Supporting Information

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SI Methods

Reagents. The chemicals (*R,R*)-2,3-butanediol [(*R,R*)-23BD], *meso*-23BD, (*S,S*)-23BD, and acetoin were obtained from Sigma-Aldrich. NADH and isopropyl-β-D-thiogalactoside (IPTG) were obtained from Fisher Scientific. Phusion polymerase was purchased from New England Biolabs. KOD polymerase and NADPH were purchased from EMD4Biosciences. Gentamicin was purchased from Teknova. Oligonucleotides were synthesized by Integrated DNA Technologies.

Culture Conditions. Unless otherwise specified, *Synechococcus elongatus* strains were cultured in BG-11 medium (1) with the addition of 50 mM $NaHCO_3$. Cells were grown at 30 °C with rotary shaking (100 rpm) and light (55 $\mu E \cdot s^{-1} \cdot m^{-2}$) provided by four 86-cm 20 W fluorescent tubes 5 cm above the cell cultures. Cell growth was monitored by measuring OD_{730}.

For acetoin and 23BD production in *S. elongatus*, cells in exponential phase were diluted to an OD_{730} of 0.1 in 25 mL BG-11 medium including 50 mM $NaHCO_3$, 10 mg/L thiamine, and 10 mg/L gentamicin in 125 mL baffled shake flasks. Cultures were grown to an OD_{730} of 0.3–0.5 before induction with 1 mM IPTG. Every 24 h 10% of the culture volume was removed, the pH was adjusted to 7.5 ± 0.4 with 10 N HCl, and volume was replaced with BG-11 containing 0.5 M $NaHCO_3$, achieving a final concentration of 50 mM $NaHCO_3$ in the culture.

For acetoin and 23BD production in *Escherichia coli*, overnight cultures were diluted 1:100 into 5 mL of modified M9 medium (2) containing 50 g/L glucose, 5 g/L yeast extract, and 5 mg/L gentamicin in 30-mL culture tubes. Cells were grown at 37 °C to an OD of 0.2–0.4 followed by addition of 0.1 mM IPTG. Production was continued at 30 °C on a rotary shaker (250 rpm) for 40 h.

Plasmid Construction. Plasmids used and constructed are listed in Table S1. All plasmids except pAL60 were constructed using sequence and ligase independent cloning (SLIC) (3) in *E. coli* XL1-Blue (Agilent Technologies). Primers for constructions and genotype verifications are listed in Table S2.

A neutral site (NS) located between Synpcc7942_0893 (903,564–904,283 bp) and Synpcc7942_0894 (904,845–905,417 bp) in the *S. elongatus* chromosome was used for insertion of an expression cassette. This region was amplified with primers MC173 and MC176. PCR products were digested with AatII and AvrII and cloned into pZE12-luc (4) cut with the same enzyme, creating pAL60.

The fragment containing P_{LlacO1} and *alsS* (*Bacillus subtilis*) was amplified with primers IM103 and IM11 and $lacI^q$ was amplified with primers IM39 and AK3 from pSA69 (2). The resulting fragments were inserted into pAL60 by SLIC, creating pAL301.

