

Evaluation of engineered low-lignin poplar for conversion into advanced bioproducts

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Additional File 1

Table S1. Primers used for RT-PCR

Figure S1. The QsuB plant engineering strategy.

Figure S2. Metabolic routes for microbial conversion of fermentable sugars from lignocellulosic hydrolysates into sesquiterpenes (epi-isozizaene and α -bisabolene) and fatty alcohols.

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Table S1. Primers used for RT-PCR

Primer name	Sequence (5' to 3')	Target gene
QsuB_qRT-F	ACATGGTGGCTCCG TTCACC	3-Dehydroshikimate
QsuB_qRT-R	GTTGCTGGGAAGGGTGGAGA	Dehydratase (<i>QsuB</i>)
PtTIF5A_qRT_F	GGCATTAAAGTTTTGTCGGTCTG	Translation Initiation
PtTIF5A_qRT_R	GCGGTTTCATCATTTTCATCTGG	Factor 5A (<i>PtTIF5A</i>)

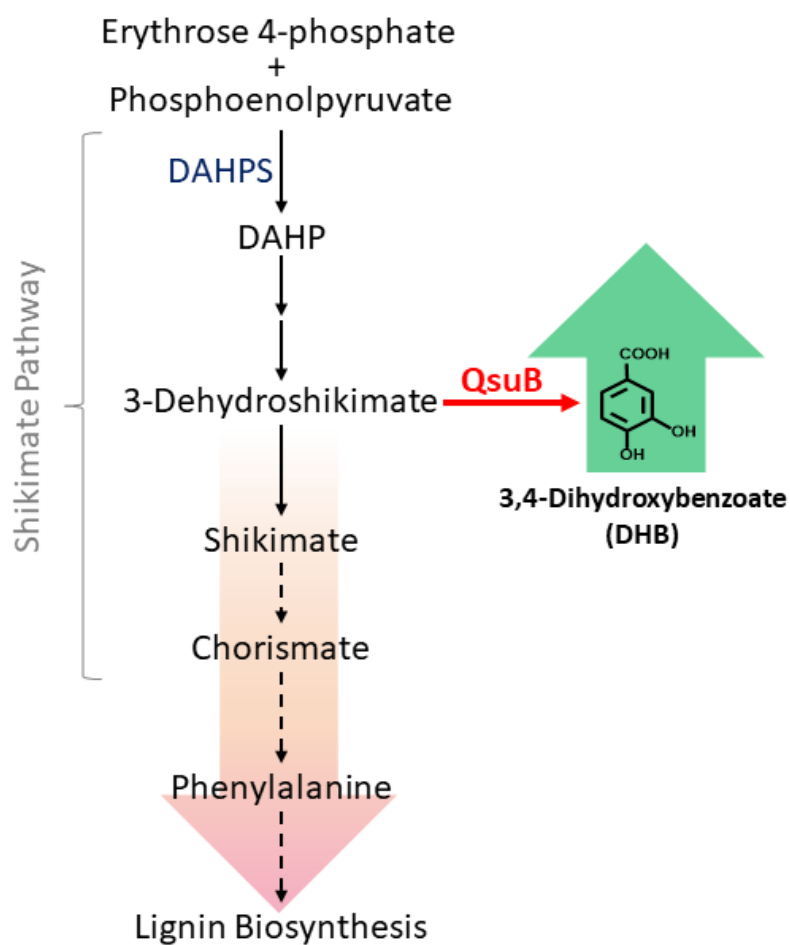


Figure S1. The QsuB engineering strategy. QsuB converts 3-dehydroshikimate into 3,4-dihydroxybenzoate (DHB) and thereby reduces the carbon flux towards shikimate and phenylalanine used for lignin biosynthesis. DAHP: 3-deoxy-D-arabinoheptulosonate 7-phosphate; DAHPS, DAHP synthase.

