

Table S1. Microorganisms and plasmids used in this study.

| Strain | Relevant characteristic(s) | References |
|----------------------------|--|---|
| <i>Sphingomonas</i> sp. A1 | | Murata <i>et al.</i> 2008 |
| Wild-type strain A1 | Wild type | |
| EPv1 | Plasmid 7 in wild-type strain A1 | This study |
| EPv14 | Plasmid 11 in wild-type strain A1 | This study |
| EPv87 | Plasmid 13 in wild-type strain A1 | This study |
| EPv88 | Plasmid 15 in wild-type strain A1 | This study |
| EPv90 | Plasmid 16 in wild-type strain A1 | This study |
| EPv98 | Plasmid 17 in wild-type strain A1 | This study |
| Δdh | <i>ldh::Km^r</i> | This study |
| EPv104 | Plasmid 17 in strain A1 Δdh | This study |
| <i>E. coli</i> | | |
| DH5 α | General cloning host strain | |
| HB101 | General cloning host strain | |
| JM110 | <i>dam, dcm, rpsL(strr), thr, leu, thi, hsdR17(r_K⁻, m_K⁺), lacY, galK, galT, ara, tonA, tsx, supE44, $\Delta(lac-proAB)$, [F', <i>traD36, proAB, lacIqZΔM15]</i></i> | |
| <i>Z. mobilis</i> ZM4 | Ethanologenic bacteria | ATCC 31821 |
| Plasmid | Plasmid name | |
| Plasmid 1 | pUC18 | Amp ^r , lacZ', pMB9 replicon |
| Plasmid 2 | pKS13 | Tet ^r , mob, cos, RK2 replicon |
| Plasmid 3 | pRK2013 | Km ^r , tra ^r , ColE1 replicon |
| Plasmid 4 | pUC18- <i>pdC</i> | <i>Z. mobilis pdc</i> in pUC18 |
| Plasmid 5 | pUC18- <i>adh</i> | <i>Z. mobilis adhB</i> in pUC18 |
| Plasmid 6 | pUC18- <i>pdC-adh</i> | <i>pdC</i> native promoter- <i>pdC</i> ORF and <i>adhB</i> native promoter- <i>adhB</i> ORF in pUC18 |
| Plasmid 7 | pKS13- <i>pdC-adh</i> | <i>pdC</i> native promoter- <i>pdC</i> ORF and <i>adhB</i> native promoter- <i>adhB</i> ORF in pKS13 |
| Plasmid 8 | pUC18- <i>sph2987p-adh</i> | Strain A1 <i>sph2987</i> promoter (2 kb)- <i>adhB</i> in pUC18 |
| Plasmid 9 | pKS13- <i>sph2987p-adh</i> | <i>sph2987</i> promoter (2 kb)- <i>adhB</i> in pKS13 |
| Plasmid 10 | pUC18- <i>sph2987p-pdC</i> | <i>sph2987</i> promoter (2 kb)- <i>pdC</i> in pUC18 |
| Plasmid 11 | pKS13- <i>sph2987p-pdC-sph2987p-adh</i> | <i>sph2987</i> promoter (2 kb)- <i>pdC</i> and <i>sph2987</i> promoter (2 kb)- <i>adhB</i> in pUC18 |
| Plasmid 12 | pUC18- <i>sph2987p(s)-pdC-sph2987p(s)-adh</i> | <i>sph2987</i> promoter (250 b)- <i>pdC</i> and <i>sph2987</i> promoter (250b)- <i>adhB</i> in pUC18 |
| Plasmid 13 | pKS13- <i>sph2987p(s)-pdC-sph2987p(s)-adh</i> | <i>sph2987</i> promoter (250 b)- <i>pdC</i> and <i>sph2987</i> promoter (250b)- <i>adhB</i> in pKS13 |
| Plasmid 14 | pUC18- <i>sph2987p(s)-pdC</i> | <i>sph2987</i> promoter (250b)- <i>pdC</i> in pUC18 |
| Plasmid 15 | pKS13- <i>[sph2987p(s)-pdC]₂-sph2987p(s)-adh</i> | Two copies of [<i>sph2987</i> promoter (250 b)- <i>pdC</i>] and single [<i>sph2987</i> promoter (250b)- <i>adhB</i>] in pKS13 |
| Plasmid 16 | pKS13- <i>[sph2987p(s)-pdC]₄-sph2987p(s)-adh</i> | Four copies of [<i>sph2987</i> promoter (250 b)- <i>pdC</i>] and single [<i>sph2987</i> promoter (250b)- <i>adhB</i>] in pKS13 |
| Plasmid 17 | pKS13- <i>[sph2987p(s)-pdC]₈-sph2987p(s)-adh</i> | Eight copies of [<i>sph2987</i> promoter (250 b)- <i>pdC</i>] and single [<i>sph2987</i> promoter (250b)- <i>adhB</i>] in pKS13 |
| Plasmid 18 | pUC4K | Amp ^r , Km ^r , ColE1 replicon |
| Plasmid 19 | pKTY320 | Amp ^r , Cm ^r , Mob, p15A replicon |
| Plasmid 20 | pKTY320- <i>ldh::Km^r</i> | Strain A1 <i>ldh::Km^r</i> in pKTY320 |

Table S2. Oligonucleotides used in this study as primer.