To clone *alsD* (*Enterobacter aerogenes*), we used genomic DNA of *E. aerogenes* ATCC13048 (ATCC) as a PCR template with primers IM16 and IM17. To clone *alsD* (*Enterobacter cloacae*), genomic DNA of *E. cloacae* ATCC13047 (ATCC) was used as a PCR template with primers IM19 and IM20. To clone *alsD* (*Bacillus licheniformis*), genomic DNA of *B. licheniformis* ATCC14580 (ATCC) was used as a PCR template with primers IM23 and IM24. To clone *alsD* (*Gluconacetobacter xylinus*), genomic DNA of *G. xylinus* (NBRC3288) was used as a PCR template with primers IM21 and IM22. *alsD* (*B. subtilis*) and *alsD* (*Aeromonas hydrophila*) were chemically synthesized by DNA2.0 to optimize codon use for *S. elongatus*. Each *alsD* gene was cloned downstream of *alsS* (*B. subtilis*) on pAL301 by SLIC, creating pAL302, pAL303, pAL304, pAL305, pAL306, and pAL307 (Table S1). To construct plasmid pAL312, we used plasmid pAL301 as a PCR template and primers IM114 and IM11 to amplify the entire plasmid, without *alsS*. The resulting fragment was assembled by SLIC. All four *adh* were chemically synthesized by DNA2.0 to optimize codon use for S. *elongatus*. Each *adh* was cloned downstream of *alsD* (*E. aerogenes*) on pAL302 by SLIC, creating pAL308, pAL309, pAL310, and pAL315 (Table S1). The *adh* (*Thermoanaerobium brockii*) and *adh* (*Clostridium beijerinckii*) were cloned downstream of *alsD* (*A. hydrophila*) on pAL306 by SLIC, creating pAL299 and pAL300, respectively.

Transformation of *S. elongatus*. Transformation of *S. elongatus* was performed as previously described (5). Strains were segregated several times by transferring colonies to fresh selective plates. Correct recombinants were confirmed by PCR to verify integration of target genes into the chromosome (Fig. S1). The strains used and constructed are listed in Table S1. NS between Synpcc7942_0893 (903,564–904,283 bp) and Synpcc7942_0894 (904,845–905,417 bp) on the *S. elongatus* chromosome was used as a targeting site for recombination in this study. It was confirmed that insertion of the gentamicin resistance gene at this site does not affect the growth of cells.

Acetoin Quantification. Acetoin was quantified by the method of Voges and Proskauer as optimized by Westerfeld (6, 7), adapted to small volume on 96-well plates. Sample concentration was varied between 1% (vol/vol) and 10% (vol/vol) of final volume to achieve a result within the linear range of detection. This result was achieved by dilution in H_2O to 100 μL initial volume. For an assay containing 2% (vol/vol) sample (most common), 96 μL water and 4 μL of the supernatant were added to wells and mixed. To this mixture was added 100 μL of a solution, prepared at the time of use, consisting of one part 5% (wt/vol) naphthol dissolved in 2.5 N NaOH and one part 0.5% (wt/vol) creatine in water. The assay was monitored every 5 min and final readings were taken after 40 min, when the slope of the absorbance curve matched the background oxidation rate of naphthol. Triplicate measurements of no less than three standards, including at least one value each above, below, and within the desired range, were included in every assay.

Quantification of 23BD. Supernatant samples from cultures were analyzed with gas chromatography (GC) (Shimadzu) equipped with a flame-ionization detector and an HP-chiral 20b column (30 m, 0.32-mm internal diameter, 0.25-mm film thickness; Agilent Technologies). Samples were prepared by mixing nine parts supernatant (diluted as necessary in H_2O) with one part internal standard. For each analysis the GC oven temperature was held at 40 °C for 4 min, increased with a gradient of 15 °C min^{-1} until 235 °C, and held for 4 min. Ultrahigh purity Helium was used as the carrier gas. The temperature of the injector and detector were set at 250 °C. The stereoisomers were identified by matching retention time to standards for (*R,R*)-23BD, *meso*-23BD, and (*S,S*)-23BD.

O_2 Evolution. Evolution of O_2 was measured using a clark-type electrode with the Oxygraph system (Hansatech Instruments). Under ambient light conditions, 1 mL of cells was transferred to the 4-mL borosilicate glass chamber and headspace gas was expelled using a center bored contact plunger with rubber cap. Cells were stirred at 100 rpm using a magnetic flea, and subjected to 2 min of darkness to allow the cells to equilibrate with the surrounding water jacket to 25 °C. A constant negative rate over at least 30 s was recorded after equilibration. Cells were then subjected to excess light (60 $\mu E \cdot s^{-1} \cdot m^{-2}$) and allowed to

equilibrate for at least 2 min until a constant rate could be measured over at least 60 s.