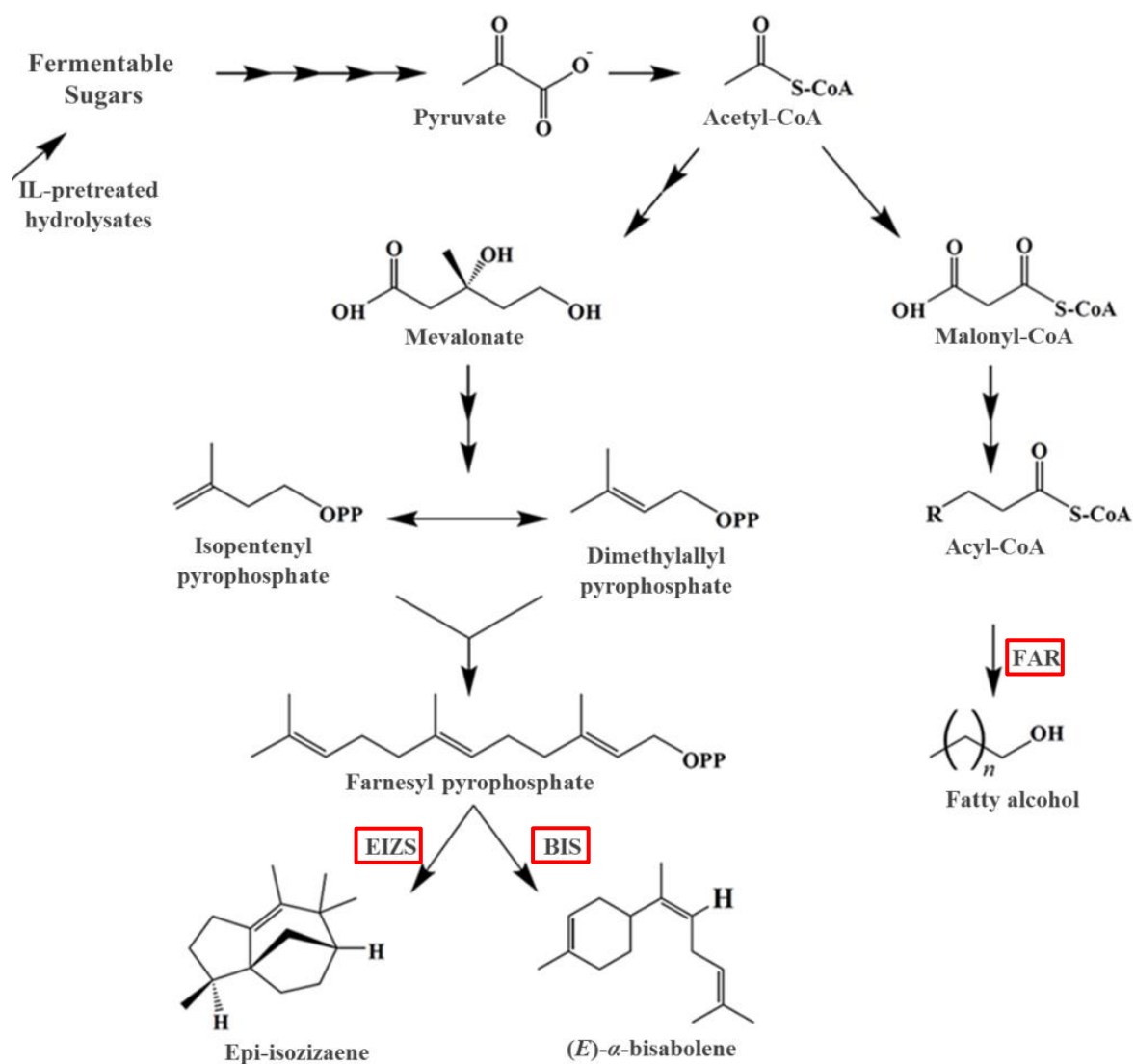


Figure S2. Metabolic routes for microbial conversion of fermentable sugars from lignocellulosic hydrolysates into sesquiterpenes (epi-isozizaene and α -bisabolene) and fatty alcohols. Engineered strains of *Rhodospiridium toruloides* containing an epi-isozizaene synthase (EIZS), an α -bisabolene synthase (BIS), or a fatty acyl-CoA reductases (FAR) are used for fermentation.