| Primer number | Sequence (5' to 3') | Purpose | Notes |
|---------------|--|---|--|
| Primer 1 | GAGGATCCTCACTTAATCCAGAAACGGGCG | <i>Z. mobilis pdc</i> cloning | Underline: <i>Bam</i> HI site |
| Primer 2 | GACTGCAGACGGGCTTTTCGCCTTAAGC | <i>pdc</i> cloning | Underline: <i>Pst</i> I site |
| Primer 3 | GACTGCAGAAAGGCCAAAATCGGTAACCACATCTC | <i>Z. mobilis adhB</i> cloning | Underline: <i>Pst</i> I site |
| Primer 4 | GTTCCTAGATTATGACGGTAGGCTTAATAGCCTG | <i>adhB</i> cloning | Underline: <i>Xba</i> I site |
| Primer 5 | CGGGATCCCCGGGTACCGAGCTCGAATTC | Inverse PCR of pUC18- <i>adhB</i> | |
| Primer 6 | GCTTCTTCAACTTTTTATATTCCTTTTCGTC AACGAAATG | Inverse PCR of pUC18- <i>adhB</i> | |
| Primer 7 | TACCCGGGGATCCCGAAGCGGCTCCGGGATAGAAC | Strain A1 <i>sph2987</i> promoter cloning | Underline: overlapping sequence for In-Fusion cloning |
| Primer 8 | AAAAGTTGAAGAAGCCATGTTGTCTGCCCTTTTACATAG | Strain A1 <i>sph2987</i> promoter cloning | Underline: overlapping sequence for In-Fusion cloning |
| Primer 9 | AGTTATACTGTGGTACCTATTTAGCGGAGC | <i>pdc</i> subcloning | |
| Primer 10 | TGCTCTAGAACGGGCTTTTCGCCTTAAGCTCTAAG | <i>pdc</i> subcloning | Underline: <i>Xba</i> I site |
| Primer 11 | CATGGTTGTCTGCCCTTTTACATAGTATGCGTTGAACAC | Inverse PCR of pUC18- <i>sph2987p-adh</i> | |
| Primer 12 | TGCTCTAGAGGATCCGTCGACCTGCAGGCATGCAAGCTTGG | Inverse PCR of pUC18- <i>sph2987p-adh</i> | Underline: <i>Xba</i> I and <i>Bam</i> HI sites |
| Primer 13 | CCGGAATTCTCTAGATCCCTTATGGGAGAAGACTTCTGATGGGCATC | Subcloning of <i>sph2987</i> promoter (250 b) and <i>pdc</i> | Underline: <i>Eco</i> RI and <i>Xba</i> I sites. Bold: dam methylation site. |
| Primer 14 | AACGCGTCGACACGGGCTTTTCGCCTTAAGCTCTAAGTT | Subcloning of <i>sph2987</i> promoter (250 b) and <i>pdc</i> | Underline: <i>Sal</i> I site |
| Primer 15 | AAAAGTGCAGTCTAGACCCTTATGGGAGAAGACTTCTGATGGGCATC | Subcloning of <i>sph2987</i> promoter (250 b) and <i>adhB</i> | Underline: <i>Pst</i> I and <i>Xba</i> I sites |
| Primer 16 | AACGCGTCGACTTATGACGGTAGGCTTAATAGCCTGTAAAAATTTGTAAC | Subcloning of <i>sph2987</i> promoter (250 b) and <i>adhB</i> | Underline: <i>Sal</i> I site |
| Primer 17 | TTTTCTGCAGTCTAGAACGGGCTTTTCGCCTTAAGCTCTAAGTT | Subcloning of <i>sph2987</i> promoter (250 b) and <i>pdc</i> | Underline: <i>Pst</i> I and <i>Xba</i> I sites |
| Primer 18 | CCGTCTAGATGCACGGCTCCTGTTGGCCGTCAG | Strain A1 <i>ldh</i> cloning | Underline: <i>Xba</i> I site |
| Primer 19 | CGCGGATCCAGATCACCTCGTTGGCCAGCGGTTCCCTTG | <i>ldh</i> cloning | Underline: <i>Bam</i> HI site |
| Primer 20 | GGCCGCATTGGCCAATGCTCGATGC | Kanamycin cassette insertion | |
| Primer 21 | TGCGCCGAGCGAACCGAAGTCTTG | Kanamycin cassette insertion | |

Table S4. Metabolome analysis of strain A1.