Enzyme Assays. *S. elongatus* cells were collected 72 h after induction by centrifugation (4,000 × *g*, 5 min), washed in 50 mM potassium phosphate buffer (pH 7.5), and resuspended in the same buffer. Crude extracts were prepared with 0.1-mm glass beads and a Mini bead beater (Mini Bead Beater 8; BioSpec Products). The total protein determination was performed using Advanced Protein Assay Reagent from Cytoskeleton.

Acetolactate synthase (ALS) activity was determined as previously described (8). The concentration of acetoin produced was measured by a standard curve using pure acetoin. One specific unit of ALS activity corresponds to the formation of 1 nmol of acetoin per milligram of protein per minute.

Alcohol dehydrogenase (ADH) activity was determined by measuring the oxidation of NAD(P)H. The reaction mixture contained 50 mM 3-(*N*-morpholino) propanesulfonic acid (Mops) pH 7.0, 25 mM acetoin and 0.2 mM NAD(P)H. The consumption of NAD(P)H was monitored at 340 nm. One specific unit of ADH activity corresponds to the oxidation of 1 nmol of NAD(P)H per minute per milligram of protein. Background activity was determined for each sample by addition of MilliQ water in place of substrate, and this rate was subtracted from final rates measured with substrate. For use of acetoin as a substrate by native enzymes, activity of the host strain is published as a comparison. The rate without substrate across all *S. elongatus* samples was 8.7 ± 3.9 for NADPH, and 4.8 ± 3.0 (nmol·min^{-1}·mg^{-1}) for NADH. Across *E. coli* samples the rate without substrate was 4.3 ± 5.6 nmol·min^{-1}·mg^{-1} measured with NADPH. This sampling does not include the *E. coli* strain containing sADH (*C. beijerinckii*), which displayed a higher rate of 69 nmol·min^{-1}·mg^{-1} in the absence of substrate, and is given independently.

2-Acetolactate decarboxylase (ALDC) was determined in crude lysate by coexpression with ALS. Activity was determined as described for ALS (8), with the exception that acidification is omitted. Absence of acidification prevents conversion of 2-acetolactate to acetoin in the absence of ALDC. The concentration of acetoin produced by ALDC was measured by a standard curve using pure acetoin. A concurrent background rate of 2-acetolactate conversion in the absence of ALDC was substracted from each activity. One specific unit of ALDC activity corresponds to the formation of 1 nmol of acetoin per milligram of total protein per minute.

SDS/PAGE Analysis. To estimate the amount of the installed enzymes translated in AL757, cell extracts of AL757 were analyzed with Coomassie Brilliant blue-G250 staining of SDS/PAGE gels. AL757 and the wild-type were cultured for 72 h with or without IPTG. The cells were centrifuged, washed, and resuspended in potassium phosphate buffer (pH 7.5). The cells were lysed with a bead beater (BioSpec Products) and centrifuged at 16,000 × *g* for 20 min at 4 °C. The soluble protein concentration was determined by Advanced Protein Assay Reagent (Cytoskeleton) and 50 μg of protein was run per lane on 4–20% Mini-Protean TGX acrylamide gel (Bio-Rad). ImageJ (National Institutes of Health) was used to quantify bands on the gel.

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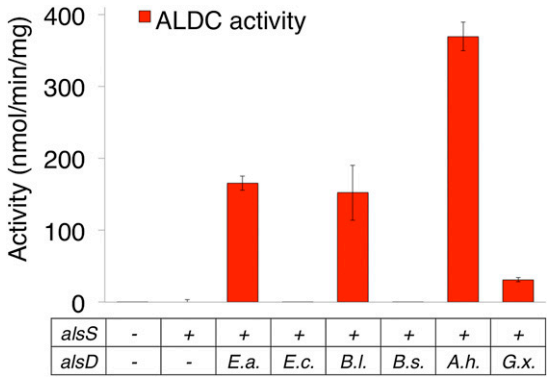

