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# Pathway Compartmentalization in Peroxisome of *Saccharomyces cerevisiae* to Produce Versatile Medium Chain Fatty Alcohols

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Figure S1

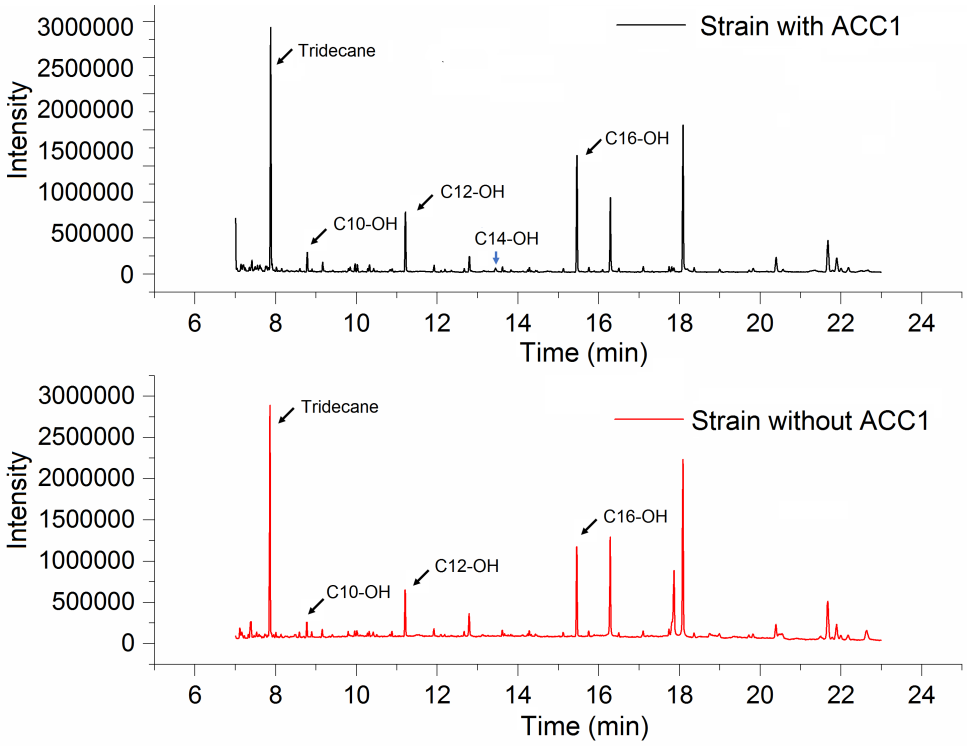


Figure S1: The GC spectrum of fatty alcohols produced by the strains with or without ACC1 overexpressed. Both of the strains were constructed in *S. cerevisiae* BY4741 with TaFAR expressed under constitutive TEF1 promoter and tagged with the PTS2 peptide (KL-QL) and the PEX7 was expressed under constitutive TPI1 promoter.

