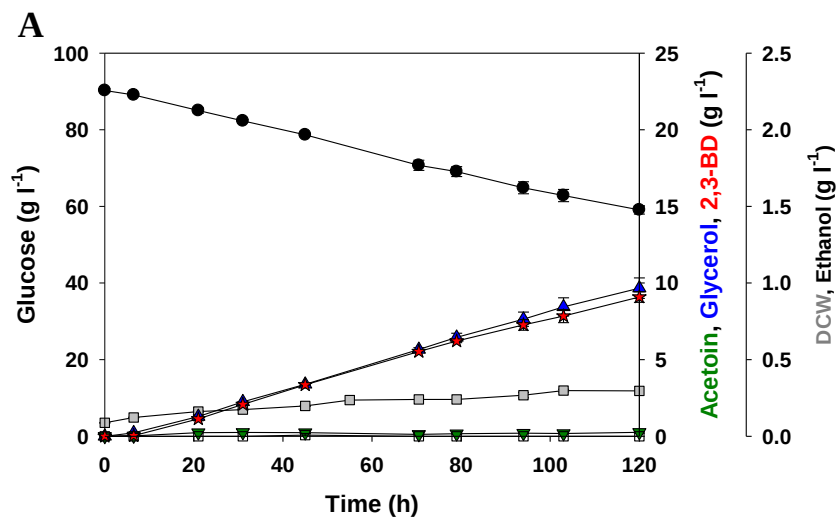


Figure S4. Fermentation profiles of the (A) BD5_Con, (B) BD5_C1CtPDC1, (C) BD5_G1CtPDC1, (D) BD5_C2CtPDC1, (E) BD5_T2CtPDC1 strains with 90 g/L glucose as a sole carbon source in minimal medium. Symbols: Glucose (filled circle), dry cell weight (grey square), glycerol (blue triangle), acetoin (green inverted triangle), 2,3-BD (red star), and ethanol (open square). Results are the averages of duplicate experiments and error bars indicate standard deviation.