Fig. S1. Activity of ALDC coexpressed with ALS. Strains containing *alsS* and *alsD* were induced for 4 h and activity was tested in crude lysate. This is an approximation of ALDC activity for internal comparison only. An excess of substrate is assumed because of high ALS activity (300–600 nmol·min^{-1}·mg^{-1}) and not quantified. In the case of ALDC (*A. hydrophila*) substrate may be limiting. Error represents SD between $n = 3$ cultures. *A. h.*, *A. hydrophila*; *B. l.*, *B. licheniformis*; *B. s.*, *B. subtilis*; *E. a.*, *E. aerogenes*; *E. c.*, *E. cloacae*; *G. x.*, *G. xylinus*.

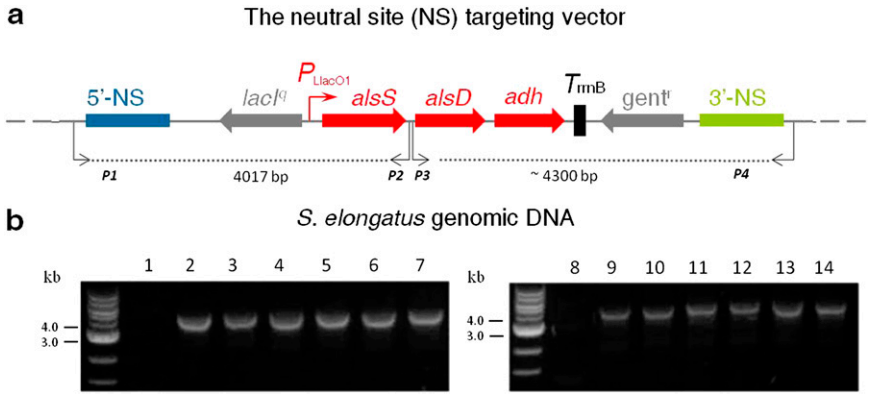

Fig. S2. PCR confirmation of the integration of 23BD pathway genes into the *S. elongatus* chromosome. (*A*) Schematic representation showing the integration of the 23BD pathway genes *alsS*, *alsD*, and *adh* into NS on the *S. elongatus* chromosome. (*B*) Confirmation of correct recombinants. PCR performed with specific primers listed in Table S2, with genomic DNA of *S. elongatus* strains (Table S1) as a template. (*Left*) Result of PCR with P1 and P2 to demonstrate integration of *lacI*^{q} and *alsS*. (lane 1) Wild-type, (lane 2) AL769, (lane 3) AL770, (lane 4) AL771, (lane 5) AL753, (lane 6) AL756, and (lane 7) AL757. (*Right*) Result of PCR with P3 and P4 to demonstrate integration of *alsD* and *adh* genes. (lane 8) Wild-type, (lane 9) AL769, (lane 10) AL770, (lane 11) AL771, (lane 12) AL753, (lane 13) AL756, and (lane 14) AL757.

