**Supplementary Information**

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**Table S1** Fermentation profiles of consortia consisting of *S. stipitis* CICC1960 and *Z. mobilis* 8b in 80G40XRM

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| a *S. stipitis* CICC1960 : *Z. mobilis* 8b | Glucose consumption (g/L) | Xylose consumption (g/L) | Ethanol | | |
| Titer  (g/L) | Yield  (g/g) | Productivity (g/L/h) |
| *S. stipitis* CICC1960 | 76.29 ± 2.50 | 12.71 ± 0.81 | 38.56 ± 0.29 | 0.43 ± 0.01 | 4.28 ± 0.03 |
| *Z. mobilis* 8b | 76.29 ± 2.50 | 34.81 ± 0.55 | 53.71 ± 2.03 | 0.48 ± 0.02 | 5.10 ± 0.17 |
| 3:1 | 76.29 ± 2.50 | 21.93 ± 1.03 | 49.22 ± 0.49 | 0.50 ± 0.01 | 4.20 ± 0.08 |
| 2:1 | 76.29 ± 2.50 | 28.23 ± 0.54 | 52.70 ± 0.75 | 0.50 ± 0.00 | 4.47 ± 0.10 |
| 1:1 | 76.29 ± 2.50 | 36.60 ± 0.48\*\* | 55.76 ± 1.11 | 0.49 ± 0.01 | 4.86 ± 0.16 |
| 1:2 | 76.29 ± 2.50 | 37.06 ± 0.17\*\* | 54.84 ± 2.18 | 0.48 ± 0.02 | 5.02 ± 0.04 |
| 1:3 | 76.29 ± 2.50 | 36.73 ± 0.48\*\* | 57.21 ± 1.73 | 0.50 ± 0.01 | 4.99 ± 0.09 |

Data are mean ± standard error from four replicates. A significant difference was determined using a one-way ANOVA followed by the Turkey test. Asterisks indicate that this group data was significantly higher than that in *Z. mobilis* 8b mono-fermentation (none, P > 0.05; \*\*, P < 0.01)

a *S. stipitis* CICC1960 : *Z. mobilis* 8b represent the initial inoculum proportion between the two species

**Table S2** Fermentation profiles of *Z. mobilis* 8b, *Z. mobilis* FR1, and *Z. mobilis* FR2 in 80G40XRM

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Strain | Glucose consumed (g/L) | Xylose consumed (g/L) | Ethanol | | |
| Titer (g/L) | Yield (g/g) | Productivity (g/L/h) |
| *Z. mobilis* 8b | 72.95 ± 0.73 | 31.38 ± 0.42 | 49.75 ± 0.16 | 0.48 ± 0.00 | 4.84 ± 0.01 |
| *Z. mobilis* FR1 | 72.95 ± 0.73 | 30.99 ± 0.33 | 47.94 ± 1.56 | 0.46 ± 0.01 | 4.87 ± 0.00 |
| *Z. mobilis* FR2 | 72.95 ± 0.73 | 36.20 ± 0.33\*\* | 53.14 ± 1.06 | 0.49 ± 0.01 | 5.08 ± 0.00\*\* |

Data are mean ± standard error from four replicates. A significant difference was determined using a one-way ANOVA followed by the Turkey test, if P > 0.05 in the test of homogeneity of variances. Otherwise, significant difference was determined by a nonparametric test (Kruskal-Wallis H). Asterisks indicate that the group data was significantly higher than that in *Z. mobilis* 8b fermentation (none, P > 0.05; \*\*, P < 0.01)

**Table S3** Strains, plasmids, and sgRNAs used in this study

|  |  |  |  |
| --- | --- | --- | --- |
| Strain, plasmid or sgRNA | Characteristic | | Source |
| Strain |  | |  |
| *Z. mobilis* 8b | Having exogenous xylose-metabolic pathway | Kindly given by Shihui Yang, Hubei University (14) | |
| *Z. mobilis* FR1 | Derived from *Z. mobilis* 8b; ZMO0256::P*pdc*-*talB*-*tktA* | | This study |
| *Z. mobilis* FR2 | Derived from *Z. mobilis* FR1; ZMO0689::P*pdc*-*xylA*-*xylB* | | This study |
| *S. stipitis* CICC1960 |  | | China Center of Industrial Culture Collection |
| *E. coli* trans110 |  | | TransGen Biotech |
| Plasmid |  | |  |
| Pmini | addA | | Kindly given by Nan Peng, Huazhong Agricultural University (39) |
| Pmini-P*pdc*-*talB*-*tktA* | Derived from Pmini; carrying sgRNA (ZMO0256), ZMO0256up500, P*pdc*-*talB*-*tktA* and ZMO0256down500 | | This study |
| Pmini-P*pdc*-*xylA*-*xylB* | Derived from Pmini; carrying sgRNA (ZMO0689), ZMO0689up540, P*pdc*-*xylA*-*xylB* and ZMO0689down500 | | This study |

