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Supplementary materials to:

# An Optimized *Ustilago Maydis* for Itaconic Acid Production at Maximal Theoretical Yield

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**Table S1.** Oligonucleotides used for deletion and overexpression constructs.

| primer name | sequence (5′-3′) & description |
|---|---|
| JB-89 | ctcgagtttttcagcaagatCCGATCGCTGTTAGGACAC<br>Amplification of 5′-UTR flank for generation of *fuz7* deletion construct |
| JB-90 | acttctggccCGTGAAACGTTGCAAAACAG<br>Amplification of 5′-UTR flank for generation of *fuz7* deletion construct |
| JB-91 | acgtttcacgGGCCAGAAGTTCCTATTC<br>Amplification of FRT_m1-HygR-FRT_m1 cassette for generation of *fuz7* deletion construct |
| JB-92 | tctcagtcggCCCGGGAAGTTCCTATAC<br>Amplification of FRT_m1-HygR-FRT_m1 cassette for generation of *fuz7* deletion construct |
| JB-93 | acttcccgggCCGACTGAGAGATTATGGTC<br>Amplification of 3′-UTR flank for generation of *fuz7* deletion construct |
| JB-94 | aggagatcttctagaaagatAATCGGAACCGTGTACCTG<br>Amplification of 3′-UTR flank for generation of *fuz7* deletion construct |
| JB-126_fwd | ATGGCTTCTCAATCGCAC<br>Amplification of reference gene UMAG_02592 during qRT-PCR |
| JB-127_rev | CCTGGTGTTGAGGATGAG<br>Amplification of reference gene UMAG_02592 during qRT-PCR |
| JB-128_fwd | ACATCGTCAAGGCTATCG<br>Amplification of reference gene UMAG_03726 during qRT-PCR |
| JB-129_rev | AAAGAACACCGGACTTGG<br>Amplification of reference gene UMAG_03726 during qRT-PCR |
| JB-132_fwd | AACACGTTCAACTGCGTCAA<br>Amplification of *mttA* during qRT-PCR |
| JB-133_rev | GAACATGATGGCCGAGGTG<br>Amplification of *mttA* during qRT-PCR |
| HT-4a_rev | ACAGACGTCGCGGTGAGTTC<br>Verification of FRT-HygR-cassette based insertions |
| HT-202_fwd | TCCTGCGTCAGTCGTCCAAC<br>Verification of $P_{etef}$*mttA* integration |
| HT-203_fwd | GTCCGAGGGCAAAGGAATAG<br>Verification of *fuz7* deletion |
| HT-210_fwd | TCGCTGTTAGGACACAACTG<br>Amplification of *fuz7* deletion construct |
| HT-210a | TCGGTGTGCGGCGATTTCTG |

| | Verification of *fuz7* deletion |
|---|---|
| HT-211 | CCGTGTACCTGGCTGTGTAG<br>Amplification of *fuz7* deletion construct |
| HT-220 | GATTCTGTGGGACAAGAAGC<br>Verification of *fuz7* deletion |
| Tnos | CAAGACCGGCAACAGGATTC<br>Verification of $P_{etef}$*mttA* integration |

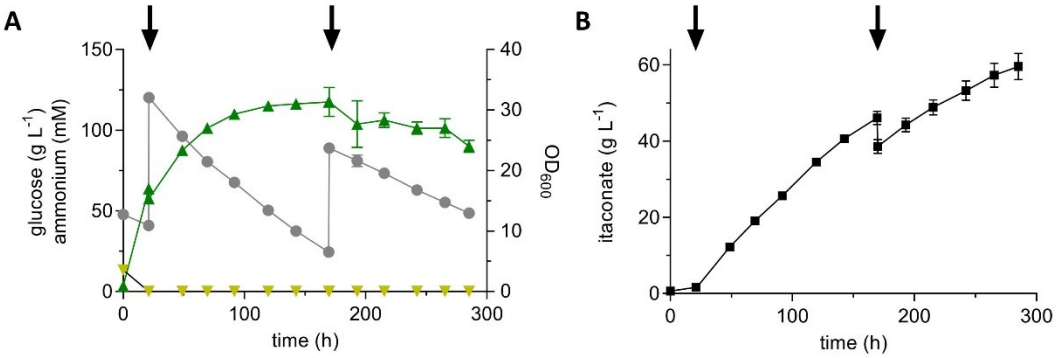


**Figure S1.** Low-density pulsed fed-batch fermentation of *U. maydis* strain K14. (A) concentration of glucose (●), ammonium (▼) and $OD_{600}$ (▲) and (B) concentration of itaconate (■) during fermentation in a bioreactor containing batch medium with 50 g $L^{-1}$ glucose and 0.8 g $L^{-1}$ $NH_4Cl$. The pH was kept at 6.5 by automatic titration with NaOH. Arrows indicate the addition of 80 g glucose. Error bars indicate the standard error of the mean (n = 3).

**Table S2.** Production parameters of two engineered *U. maydis* MB215 strains in two different types of fed-batch fermentations. ±values indicate the standard error of the mean (n = 3 for 50 g $L^{-1}$ glucose fermentation) and the deviation from the mean (n = 2 for 200 g $L^{-1}$ glucose fermentation).

| Fermentation conditions | Feed | Strain | ITA $titer_{max}$[a] (g $L^{-1}$) | $q_P$[b] (g $L^{-1}$ $h^{-1}$) | $y_{P/S}$[c] ($g_{ITA}$ $g_{glu}^{-1}$) |
|---|---|---|---|---|---|
| 200 g $L^{-1}$ glucose<br>4 g $L^{-1}$ $NH_4Cl$<br>$CaCO_3$ | Pulsed | *U. maydis* MB215 Δ*cyp3* Δ*fuz7* Δ$P_{ria1}$::$P_{etef}$ $P_{etef}$*mttA* | 220.3 | 0.46 | 0.33 |
| | Pulsed | *U. maydis* strain K14 | 205.6 ± 1.1 | 0.43 ± 0.00 | 0.32 ± 0.00 |
| 50 g $L^{-1}$ glucose<br>0.8 g $L^{-1}$ $NH_4Cl$<br>NaOH | Pulsed | *U. maydis* MB215 Δ*cyp3* Δ*fuz7* Δ$P_{ria1}$::$P_{etef}$ $P_{etef}$*mttA* | 35.9 ± 1.5 | 0.12 ± 0.00 | 0.20 ± 0.01 |
| | Pulsed | *U. maydis* strain K14 | 59.6 ± 5.9 | 0.21 ± 0.02 | 0.42 ± 0.02 |
| | Continuous | *U. maydis* strain K14 | 75.7 ± 1.3 | 0.24 ± 0.01 | 0.66 ± 0.02 |

a. Maximum itaconate titer (g $L^{-1}$).
b. Overall itaconate production rate ([glucose] > 5.5 g $L^{-1}$).
c. Yield itaconate per consumed glucose.