| Initial alginate conc. in the culture medium (% w/v) | Concentration (pmol / 8 × 10 ⁸ cells) | | | | | | | | | | | | | | | |
|--|--|-------|-----------|------|-----------|-------|-----------|------|------------------|------|------------------|------|------------------|-------|------------------|-------|
| | 0.5 | | 3.0 | | 0.5 | | 3.0 | | 0.5 | | 3.0 | | 0.5 | | 3.0 | |
| | 50 | | 50 | | 100 | | 100 | | 50 | | 50 | | 100 | | 100 | |
| Shaking speed (rpm) | Log phase | | Log phase | | Log phase | | Log phase | | Stationary phase | | Stationary phase | | Stationary phase | | Stationary phase | |
| Metabolites | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| ATP | 365.5 | 253.7 | 62.0 | 16.0 | 191.6 | 232.2 | 21.5 | 17.7 | 601.5 | 82.2 | 91.3 | 51.5 | 865.6 | 163.6 | 262.8 | 151.6 |
| Lactic acid | 329.7 | 187.9 | 263.6 | 52.6 | 193.0 | 124.8 | 323.5 | 59.9 | 271.2 | 56.5 | 523.6 | 63.9 | 353.0 | 107.3 | 333.5 | 108.5 |
| ADP | 210.9 | 40.8 | 75.0 | 14.8 | 136.6 | 49.3 | 63.0 | 28.7 | 204.3 | 36.3 | 103.7 | 19.6 | 253.8 | 27.7 | 215.2 | 19.9 |
| dTMP | 123.3 | 63.0 | 7.6 | 3.4 | 109.4 | 81.5 | 13.3 | 14.0 | 21.3 | 7.1 | 3.2 | 1.5 | 18.9 | 4.7 | 7.4 | 1.4 |
| UTP | 120.1 | 110.9 | 13.4 | 6.5 | 68.6 | 116.2 | 9.7 | 3.3 | 166.3 | 30.4 | 23.0 | 17.6 | 258.6 | 56.8 | 171.4 | 104.2 |
| AMP | 108.8 | 22.1 | 56.4 | 11.2 | 83.6 | 11.9 | 75.7 | 36.0 | 100.8 | 16.5 | 77.4 | 8.3 | 127.1 | 13.7 | 152.5 | 17.2 |
| Acetyl CoA_divalent | 78.7 | 19.4 | 44.2 | 20.2 | 84.2 | 53.3 | 34.9 | 37.1 | 173.1 | 36.1 | 53.9 | 26.3 | 119.4 | 66.2 | 93.3 | 34.8 |
| dTDP | 73.8 | 36.1 | 3.4 | 1.8 | 60.1 | 43.7 | 8.6 | 8.2 | 7.9 | 2.1 | 1.6 | 0.3 | 13.5 | 2.0 | 4.6 | 1.2 |
| GDP | 70.2 | 11.2 | 29.4 | 4.6 | 50.8 | 9.6 | 29.1 | 5.9 | 46.2 | 9.0 | 41.7 | 11.0 | 46.5 | 10.4 | 33.5 | 3.7 |
| Succinic acid | 40.6 | 14.9 | 26.8 | 6.7 | 32.7 | 10.4 | 24.2 | 15.8 | 66.6 | 20.6 | 27.6 | 4.1 | 86.0 | 25.5 | 52.3 | 11.7 |
| CMP | 39.7 | 15.4 | 5.3 | 2.0 | 39.9 | 26.6 | 8.6 | 7.7 | 17.1 | 1.1 | 3.9 | 0.6 | 30.0 | 8.5 | 31.5 | 3.6 |
| CTP | 36.7 | 34.7 | 3.7 | 1.5 | 27.0 | 50.8 | 2.1 | 1.2 | 69.3 | 17.6 | 6.5 | 5.6 | 158.8 | 36.1 | 41.3 | 25.2 |
| Glycolic acid | 34.6 | 22.9 | 27.3 | 6.6 | 17.3 | 4.1 | 18.1 | 3.1 | 33.3 | 6.9 | 45.1 | 11.5 | 23.3 | 4.6 | 27.1 | 5.5 |