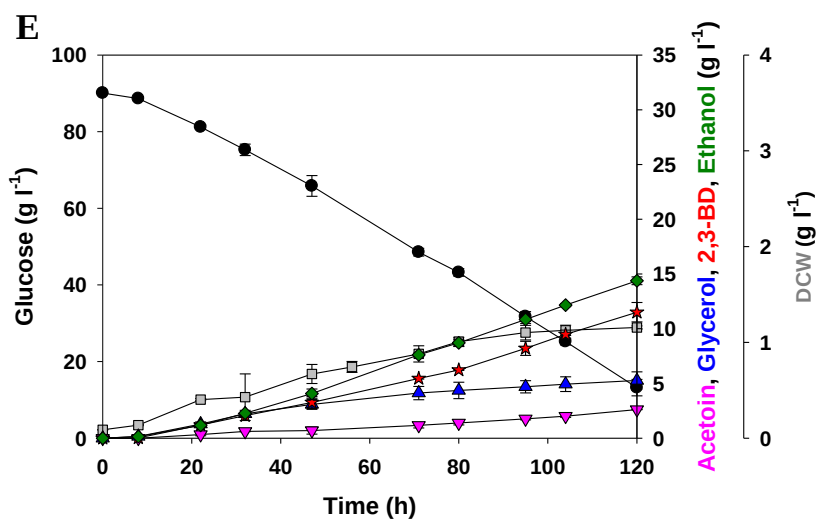
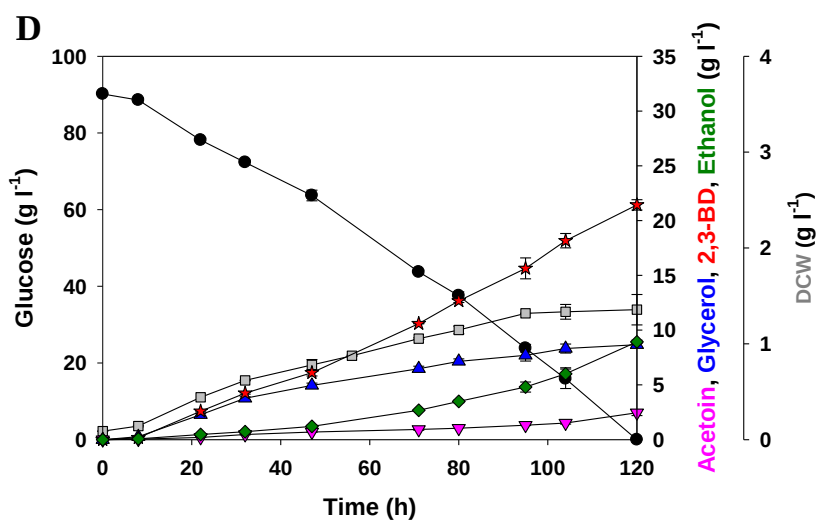
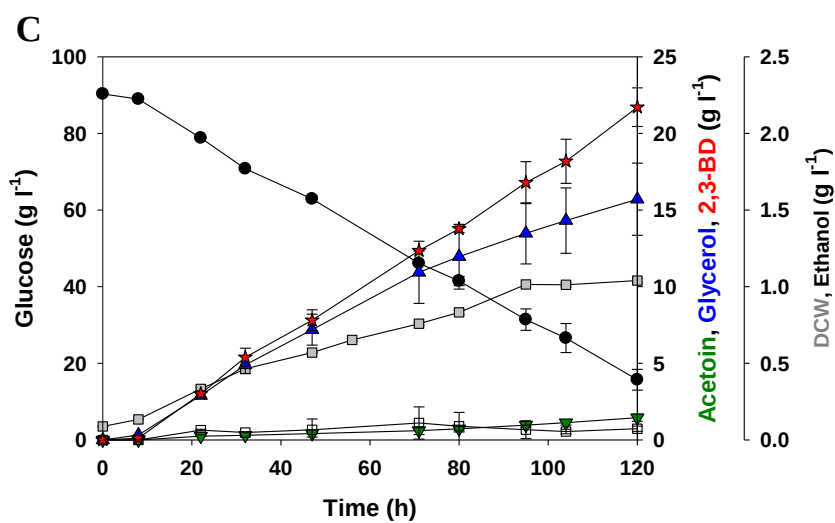


Figure S5. The NADH and NAD⁺ concentrations in the BD5_G1CtPDC1_nox strain with various aeration conditions. The cells from the batch cultivations at 20 h in Figure 4 were analyzed.