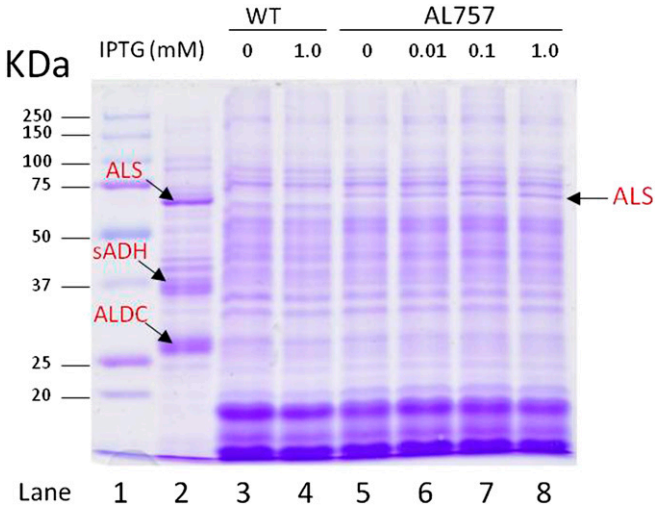

Fig. S3. SDS/PAGE analysis of the 23BD strain. Cell extracts of the wild-type and AL757 were analyzed with SDS/PAGE. (lane 1) Size ladder; (lane 2) the mixture of cell extracts of *E. coli* overexpressing *alsS*, *alsD* (*A. hydrophila*), and *adh* (*C. beijerinckii*); (lanes 3 and 4) cell extracts of the wild-type without (lane 3) and with 1.0 mM IPTG (lane 4); (lanes 5–8) cell extracts of AL757 without IPTG (lane 5), and with 0.01 (lane 6), 0.1 (lane 7), and 1.0 mM (lane 8) IPTG. The calculated molecular weights of ALS, ALDC, and sADH are 62.1, 29.0, and 37.7 kDa, respectively.

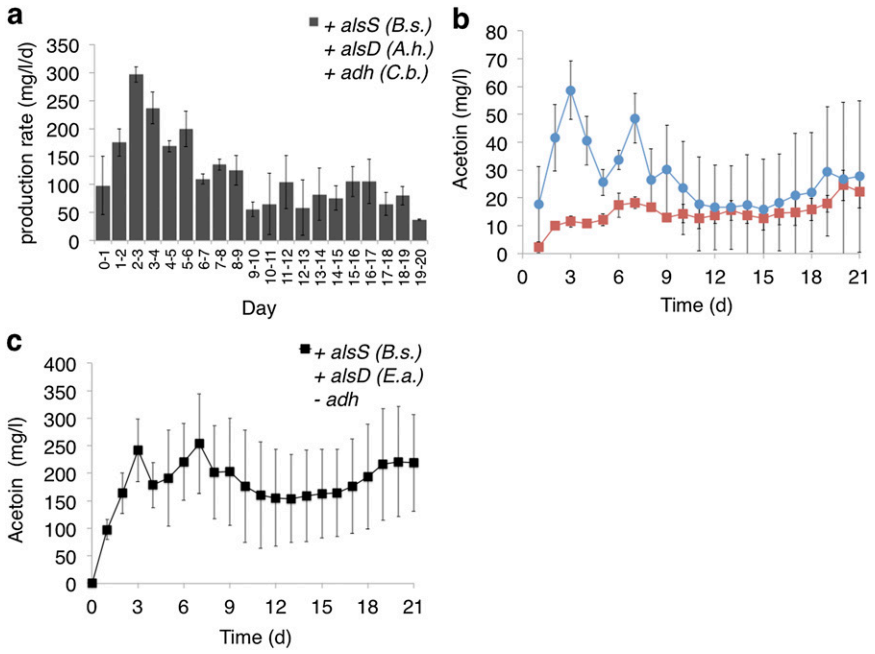

Fig. S4. 23 BD and acetoin production in *S. elongatus*. (*A*) 23BD daily production by AL757 [with integrated *alsS, alsD* (*A. hydrophila*), and *adh* (*C. beijerinckii*)] (Fig. 5). (*B*) Acetoin concentration during long-term 23BD production experiments (Fig. 5). Red squares: AL757 [with integrated *alsS* (*B. subtilis*), *alsD* (*A. hydrophila*), and *adh* (*C. beijerinckii*)]. Blue circles: AL756 [with integrated *alsS* (*B. subtilis*), *alsD* (*A. hydrophila*), and *adh* (*T. brockii*)]. (*C*) Long-term acetoin production by AL763 [with integrated *alsS* and *alsD* (*E. aerogenes*)].

