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# Metabolic flux pattern of glucose utilization by the phytopathogen and xanthan-producer *Xanthomonas campestris* pv. campestris: prevalent role of the Entner-Doudoroff pathway and minor fluxes through the pentose phosphate pathway and through glycolysis

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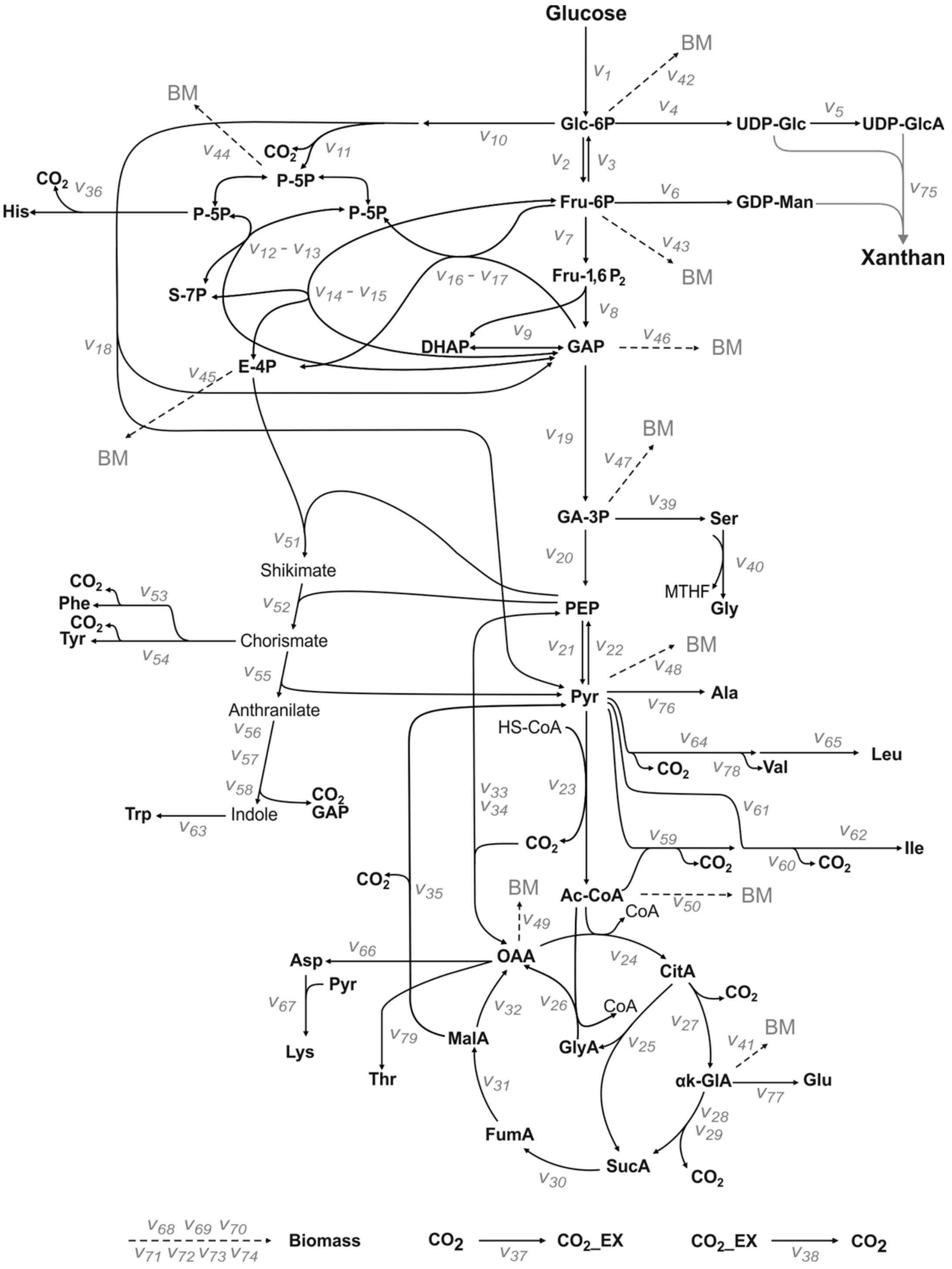
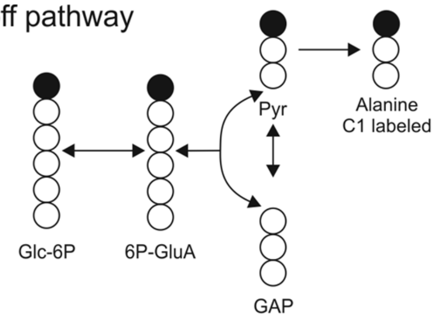
## Supplementary Data