Figure S2

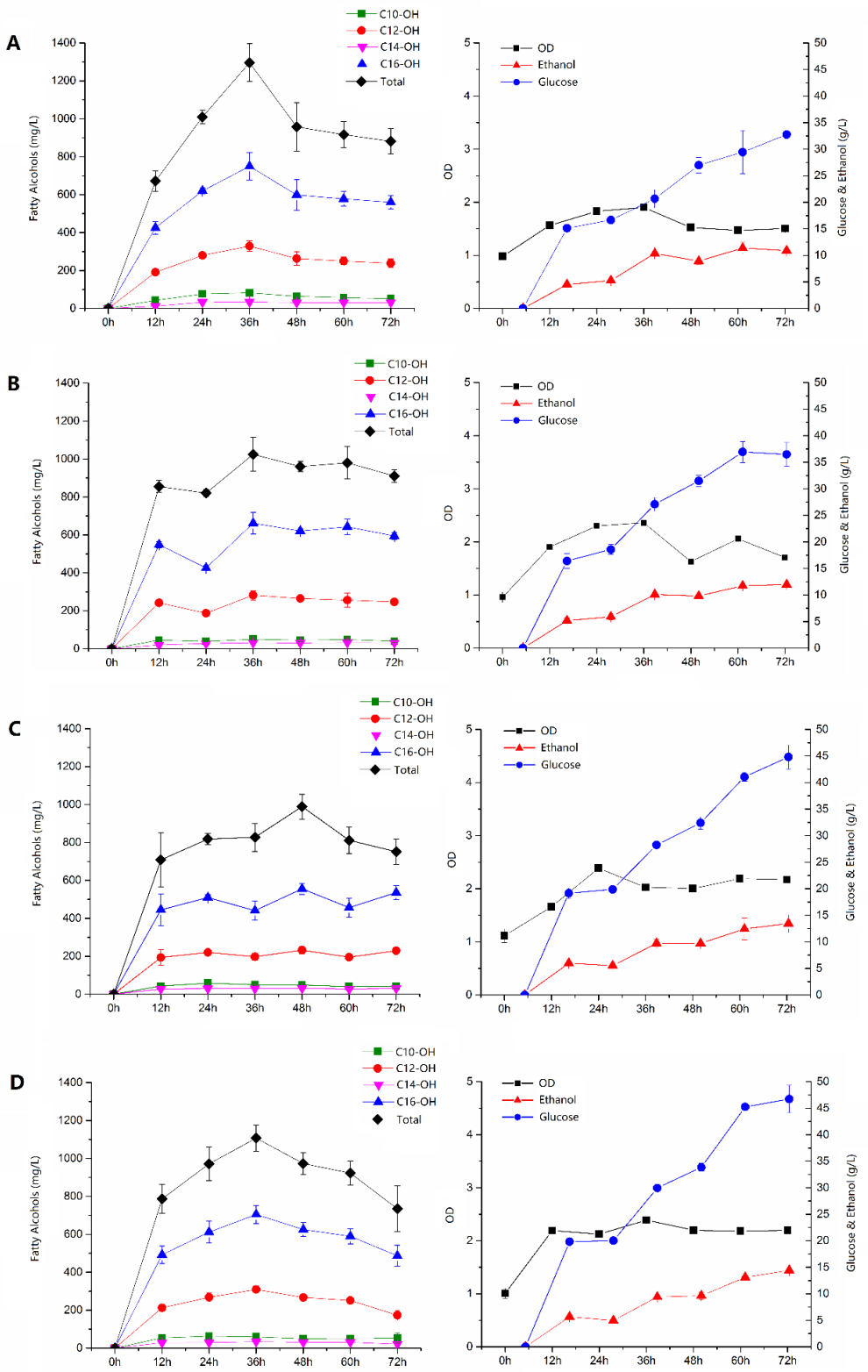


Figure S2. Medium optimization and fed-batch fermentation. (A) Nitrogen limited SC medium with C:N ratio at 50:1; (B) Nitrogen limited SC medium with C:N ratio at 50:1, supplement of 40 mM KCl and 10 mM KOH.; (C) Normal SC medium (control condition with C:N ratio at 3:1); and (D) Normal SC medium with supplement of 40 mM KCl and 10 mM KOH.