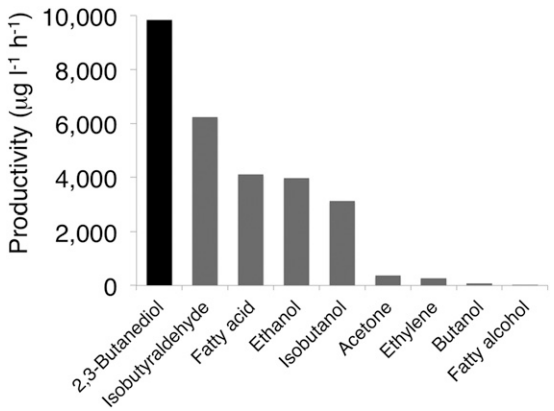

Fig. S5. Comparison of productivities for various chemicals produced from exogenous pathways in cyanobacteria. Source data are obtained from: 23BD (present work), isobutyraldehyde, and isobutanol (1), fatty acid (2), ethanol (3), acetone (4), ethylene (5), butanol (6), fatty alcohol (7). The detailed calculation is as follows (the productivities were calculated as described in ref. 8 for isobutyraldehyde): (*i*) 23BD: Productivity per day was averaged over 3 d. Per day yields were 175 mg·L^{-1}, 297 mg·L^{-1}, and 237 mg·L^{-1} for the time periods from 24–48 h, 48–72 h, and 72–96 h, respectively. By converting units, this becomes 7,292 μg·L^{-1}·h^{-1}, 12,375 μg·L^{-1}·h^{-1}, and 9,875 μg·L^{-1} h^{-1}, respectively. The average of these rates is 9,847 μg·L^{-1}·h^{-1} (Fig. 5 and Fig. S2). (*ii*) Isobutyraldehyde: The rate is calculated as in ref. 1. (*iii*) Fatty acids: The apparent maximum titer was 197 mg·L^{-1}, which was produced over a minimum of 2 d, converting units gives 4104.2 μg·L^{-1}·h^{-1} (2). (*iv*) Ethanol: The apparent maximum is 13 mM·L^{-1} produced in 145 h. Using the Molar mass of ethanol (46.07 g·mol^{-1}), this becomes 575.9 mg·L^{-1} over 145 h. Converting units gives 3,972 μg·L^{-1}·h^{-1} (3). (*v*) Isobutanol: Published titer is 450 mg·L^{-1} over 6 d. Converting units gives 3,125 μg·L^{-1}·h^{-1} (1). (*vi*) Acetone: The apparent maximum titer was 36 mg·L^{-1}over 4 d. Converting units gives 375 μg·L^{-1}·h^{-1} (4). (*vii*) Ethylene: The apparent maximum rate was 240 nL·ml^{-1}·h^{-1}. This rate can be approximated as 9.81 μmol·L^{-1}·h^{-1} using the molar volume of an ideal gas at ambient temperature and pressure (24.465 L·mol^{-1} at 25 °C and 1 atm) and converting units. Using the molar mass of ethylene (28.05 g·mol^{-1}), this becomes 275.17 μg·L^{-1}·h^{-1} (5). (*viii*) 1-Butanol: The apparent maximum titer was 19 mg·L^{-1}over 10 d. Converting units gives 79.2 μg·L^{-1}·h^{-1} (6). (*ix*) Fatty alcohol: The apparent maximum titer was 137.63 μg·L^{-1}over 4 d. Converting units gives 0.48 μg·L^{-1}·h^{-1} (7).

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Table S1. Strains and plasmids used in this study