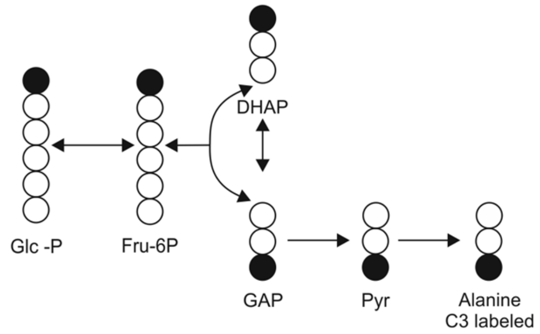


**Supplementary Figure 1. Graphical display of the *X. campestris* pv. campestris B100 metabolic network employed to establish a model for $^{13}$C metabolic flux analysis.** The metabolic network underlying the model includes the Embden-Meyerhof-Parnas pathway, the pentose phosphate pathway, the Entner-Doudoroff pathway, gluconeogenesis, the tricaboxylic acid cycle, biosynthesis of xanthan precursors, and lumped reactions for the amino acids biosyntheses of alanine (Ala), valine (Val), aspartate (Asp), glutamate (Glu), serine (Ser), phenylalanine (Phe), glycine (Gly), tyrosine (Tyr), threonine (Thr), tryptophan (Trp), isoleucine (Ile), histidine (His), lysine (Lys) and leucine (Leu). The reactions with ID numbers colored in grey are detailed in Supplementary Table 1. Glc-6P, glucose 6-phosphate; Fru-6P, fructose 6-phosphate; Fru-1,6P$_2$, fructose 1,6-bisphosphate; P-5P, pentose 5-phosphate; S-7P, sedoheptulose 7-phosphate; E-4P, erythrose 4-phosphate, GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; GA3P, 3-Phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate; Ac-CoA, acetyl-CoA; CitA, citrate; αk-GlA, α-ketoglutarate (2-oxoglutarate); SucA, succinate; FumA, fumarate, MalA, malate; OAA, oxaloacetate; CO$_2$, carbon dioxide.