Figure S3

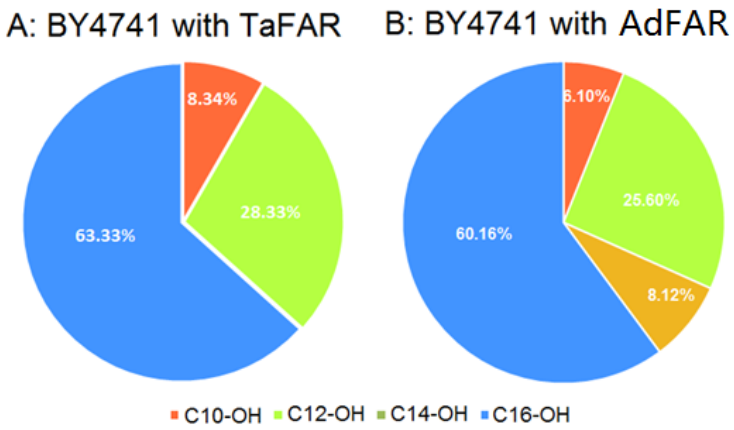


Figure S3: Profiles of fatty alcohols produced by expressing different FAR enzymes in peroxisome. (A) Strain MFAOH31 with TaFAR expressed in peroxisome (8.34% 1-decanol, 28.33% 1-dodecanol and 63.33% 1-hexadecanol); (B) Strain MFAOH38 with AdFAR1 expressed in peroxisome (6.10% 1-decanol, 26.60% 1-dodecanol, 8.12% 1-tetradecanol and 60.16% 1-hexadecanol).

**Table S1** The primers used in this study.

| Name | Sequence (5'-3') |
|---|---|
| TEF1p F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGTTTCCATACCTGAATA |
| TEF1t R | GCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCGATAGCGCCGATCAAAGTA |
| TaFAR-SKL R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCATAGCTTGGAGTATCTCA |
| TaFAR-SKF R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCAGAACTTGGAGTATCTCA |
| TaFAR-SFL R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCATAGGAAGGAGTATCTCA |
| TaFAR-SKV R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCAAACCTTGGAGTATCTCA |
| TaFAR-LKL R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCATAGCTTTAGGTATCTCA |
| TaFAR-FKL R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCATAGCTTGAAGTATCTCA |
| TaFAR-SHL R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCATAGATGGGAGTATCTCA |
| TEF1t-SKL F | CTTCAGAGCCTCCAGTACTATGAGATACTCCAAGCTATGAGGAGATTGATAAGACTTTTC |
| TEF1t-SKF F | CTTCAGAGCCTCCAGTACTATGAGATACTCCAAGTTCTGAGGAGATTGATAAGACTTTTC |
| TEF1t-SFL F | CTTCAGAGCCTCCAGTACTATGAGATACTCCTTCCTATGAGGAGATTGATAAGACTTTTC |
| TEF1t-SKV F | CTTCAGAGCCTCCAGTACTATGAGATACTCCAAGGTTTGAGGAGATTGATAAGACTTTTC |
| TEF1t-LKL F | CTTCAGAGCCTCCAGTACTATGAGATACCTAAAGCTATGAGGAGATTGATAAGACTTTTC |
| TEF1t-FKL F | CTTCAGAGCCTCCAGTACTATGAGATACTTCAAGCTATGAGGAGATTGATAAGACTTTTC |
| TEF1t-SHL F | CTTCAGAGCCTCCAGTACTATGAGATACTCCCATCTATGAGGAGATTGATAAGACTTTTC |
| RV-QL F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAACGTGTTCAAAGTATTA AAGATCAACTTGTTTCCATACCTGAATATTAT |
| KL-QL F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAAAAACTTCAAAGTATTA AAGATCAACTTGTTTCCATACCTGAATATTAT |
| KI-QL F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAAAAAATTCAAAGTATTA AAGATCAACTTGTTTCCATACCTGAATATTAT |
| KV-HL F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAAAAAGTTCAAAGTATTA AAGATCATCTTGTTTCCATACCTGAATATTAT |
| TEF1p RV-QL R | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAACGTGTTCAAAG |
| TEF1p KL-QL R | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAAAAACTTCAAAG |
| TEF1p KI-QL R | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAAAAAATTCAAAG |
| TEF1p KV-HL R | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTCAAAAAGTTCAAAG |
| PEX5 F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGACGTAGGAAGTTGCTC |
| PEX5 R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTTAAAACGAAAATTCTCCTT |
| TEP1p PEX5 R | CAAGCGGATTATTTCCCACTGAGCAACTTCCTACGTCCATTTTGTAATTAAAACTTAGAT |
| TEP1t PEX5 F | CATGGACCTGAAAAGATTTAAAGGAGAATTTTCGTTTTAAGGAGATTGATAAGACTTTTC |
| PEX7 F1 | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGCTCAGATATCATATGCA |
| PEX7 R1 | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTCAACCTAAGCCGTTCCATA |
| TEF1p PEX7 R | CACCGTACCCACTAAAACCTTGCATATGATATCTGAGCATTTTGTAATTAAAACTTAGAT |
| TEF1t PEX7 F | ATGGGATGGAAATTTATTTGTATGGAACGGCTTAGGTTGAGGAGATTGATAAGACTTTTC |
| TPI1p F | ATTCGATATTGTCGTAACAAATACTTTGATCGGCGCTATCTATATCTAGGAACCCATCAG |
| TPI1p PEX7 R | CACCGTACCCACTAAAACCTTGCATATGATATCTGAGCATTTTTAGTTTATGTATGTGTT |
| PEX7 F2 | TTAAATCTATAACTACAAAAAACACATACATAAACTAAAAATGCTCAGATATCATATGCA |
| PEX7 R2 | TAACTAATTACATGATATCGACAAAGGAAAAGGGGCCTGTTCAACCTAAGCCGTTCCATA |