| FAD_divalent | 25.9 | 4.9 | 19.7 | 5.4 | 28.7 | 5.4 | 22.0 | 4.6 | 21.1 | 3.2 | 23.0 | 3.0 | 21.0 | 4.3 | 26.2 | 6.5 |
| dATP | 21.1 | 11.9 | 2.0 | 0.6 | 21.4 | 10.1 | 1.5 | 0.3 | 14.8 | 2.1 | 1.8 | 0.7 | 24.3 | 2.9 | 11.8 | 5.3 |
| CDP | 21.0 | 5.3 | 4.7 | 1.7 | 10.6 | 11.5 | 2.1 | 2.3 | 21.4 | 3.9 | 6.5 | 2.4 | 32.9 | 9.2 | 20.3 | 4.5 |
| 2-Oxoisovaleric acid | 15.8 | 10.3 | 13.2 | 1.9 | 6.3 | 2.3 | 23.8 | 4.1 | 11.8 | 2.7 | 13.6 | 1.8 | 10.4 | 3.4 | 22.9 | 7.5 |
| Sedoheptulose 7-phosphate | 10.5 | 4.7 | 1.8 | 0.6 | 12.9 | 12.8 | 1.4 | 1.1 | 25.6 | 3.9 | 1.7 | 0.5 | 35.2 | 17.8 | 15.2 | 5.1 |
| dTTP | 10.5 | 6.1 | 1.7 | 0.3 | 5.9 | 4.5 | 1.3 | 0.4 | 13.9 | 2.0 | 1.5 | 0.4 | 28.6 | 8.9 | 3.6 | 1.4 |
| Citric acid | 8.3 | 5.3 | 1.3 | 0.3 | 28.8 | 43.5 | N.D. | N.D. | 43.3 | 7.0 | 1.6 | 0.9 | 116.4 | 24.7 | 8.0 | 2.5 |
| Malic acid | 7.2 | 5.7 | 3.4 | 0.7 | 3.9 | 1.6 | 2.1 | 1.1 | 7.9 | 1.5 | 4.8 | 1.5 | 11.7 | 2.7 | 20.5 | 5.6 |
| 3-Phosphoglyceric acid | 5.2 | 3.0 | N.D. | N.D. | 2.0 | 1.0 | N.D. | N.D. | 4.5 | 1.4 | N.D. | N.D. | 4.9 | 1.0 | 1.2 | 0.4 |
| Fumaric acid | 5.1 | 2.0 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 5.7 | 1.2 | N.D. | N.D. | 5.6 | 1.6 | 12.5 | 4.9 |
| Glycerol 3-phosphate | 4.7 | 2.1 | 2.9 | 0.5 | 2.5 | 0.5 | 2.7 | 0.7 | 12.6 | 2.7 | 6.1 | 1.9 | 9.8 | 1.8 | 17.0 | 6.5 |
| Dihydroxyacetone phosphate | 4.3 | 2.4 | N.D. | N.D. | 4.3 | 1.1 | 1.4 | N.D. | 17.2 | 3.0 | 5.3 | N.D. | 10.6 | 5.4 | 4.6 | 1.9 |
| dCTP | 4.2 | 2.3 | 0.4 | 0.11 | 2.8 | 3.3 | N.D. | N.D. | 4.6 | 0.8 | 0.6 | N.D. | 9.0 | 2.5 | 3.5 | 2.2 |
| Phosphoenolpyruvate | 4.1 | 2.4 | 1.7 | N.D. | 1.8 | 0.6 | 1.4 | 0.6 | 3.5 | 1.0 | 3.4 | 1.0 | 3.1 | 1.2 | 2.7 | 0.6 |
| GMP | 3.7 | 1.1 | 1.9 | 0.4 | 2.0 | 0.6 | 2.0 | 1.5 | 8.3 | 1.9 | 2.4 | 0.3 | 10.8 | 1.8 | 4.1 | 1.3 |
| G6P | 3.5 | 1.9 | 0.9 | 0.2 | 3.7 | 2.4 | 1.2 | 0.3 | 5.6 | 4.3 | 0.6 | 0.14 | 22.2 | 14.5 | 30.3 | 18.6 |
| CoA_divalent | 2.4 | 1.5 | 1.5 | 0.9 | 2.3 | 1.5 | 5.8 | 9.7 | 3.7 | 2.4 | 5.0 | 5.4 | 2.1 | 1.5 | 11.1 | 7.0 |
| Ribose 5-phosphate | 2.0 | 0.9 | 0.5 | 0.07 | 2.0 | 1.3 | 0.6 | 0.4 | 1.9 | 0.6 | 0.7 | 0.2 | 3.1 | 2.0 | 2.4 | 1.1 |