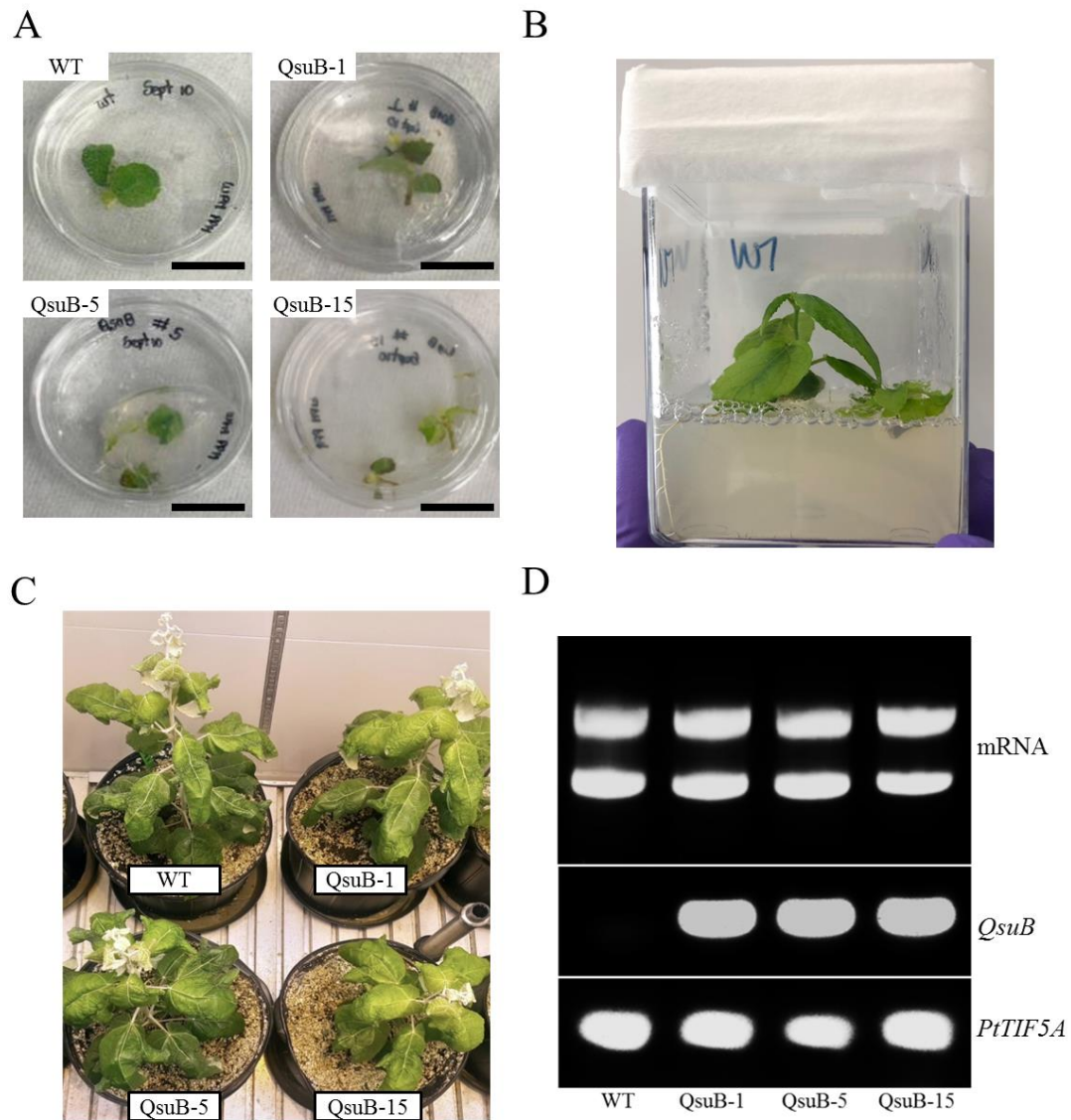


Figure S3. Propagation and characterization of WT and QsuB poplar lines. **(A)** Leaf explants **(B)** One-month-old propagated seedling with elongated roots in the rooting medium (RM). **(C)** Plants in the growth chamber one month after transfer to soil **(D)** Validation of *QsuB* expression in the stem of the QsuB transgenic lines by RT-PCR.