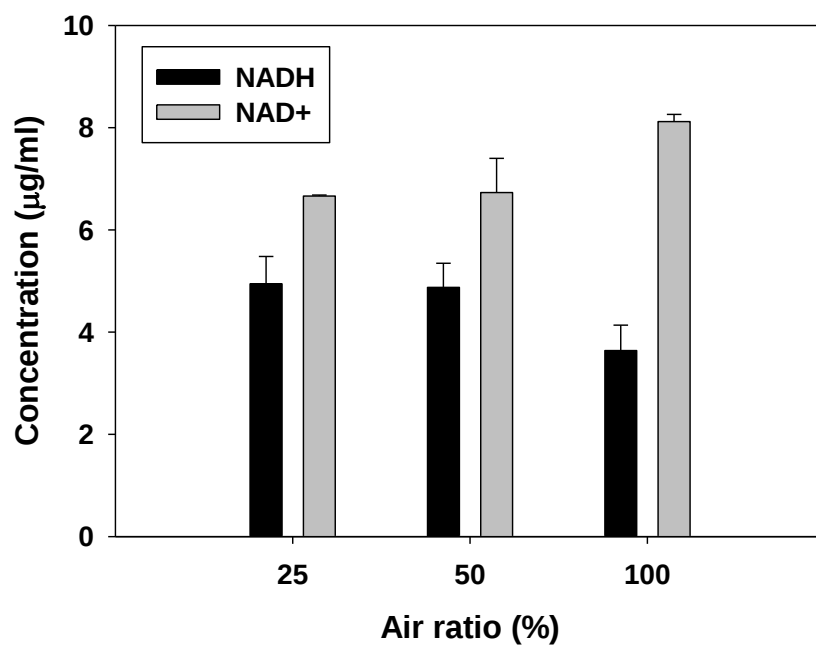
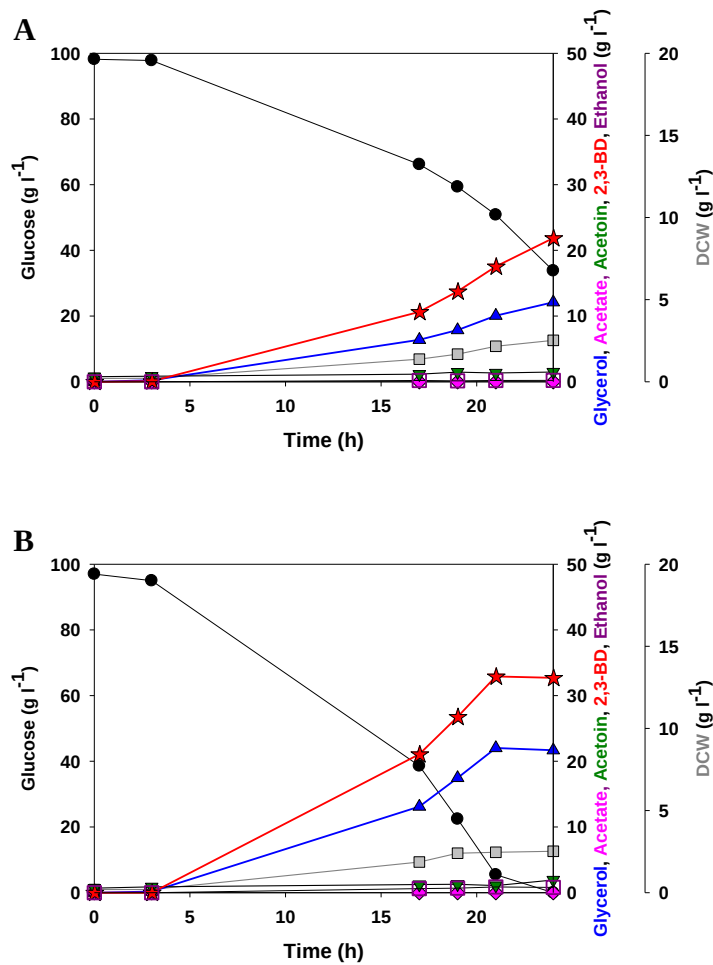


Figure S6. Profiles of batch cultivations for metabolomic analysis. The YP medium containing 100 g/L glucose was used and aeration condition was controlled by ratios of mixed inlet gas at constant agitation speed (500 rpm) and air flow rate (2 vvm). Cells were harvested at 22 h cultivation for analysis. (A) the BD5_G1CtPDC1 strain with pure air (Ct100), (B) the BD5_G1CtPDC1_nox strain with 1:1 mixture of air and nitrogen gas (N50), (C) the BD5_G1CtPDC1_nox strain with pure air (N100).