Strains	Genotype	Source
E. coli strain		
XL-1 Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [*F′ proAB lacIqZΔM15 Tn10* (*Tetr*)]	Agilent Technologies
S. elongatus strains		
PCC7942	Wild-type	S. Golden*
AL723	P_{LlacO1} and Gent^R integrated at NS	Present study
AL756	*alsS* (*B. s.*)-*alsD* (*A. h.*)-*adh* (*T. b.*) integrated at NS	Present study
AL757	*alsS* (*B. s.*)-*alsD* (*A. h.*)-*adh* (*C. b.*) integrated at NS	Present study
AL762	*alsS* (*B. s.*) integrated at NS	Present study
AL763	*alsS* (*B. s.*)-*alsD* (*E. a.*) integrated at NS	Present study
AL764	*alsS* (*B. s.*)-*alsD* (*E. c.*) integrated at NS	Present study
AL765	*alsS* (*B. s.*)-*alsD* (*B. l.*) integrated at NS	Present study
AL766	*alsS* (*B. s.*)-*alsD* (*B. s.*) integrated at NS	Present study
AL767	*alsS* (*B. s.*)-*alsD* (*A. h.*) integrated at NS	Present study
AL768	*alsS* (*B. s.*)-*alsD* (*G. x.*) integrated at NS	Present study
AL769	*alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*C. p.*) integrated at NS	Present study
AL770	*alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*L. p.*) integrated at NS	Present study
AL771	*alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*T. b.*) integrated at NS	Present study
AL772	*alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*C. b.*) integrated at NS	Present study
Plasmids		
pAL60	NS targeting vector; ColE1 ori; *P*trc; Amp^R	Present study
pSA69	P15A ori; Kan^R, $P_L$lacO$_1$:*alsS* (*B. s.*)-*ilvC* (*E. coli*)-*ilvD* (*E. coli*)	(1)
pAL299	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*A. h.*)-*adh* (*T. b.*); Gent^R	Present study
pAL300	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*A. h.*)-*adh* (*C. b.*); Gent^R	Present study
pAL301	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*); Gent^R	Present study
pAL302	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*E. a.*); Gent^R	Present study
pAL303	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*E. c.*); Gent^R	Present study
pAL304	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*B. l.*); Gent^R	Present study
pAL305	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*B. s.*); Gent^R	Present study
pAL306	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*A. h.*); Gent^R	Present study
pAL307	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*G. x.*); Gent^R	Present study
pAL308	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*C. p.*); Gent^R	Present study
pAL309	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*L. p.*); Gent^R	Present study
pAL310	As pAL60, but P_{LlacO1}: *alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*C. b.*); Gent^R	Present study
pAL312	As pAL60, but P_{LlacO1}: Gent^R	Present study
pAL315	As pAL60, but P$_{LlacO1}$: *alsS* (*B. s.*)-*alsD* (*E. a.*)-*adh* (*T. b.*); Gent^R	Present study

A. h., *A. hydrophila*; *B. l.*, *B. licheniformis*; *B. s.*, *B. subtilis*; *C. p.*, *C. parapsilosis*; *C. b.*, *C. beijerinckii*; *E. a.*, *E. aerogenes*; *E. c.*, *E. cloacae*; *G. x.*, *G. xylinus*; *L. p.*, *Leuconostoc pseudomesenteroides*; *T. b.*, *T. brockii*.

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Table S2. Oligonucleotides used in this study