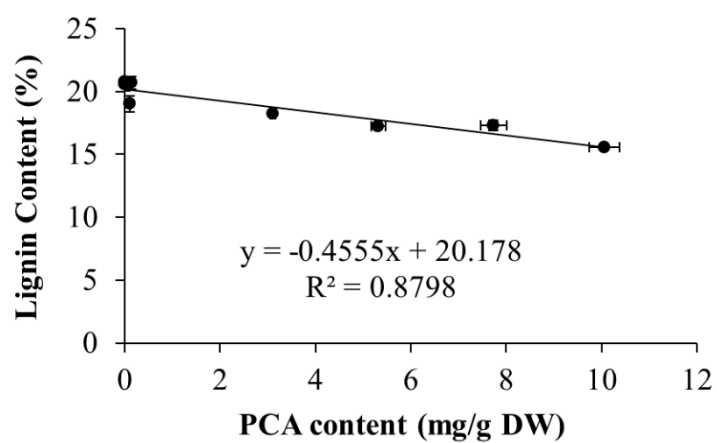


Figure S4. Correlation between total lignin content and DHB content in WT and QsuB biomass. Error bars represent the standard error of three technical replicates of the analysis of pooled biomass from eight plants.

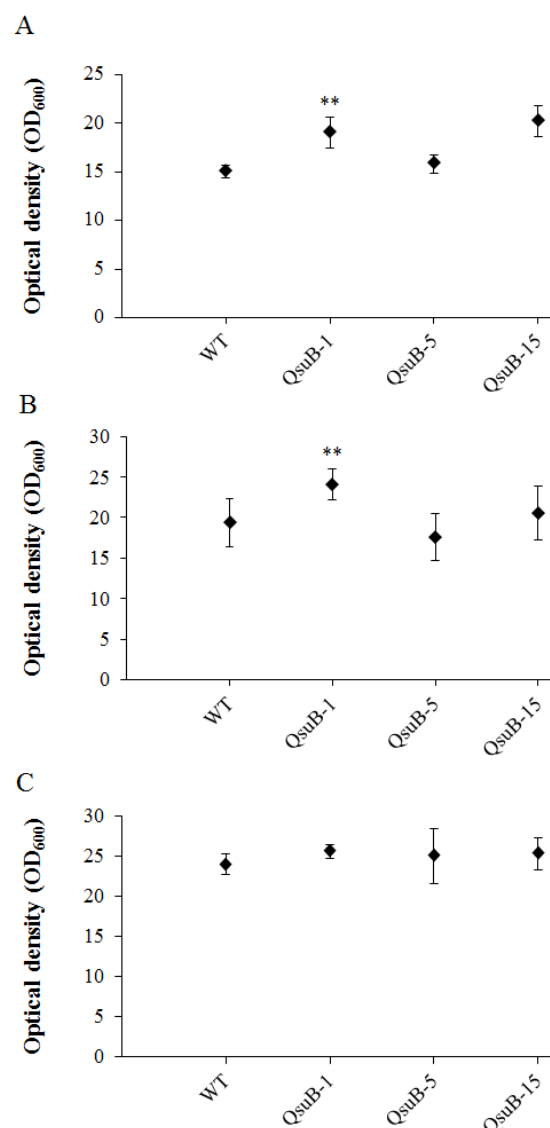


Figure S5. Optical density (OD₆₀₀) of *R. toruloides* cultures after cultivation on large-scale poplar hydrolysates. The strains are (A) GB2, (B) EIZS2, and (C) maquFOH. Error bars represent the standard error of three biological replicates from independent cultures. Asterisks indicate significant differences from the WT hydrolysate using the unpaired Student's t-test (* $p < 0.05$; ** $p < 0.01$).