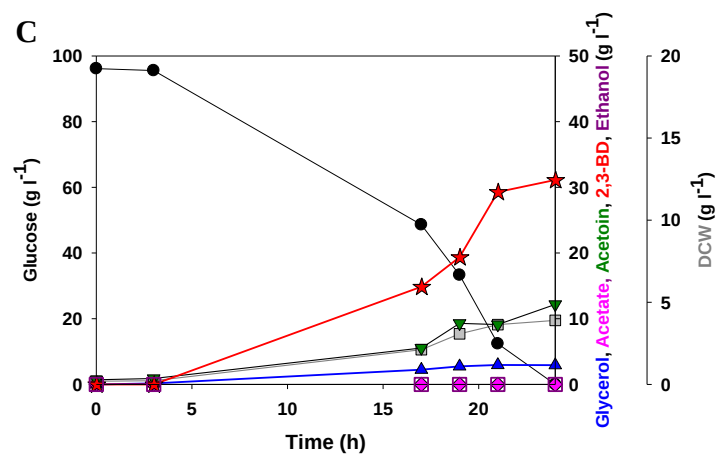


Figure S7. Principal component analysis (PCA) of intracellular metabolite profiles of BD5_G1CtPDC1 and BD5_G1CtPDC1_nox in different aerobic conditions.

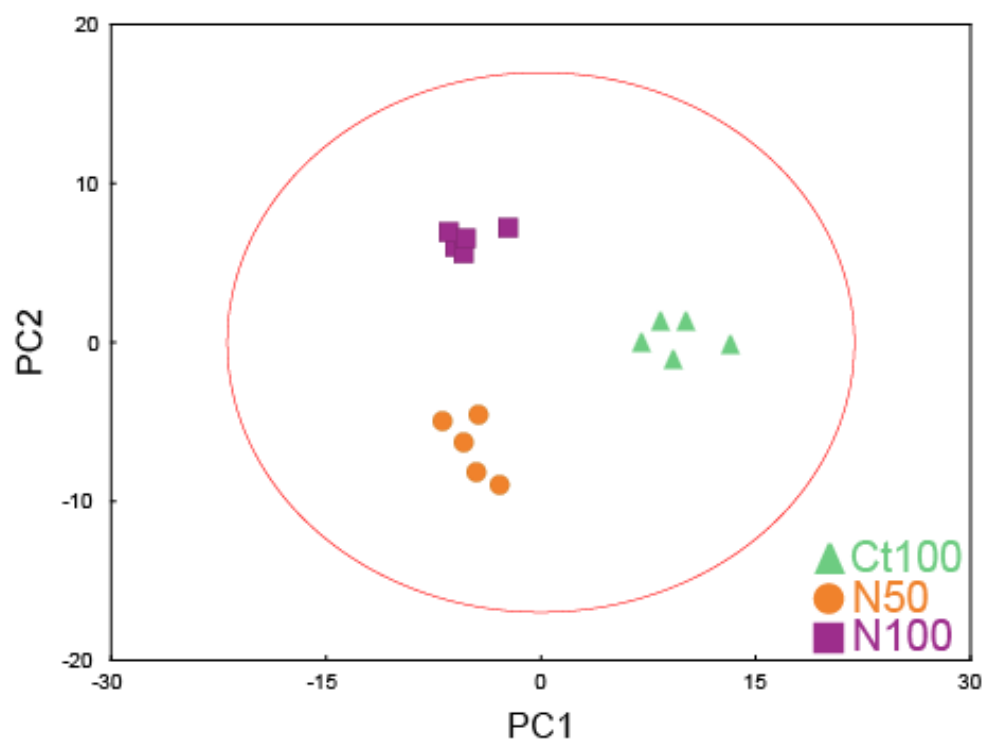


Figure S8. Hierarchical clustering analysis (HCA) of metabolite profiles of BD5_G1CtPDC1 and BD5_G1CtPDC1_nox in different aerobic conditions. Red-bars represent the increased metabolites and blue-bars represent the decreased metabolites.