**Table S3** (continued)

|  |  |  |
| --- | --- | --- |
| sgRNA |  |  |
| sgRNA (ZMO0256) | AGATACAGAAGATTTTCTGGTACCGTTCACTGCCGCACAGGCAGCTTAGAAAGACATGGGAATGAAACAGAAAATCAGCAATTTGTTCACTGCCGCACAGGCAGCTTAGAAAGGATCCTCGAACGCGCCGAATAAG | This study |
| sgRNA (ZMO0689) | AGATACAGAAGATTTTCTGGTACCGTTCACTGCCGCACAGGCAGCTTAGAAAAATGCTGTTTCAGCAACGGATGACAACGCTTCGTTCACTGCCGCACAGGCAGCTTAGAAAGGATCCTCGAACGCGCCGAATAAG | This study |

**Table S4** Primers used in this study

|  |  |
| --- | --- |
| Primers | Sequence (5’ to 3’) |
| sgRNA-F | CCATTGTAGATACAGAAGATTTTCTGGTACCG |
| sgRNA-R | ACCTGAATTACTTATTCGGCGCGTTCGA |
| Pmini-F | GCCGAATAAGTAATTCAGGTTTTTTTATAAAGACCTG |
| Pmini-R | CCAGAAAATCTTCTGTATCTACAATGGCTAATTTTATTATTAGAATG |
| 130bp-F | ATTTACGATTGCTCGTCCTAAATAAATAAG |
| 130bp-R | CACTTCACTGACACCCTCATCAGTGCCAAC |
| Pmini-backbone-F | ACTAGTAGCGGCCGCTGCAG |
| Pmini-backbone-R | GTCGACACTTGCAGCTTGATATAGCCGGTA |
| ZMO0256up500-F | TATATCAAGCTGCAAGTGTCGACTTTGATGACGATTGTG |
| ZMO0256up500-R | AAGGAACGGGACACCTTGAATATATCGACCTTTTATTTTC |
| ZMO0256down500-F | GTTGATGCAAAGAGGCCCTGCCTTTACG |
| ZMO0256down500-R | CTGCAGCGGCCGCTACTAGTCTAAGAAAGTATCTTTTCCGT |
| *talB*-F | TTTGAATATATGGAGTAAGCAATGACGGACAAATTGACCTCCCT |
| *tktA*-R | CAGGGCCTCTTTGCATCAACTTACAGCAGTTCTTTTGC |
| P*pdc*-F | TTCAAGGTGTCCCGTTCCTT |

**Table S4** (continued)

|  |  |
| --- | --- |
| P*pdc*-R | TGCTTACTCCATATATTCAAAACACTATGTCT |
| ZMO0256-checkF | TACCACGAAAGGCGGCGTTATTCC |
| ZMO0256-checkR | GCGAAAAGCTGCGGCAAGCGATC |
| ZMO0689up540-F | GCTATATCAAGCTGCAAGTGTCGACGTCTTTGCGTCCAGAAAAGACAGCA |
| ZMO0689up540-R | GAAAAAGGAACGGGACACCTTGAAAATCCTTGTTTCTTTCTTAACTAACC |
| ZMO0689down500-F | TATCCGTGCTTTCAAAGCCGGCAAGCATGT |
| ZMO0689down500-R | CTGCAGCGGCCGCTACTAGTGCACCGAGAAACGTGAA |
| *xylA*-F | TTTGAATATATGGAGTAAGCAATGCAAGCCTATTTTGACCAGCTCGA |
| *xylB*-R | CCGGCTTTGAAAGCACGGATATTACGCCATTAATGGCAGAA |
| ZMO0689-checkF | ATCAATGGATCTCCGAAGAGGCTT |
| ZMO0689-checkR | CATCATCGACTGGTTAGCATGGC |