| | |
|---|---|
| CYC1t PEX7 F | ATGGGATGGAAATTTATTTGTATGGAACGGCTTAGGTTGAACAGGCCCCTTTTCCTTTGT |
| CYC1t R | CCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGCAAATTAAAGCCTTCGAGC |
| TEF1p PEX3 R | GTCTCTGCAGAAGCGAACGTGATCTTTGATTTGGGGCCATTTTGTAATTAAAACTTAGAT |
| PEX3 F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGCCCCAAATCAAAGATC |
| PEX3 R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTTAAGGCTTGAAGGAAAACG |
| TEF1t PEX3 F | CAACTTTGGCGTCTCCAGCTCGTTTTCCTTCAAGCCTTAAGGAGATTGATAAGACTTTTC |
| TPI1p PEX19 R | ACTCGTTTTCATTCATTACTTCGTGTTGTATGTTTGGCATTTTTAGTTTATGTATGTGTT |
| PEX19 F | TTAAATCTATAACTACAAAAAACACATACATAAACTAAAAATGCCAAACATACAACACGA |
| PEX19 R | TAACTAATTACATGATATCGACAAAGGAAAAGGGGCCTGTTTATTGTTGTTTGCAACCGT |
| CYC1t PEX19 F | CCTTGATAAGGAATTAACCGACGGTTGCAAACAACAATAAACAGGCCCCTTTTCCTTTGT |
| TEF1p PEX14 R | CGAACAATGCCTTACGATCTTTACTGACCACGTCACTCATTTTGTAATTAAAACTTAGAT |
| PEX14F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGAGTGACGTGGTCAGTAA |
| PEX14R | CTTTAGACATACCACCAGATTGAAAATACAAATTTTCACCTGGGATGGAGTCTTCGACCT |
| GFP F | CTCCATCCCAGGTGAAAATTTGTATTTTCAATCTGGTGGTATGTCTAAAGGTGAAGAATT |
| GFP R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTTATTTGTACAATTCATCCA |
| TEF1t GFP F | TGCTGGTATTACCCATGGTATGGATGAATTGTACAAATAAGGAGATTGATAAGACTTTTC |
| TaFAR mcherry R | TGCTAACCATACCACCAGATTGAAAATACAAATTTTCACCGTATCTCATAGTACTGGAGG |
| Cherry F | TATGAGATACGGTGAAAATTTGTATTTTCAATCTGGTGGTATGGTTAGCAAAGGCGAGGA |
| Cherry R | ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTTATAGCTTGGAACCACCAG |
| TEF1t cherry F | TGAAAATTTGTATTTTCAATCTGGTGGTTCCAAGCTATAAGGAGATTGATAAGACTTTTC |
| AdFAR F | AAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGTCTCAAAGACTACAAAG |
| AdFAR R | ATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTTATAGCTTGGAGTAACGCATG |
| TEF1P_AdFAR R | CCAAATGATCCTTGATACTTTGTAGTCTTTGAGACATTTTGTAATTAAAACTTAGATTAG |
| TEF1T_AdFAR F | CTTCAGGGCAAGCTCCACCATGCGTTACTCCAAGCTATAAGGAGATTGATAAGACTTTTC |