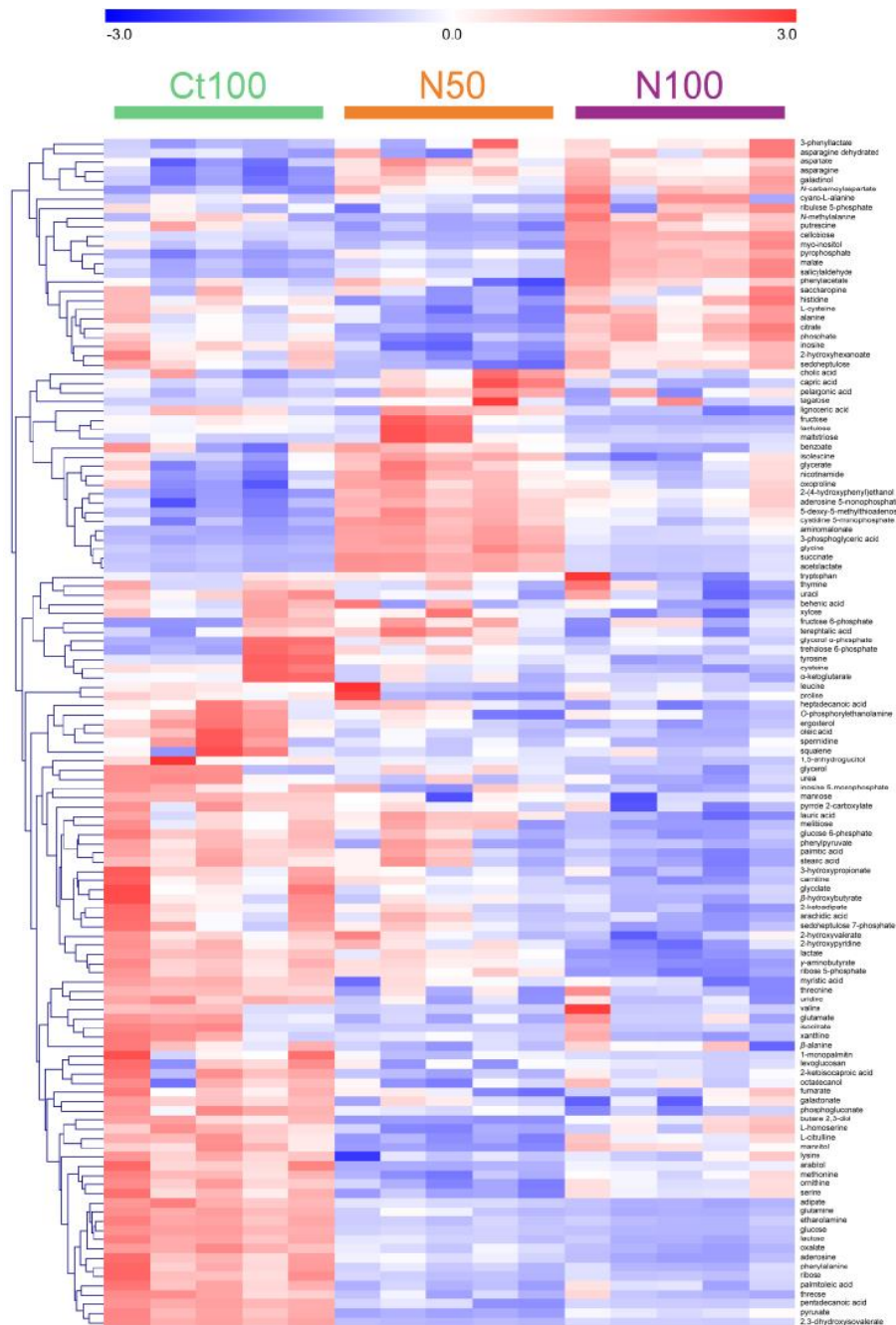


Figure S10. Specific glucose uptake rates of the wild type D452-2 and engineered strains. To determine the specific glucose uptake rate, the initial OD10 of cells were cultivated in YP medium with 100 g/L glucose. The amounts of glucose consumed in 9 h cultivation were used for calculating specific glucose uptake rates.