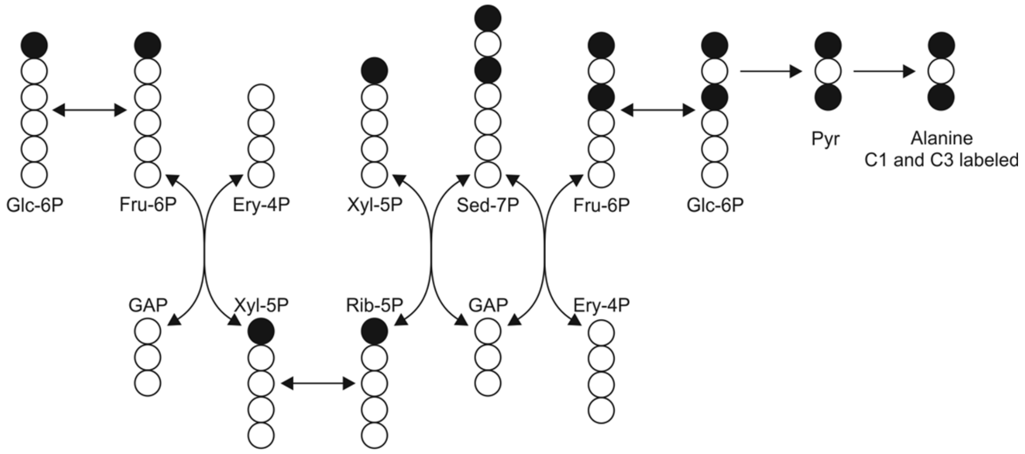
**A** Entner-Doudoroff pathway



**B** Embden-Meyerhof-Parnas pathway



**C** Pentose phosphate pathway: reversible reactions of the non-oxidative branch



**Supplementary Figure 2. Distribution of $^{13}$C labeling pattern derived from 1-$^{13}$C glucose through the three main pathways of the *X. campestris* pv. campestris carbohydrate catabolism leading to labeling of the amino acid alanine.** Carbon atoms are symbolized by open circles. Shaded circles indicate $^{13}$C labeling of the respective C atom. (**A**) The ED pathway generates alanine precursors with labeled C1. (**B**) Activity of the EMP pathway leads to labeling of the alanine C3. (**C**) In the reversible reactions of the non-oxidative branch of the PP pathway, reactions of isomerization, transketolation and epimerization can result in labeling of the C1 and the C3 of alanine. Gluconeogenesis reactions that might add further complexity to the labeling pattern were not considered in this overview as we observed no indications for gluconeogenesis activity for the cultivation conditions underlying this study. Abbreviations: DHAP, dihydroxyacetone phosphate; Ery-4P, erythrose 4-phosphate; Fru-6P, fructose 6-phosphate; Glc-6P, glucose 6-phosphate; 6P-GluA, 6-Phosphogluconic acid; GAP, glyceraldehyde 3-phosphate; Pyr, pyruvate; Rib-5P, ribose 5-phosphate; Sed-7P, sedoheptulose 7-phosphate, Xyl-5P, xylulose 5-phosphate.

**Supplementary Table 1**

OpenFLUX model of *Xanthomonas campestris* pv. campestris B100

| rxnID | rxnEQ | cTrans | rates | type | basis | deviation |
|---|---|---|---|---|---|---|
| R01 | GLC_EX + ATP = GLC6P | abcdef + X = abcdef | | F | 100 | 0 |
| R02 | GLC6P = F6P | abcdef = abcdef | | FR | | |
| R03 | F6P = GLC6P | abcdef = abcdef | | R | X | |
| R04 | GLC6P = UDPG | abcdef = abcdef | | F | | |
| R05 | UDPG = UDPGA | abcdef = abcdef | | F | | |
| R06 | F6P = GDPM | abcdef = abcdef | | F | | |
| R07 | F6P + PPi = F16BP | abcdef + X = abcdef | | F | | |
| R08 | F16BP = DHAP + G3P | abcdef = abc + def | | F | | |
| R09 | DHAP = G3P | abc = cba | | F | | |
| R10 | GLC6P = 6PG + NADH | abcdef = abcdef + X | | F | | |
| R11 | 6PG = P5P + CO2 + NADH | abcdef = bcdef + a + X | | F | X | |
| R12 | P5P + P5P = S7P + G3P | abcde + fghij = fgabcde + hij | | FR | | |
| R13 | S7P + G3P = P5P + P5P | fgabcde + hij = abcde + fghij | | R | X | |
| R14 | S7P + G3P = E4P + F6P | abcdefg + hij = defg + abchij | | FR | | |
| R15 | E4P + F6P = S7P + G3P | defg + abchij = abcdefg + hij | | R | X | |
| R16 | E4P + P5P = F6P + G3P | abcd + efghi = efabcd + ghi | | FR | | |
| R17 | F6P + G3P = E4P + P5P | efabcd + ghi = abcd + efghi | | R | X | |
| R18 | 6PG = G3P + PYR | abcdef = def + abc | | F | X | |
| R19 | G3P = 3PG + ATP + NADH | abc = abc + X + X | | F | | |
| R20 | 3PG = PEP | abc = abc | | F | | |
| R21 | PEP = PYR + ATP | abc = abc + X | | FR | | |
| R22 | PYR + ATP = PEP | abc + X = abc | | R | X | |
| R23 | PYR = ACCOA + CO2 + NADH | abc = bc + a + X | | F | | |
| R24 | ACCOA + OAA = CIT | ab + cdef = fedbac | | F | | |
| R25 | CIT = GLYOXY + 0.5 SUC + 0.5 SUC | abcdef = ab + 0.5 cdef + 0.5 fedc | | F | X | |
| R26 | ACCOA + GLYOXY = OAA | ab + cd = cdba | | F | | |
| R27 | CIT = AKG + CO2 + NADH | abcdef = abcde + f + X | | F | | |
| R28 | AKG = SUCCOA + CO2 + NADH | abcde = bcde + a + X | | F | | |
| R29 | SUCCOA = 0.5 SUC + 0.5 SUC + ATP | abcd = 0.5 abcd + 0.5 dcba + X | | F | | |
| R30 | SUC = FUM + FADH2 | abcd = dcba + X | | F | | |
| R31 | FUM = MAL | abcd = bcad | | F | | |
| R32 | MAL = OAA + NADH | abcd = acbd + X | | F | | |
| R33 | OAA = PEP + CO2 | abcd = abc + d | | FR | | |
| R34 | PEP + CO2 = OAA | abc + d = abcd | | R | X | |
| R35 | MAL = PYR + CO2 + NADH | abcd = abc + d + X | | F | X | |
| R36 | P5P + CO2 + ATP + ATP + ATP + ATP + | abcde + f + X + X + X + X + X = edcbaf | | F | | |
| R37 | CO2 = CO2_EX | a = a | | FR | | |
| R38 | CO2_EX = CO2 | a = a | | R | X | |
| R39 | 3PG = SER + NADH | abc = abc + X | | F | | |
| R40 | SER = GLY + MTHF | abc = ab + c | | F | | |
| R41 | AKG = AKG_B | | | B | 4.172 | 0.6827 |
| R42 | GLC6P + ATP = GLC6P_B | | | B | 1.1825 | 0.1935 |
| R43 | F6P = F6P_B | | | B | 0.4081 | 0.0688 |
| R44 | P5P = P5P_B | abcde = abcde | | F | 4.1565 | 0.68015 |