| Ribulose 5-phosphate | 1.8 | 0.8 | 0.8 | 0.3 | 1.9 | 1.4 | 0.4 | N.D. | 1.7 | 0.5 | 0.8 | 0.4 | 2.1 | 1.6 | 2.0 | 0.8 |
| Succinyl CoA_divalent | 1.7 | 0.7 | 2.4 | 0.7 | 1.4 | 0.3 | 3.9 | 1.9 | 2.0 | 1.1 | 1.6 | 0.9 | 1.8 | 1.3 | 4.9 | 3.2 |
| Fructose 1,6-diphosphate | 1.7 | 0.4 | 0.4 | N.D. | 3.5 | 5.7 | 0.9 | 0.2 | 2.2 | 1.3 | 0.4 | N.D. | 2.5 | 1.6 | 11.0 | 7.1 |
| PRPP | 1.1 | N.D. | N.D. | N.D. | 4.1 | 5.1 | N.D. | N.D. | 3.9 | 1.7 | N.D. | N.D. | 4.0 | 1.8 | 0.7 | N.D. |
| 6-Phosphogluconic acid | 1.1 | 0.4 | N.D. | N.D. | 1.2 | 1.3 | N.D. | N.D. | 0.4 | N.D. | N.D. | N.D. | 0.7 | 0.5 | 0.4 | 0.14 |
| F6P | 1.1 | 0.4 | N.D. | N.D. | 1.1 | 0.2 | 0.3 | 0.06 | 1.8 | 1.2 | N.D. | N.D. | 6.8 | 4.4 | 7.1 | 4.7 |
| G1P | 0.7 | 0.2 | N.D. | N.D. | 1.4 | 0.2 | N.D. | N.D. | 1.2 | 0.6 | N.D. | N.D. | 1.8 | 0.9 | 3.7 | 2.1 |
| IMP | 0.5 | 0.18 | N.D. | N.D. | 0.6 | 0.2 | 0.8 | N.D. | 1.6 | 0.5 | 0.3 | N.D. | 2.2 | 0.4 | 1.0 | 0.5 |
| Gluconic acid | 0.4 | 0.11 | 0.4 | 0.11 | 0.3 | 0.09 | 0.14 | 0.02 | 0.5 | 0.08 | 0.3 | 0.04 | 0.4 | 0.08 | 0.4 | 0.08 |
| cAMP | 0.3 | 0.2 | N.D. | N.D. | 0.2 | 0.02 | N.D. | N.D. | 0.6 | 0.4 | N.D. | N.D. | 0.8 | 0.5 | 0.2 | N.D. |
| Erythrose 4-phosphate | N.D. | N.D. | 1.6 | N.D. | 0.8 | 0.08 | 0.8 | 0.11 | 1.2 | 0.6 | N.D. | N.D. | 1.1 | 0.5 | 0.9 | 0.13 |
| cis-Aconitic acid | N.D. | N.D. | N.D. | N.D. | 3.6 | 1.7 | N.D. | N.D. | 4.3 | 0.5 | N.D. | N.D. | 4.8 | 1.3 | N.D. | N.D. |
| Glyoxylic acid | N.D. | N.D. | N.D. | N.D. | 3.2 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Pyruvic acid | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 24.0 | N.D. | 27.9 | 7.7 | N.D. | N.D. | 28.5 | 5.3 | 147.0 | 133.0 |
| 2-Oxoglutaric acid | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 80.2 | 82.7 |
| DEH | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 10.2 | N.D. |
| Isocitric acid | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| cGMP | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Malonyl CoA_divalent | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| GTP | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Acetyl-P | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |

Mean values and SDs ($n = 5$) are shown.
 "ND", not detected.