Name		Sequence 5′ → 3′	Plasmid constructed
MC173		CTATGACGTCGGCGTTTTCTGCTACATGGGCCGTGAG	pAL60
MC176		CTAACCTAGGGGAAGTCCAGCGCAATCAGCGGAGTTG	pAL60
MC181	P1	CTAAATGCATTAAGTTGTTACTAGTGCTTGGATTCTCACC	
IM15	P2	CTAGAGAGCTTTCGTTTTCATGAG	
IM88	P3	CGGGGAACTCATGAAAACGAAAGCTCTCTA	
IM89	P4	GTTGGGTAGCAGACAATGCGGGGGATCTGG	
AK1		GGTTTTGCACCAGGATCCCGCTCGAGTTGACGCGTGCTTA	pAL301
AK3		TCACTGCCCGCTTTCCAGTC	pAL301
IM11		AGATCCTCTAGAGTCGACCTG	pAL299, pAL300, pAL312
IM15		CTAGAGAGCTTTCGTTTTCATGAG	pAL302
IM16		TAGGTCGACGAGGAATCACCATGAATCATGCTTCAG	pAL302
IM17		AGGTCGACTCTAGAGGATCTCTAACTTTCTACTGAACGGA	pAL302
IM19		TAGGTCGACGAGGAATCACCATGAGCGCCCTGCTAA	pAL303
IM20		CAGGTCGACTCTAGAGGATCTTTAGTTTTCGACGGA	pAL303
IM21		TAGGTCGACGAGGAATCACCATGGAAATAGGCTTTA	pAL307
IM22		CAGGTCGACTCTAGAGGATCTTCAGCCGCCCTCGGC	pAL307
IM23		CTAGGTCGACGAGGAATCACCATGAAAAGTGCAAG	pAL304
IM24		CAGGTCGACTCTAGAGGATCTTTACTCGGGATTGCCT	pAL304
IM27		TAGGTCGACGAGGAATCACCATGAAACGTGAGTCG	pAL305
IM28		CAGGTCGACTCTAGAGGATCTCTACTCGGGAGAACC	pAL305
IM29		TAGGTCGACGAGGAATCACCATGGAAACTAATAGC	pAL306
IM30		CAGGTCGACTCTAGAGGATCTCTAACCCTCAGCCGC	pAL306
IM39		CGGGATCCTGGTGCAAAACCTTTCGCGGTA	pAL301
IM44		GTACCTTTCTCCTCTTCTAACTTTCTACTGAACGGATGGC	pAL308, pAL309, pAL310, pAL315
IM45		TAGAAGAGGAGAAAGGTACATGAAAGGTTTTGCCA	pAL310
IM46		CAGGTCGACTCTAGAGGATCTCTACAGGATTACGAC	pAL310
IM47		GTTAGAAGAGGAGAAAGGTACATGAAGGGTTTCGC	pAL315
IM48		CAGGTCGACTCTAGAGGATCTCTATGCCAAAATGAC	pAL315
IM49		TTAGAAGAGGAGAAAGGTACATGGGGGAGATTGAG	pAL308
IM50		CAGGTCGACTCTAGAGGATCTCTAGGGGCATGTGTAA	pAL308
IM51		TTAGAAGAGGAGAAAGGTACATGACAAAGAAAGT	pAL309
IM52		AGGTCGACTCTAGAGGATCTCTAGTGAAACTGCATG	pAL309
IM103		AGTTGACGCGTGCTTATCATAATTGTGAGCGGATAACAAT	pAL301
IM114		GGTCGACTCTAGAGGATCTTGTACCTTTCTCCTCTTTAA	pAL312
IM125		GTACCTTTCTCCTCTTCTAACCCTCAGCCGCACGGATAGC	pAL299, pAL300

Table S3. List of ALDC and secondary alcohol dehydrogenase-ADH used in this study

ALS, ALDC, and ADH	Strain source	Cofactor	Chirality	K_m (mM)	k_{cat} (s^{-1})	Source
ALS						
B. subtilis (*B. s.*)				13.6	121	(1)
ALDC						
E. aerogenes (*E. a.*)	ATCC 13048			NA	NA	(2)
E. cloacae (*E. c.*)	ATCC 13047			NA	NA	(2)
B. licheniformis (*B. l.*)	ATCC 14580			NA	NA	(2)
B. subtilis (*B. s.*)*	str 168			NA	NA	(2)
A. hydrophila (*A. h.*)*	ATCC 7966			NA	NA	(2)
G. xylinus (*G. x.*)	NBRC 3288			NA	NA	(2)
ADH						
C. parapsilosis (*C. p.*)*	M203011	NADPH	*S*-installing	NA	NA	(3)
L. pseudomesenteroides (*L. p.*)*	CHCC 2114	NADPH	*S*-installing	5.1†	18.3	(4)
C. beijerinckii (*C. b.*)*	NRRL B593	NADPH	*R*-installing	8.3	8.2	(5)
T. brockii (*T. b.*)*	HTD4	NADPH	*R*-installing	0.23	0.91	(5)

NA, not available.

*Genes were synthesized with codon optimized for expression in *S. elongatus*.

†Apparent K_m.

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