**Supplementary Table 1**

OpenFLUX model of *Xanthomonas campestris* pv. campestris B100

| | | | | | | |
|---|---|---|---|---|---|---|
| R45 | E4P = E4P_B | abcd = abcd | F | 1.9457 | 0.3184 | |
| R46 | G3P = G3P_B | | B | 1.1825 | 0.1935 | |
| R47 | 3PG = 3PG_B | abc = abc | F | 7.461 | 0.1935 | |
| R48 | PYR = PYR_B | abc = abc | F | 8.3069 | 1.22 | |
| R49 | OAA = OAA_B | abcd = abcd | F | 7.245 | 1.19 | |
| R50 | ACCOA = ACCOA_B | ab = ab | F | 19.717 | 1.185 | |
| R51 | E4P_B + PEP = SHKM | abcd + efg = efgabcd | F | | | |
| R52 | SHKM + PEP  = CHRM | abcdefg + hij = abcdefghij | F | | | |
| R53 | CHRM + ATP + NADH = PHEX + CO2 | abcdefghij + X + X= hijbcdefg + a | F | | | |
| R54 | CHRM + NADH + ATP = TYRX + CO2 + | abcdefghij + X + X = hijbcdefg + a + X | F | | | |
| R55 | CHRM = ANTHR + PYR | abcdefghij = abcdefg + hij | F | | | |
| R56 | ANTHR + P5P_B = CPADR5P | abcdefg + hijkl = abcdefghijkl | F | | | |
| R57 | CPADR5P = INDG + CO2 | abcdefghijkl = abcdfghijkl + e | F | | | |
| R58 | INDG = IND + G3P | abcdefghijk = abcdefgh + ijk | F | | | |
| R59 | PYR_B + ACCOA_B = 2OBUT + CO2 | abc + de = abdc + e | F | | | |
| R60 | 2OBUT + PYR_B = 2AHBUT + CO2 | abcd + efg = abcdfg + e | F | | | |
| R61 | 2AHBUT = 23DHMP | abcdef = abedcf | F | | | |
| R62 | 23DHMP + NADH = ILEX | abcdef + X = abcdef | F | | | |
| R63 | IND + 3PG_B +  ATP +  ATP +  ATP + | abcdefgh + ijk + X + X + X + X  = | F | | | |
| R64 | PYR_B + PYR_B = ISV + CO2 | abc + def = abefc + d | F | | | |
| R65 | ISV + ACCOA_B +  NADH = LEUX + | abcde + fg + X = fgbcde + a + X | F | | | |
| R66 | OAA_B = ASPX | abcd = abcd | F | | | |
| R67 | ASPX + PYR_B + ATP +  NADH +  NADH | abcd + efg + X + X  + X = abcdgf + e | F | | | |
| R68 | P5P_B = P5P_BT | | B | | | |
| R69 | E4P_B = E4P_BT | | B | | | |
| R70 | PYR_B = PYR_BT | | B | | | |
| R71 | OAA_B = OAA_BT | | B | | | |
| R72 | ACCOA_B = ACCOA_BT | | B | | | |
| R73 | 3PG_B = 3PG_BT | | B | | | |
| R74 | 0.12 HIS +  0.376 GLY + 0.34 LEUX + 0.166 ILEX  + 0.149 PHEX + 0.017 TRPX  + 0.187 | | B | 5.5 | 0.9 | |
| R75 | 0.4 GDPM +  0.4 UDPG + 0.2 UDPGA = XANTHAN | | | | B | 7.99 1.3 |
| R76 | PYR_B = ALAX | abc = abc | | | S | |
| R77 | AKG + NADH = GLUX | abcde + X  = abcde | | | S | |
| R78 | PYR_B + PYR_B  + NADH = VALX + CO2 | abc + def + X  = abefc + d | | | S | |
| R79 | OAA_B +  ATP +  ATP  + NADH + NADH = THR | abcd +  X +  X  +  X +  X= abcd | | | S | |

## excludedMetabolites
\# ATP
\# NADH
\# FADH2
\# PPi
\# MTHF
\# GLC_EX
\# THR_B
\# ASP_B
\# GLY_B
\# AKG_B

**Supplementary Table 1**

OpenFLUX model of *Xanthomonas campestris* pv. campestris B100

| | |
|---|---|
| # | ACETAL_B |
| # | GLC6P_B |
| # | F6P_B |
| # | P5P_BT |
| # | E4P_BT |
| # | G3P_B |
| # | 3PG_BT |
| # | PYR_BT |
| # | OAA_BT |
| # | ACCOA_BT |
| # | BIOMASS |
| # | XANTHAN |
| # | CO2_EX |
| | |
| ## | simulatedMDVs |
| # | ALAX#111 |
| # | VALX#11111 |
| # | ASPX#1111 |
| # | GLUX#11111 |
| # | SER#111 |
| # | PHEX#111111111 |
| # | GLY#11 |
| # | TYRX#111111111 |
| # | LEUX#011111 |
| # | THR#1111 |
| # | ALAX#011 |
| # | VALX#01111 |
| # | ASPX#1100 |
| # | HIS#111111 |
| # | SER#011 |
| # | PHEX#110000000 |
| # | GLY#01 |
| # | TYRX#110000000 |
| # | THR#0111 |
| | |
| ## | inputSubstrates |
| # | CO2_EX |
| # | GLC_EX |

**Supplementary Table 2** Confidence intervals for individual reaction rates using a nonlinear approach: calculated optimum of free fluxes and the associated 95% confidence interval

| **Reaction equation** | **fluxPar** | **opVal** | **low** | **high** |
|---|---|---|---|---|
| GLC6P = F6P/F6P = GLC6P | v(2)-v(3) | 3.6 | 2.3 | 4.4 |
| F6P + PPi = F16BP | v(7) | 2.4 | 1.7 | 2.8 |
| GLC6P = 6PG + NADH | v(10) | 90.4 | 89.6 | 91.8 |
| 6PG = P5P + CO2 + NADH | v(11) | 9.4 | 7.9 | 11.4 |
| P5P + P5P = S7P + G3P/S7P + G3P = P5P + P5P | v(12)-v(13) | 2.2 | 2.0 | 2.7 |
| S7P + G3P = E4P + F6P/E4P + F6P = S7P + G3P | v(14)-v(15) | 2.2 | 2.0 | 2.7 |
| E4P + P5P = F6P + G3P/F6P + G3P = E4P + P5P | v(16)-v(17) | 0.2 | 0.0 | 0.7 |
| GLC6P = G3P + PYR | v(18) | 81.0 | 80.5 | 81.8 |
| G3P = 3PG + ATP + NADH | v(19) | 84.9 | 84.2 | 85.3 |
| 3PG = PEP | v(20) | 75.4 | 74.7 | 75.8 |
| PEP = PYR + ATP | v(21) | 61.6 | 38.1 | 63.2 |
| PYR + ATP = PEP | v(22) | 0.2 | 0.0 | 1.0 |
| PYR = ACCOA + CO2 + NADH | v(23) | 134.2 | 132.5 | 136.6 |
| ACCOA + OAA = CIT | v(24) | 113.2 | 112.7 | 113.4 |
| CIT = GLYOXY + 0.5 SUC + 0.5 SUC | v(25) | 1.3 | 0.0 | 3.3 |
| ACCOA + GLYOXY = MAL | v(26) | 1.3 | 0.0 | 3.3 |
| CIT = AKG + CO2 + NADH | v(27) | 111.9 | 110.3 | 113.0 |
| AKG = SUCCOA + CO2 + NADH | v (28) | 107.7 | 106.1 | 108.9 |
| SUCCOA = 0.5 SUC + 0.5 SUC + ATP | v(29) | 107.7 | 106.1 | 108.9 |
| SUC = FUM + FADH2 | v(30) | 109.0 | 108.5 | 109.2 |
| FUM = MAL | v(31) | 109.0 | 108.5 | 109.2 |
| MAL = OAA + NADH | v(32) | 109.0 | 87.0 | 109.2 |
| PEP + CO2 = OAA/ OAA = PEP + CO2 | v(34)- v(33) | 10.1 | 8.2 | 33.5 |
| MAL = PYR + CO2 + NADH | v(35) | 0.0 | 0.0 | 27.4 |