Re-routing Carbon-flux to Enhance Photosynthetic Productivity

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Summary: Engineered cyanobacteria efficiently excrete sugar and exhibit enhanced photosynthetic productivity.

Abstract:

Sustainable production of fuels, chemicals, and therapeutics typically rely upon carbohydrate inputs derived from agricultural plants. Terrestrial feedstocks require arable croplands, resulting in entanglement of food and chemical commodity markets and limiting the efficacy of first-generation biofuels. We demonstrate efficient production of sucrose from a freshwater cyanobacterial species, *Synechococcus elongatus,* heterologously expressing a symporter of protons and sucrose (*cscB*). *CscB*-expressing cyanobacteria export sucrose irreversibly to concentrations >10mM without culture toxicity. Moreover, sucrose-exporting cyanobacteria exhibit increased overall biomass production rates relative to wild-type strains, accompanied by enhanced Photosystem II activity and chlorophyll content. Genetic modification of sucrose biosynthesis pathways to minimize competing glucose- or sucrose-consuming reactions can further improve sucrose production, allowing export of sucrose at rates up to 36.1 mg L^{-1} hour-illumination^{-1}. This rate of production exceeds that of previous reports of targeted, photobiological production from microbes. Importantly, engineered *S. elongatus* produce sucrose in sufficient quantities (up to ~80% of total biomass) to represent a viable alternative to sugar synthesis from terrestrial plants, including sugarcane.

Main Body:

While biologically produced fuels and chemicals hold the promise of increased sustainability and reduced CO_2 footprints, current feedstocks compete with agricultural crops for arable land. Development of biological alternatives to standard petroleum-based fuels and chemicals has therefore been criticized for its capacity to increase food cost and market instabilities (*1*). Cyanobacteria are autotrophs that could be an alternative source of sugar, as they can be

cultivated in locations that do not compete with terrestrial plants. Cyanobacteria also achieve higher photosynthetic efficiencies than plants (*2*), and are more amenable to genetic modification and selection.

We examined the carbohydrate production capacity of the freshwater cyanobacterium *Synechococcus elongatus sp. PCC 7942* (*S. elongatus*). Under osmotic stress, *S. elongatus* accumulates the compatible solute sucrose at concentrations up to ~300mM, which helps to balance osmotic pressure and exhibits other protective qualities (*3, 4*). As sucrose is naturally generated and tolerated at high concentrations, this simple carbohydrate is an attractive candidate for targeted overproduction in cyanobacteria. To export intracellular sucrose, we used the major facilitator superfamily (MFS) transporter, sucrose permease (*cscB*). Sucrose permease is a sucrose/proton symporter, typically enabling heterotrophic species to import sucrose by utilizing proton gradients established across the cell membrane (Fig. 1A). In contrast, *S. elongatus* naturally alkalinizes its environment, driving biochemical reactions from proton gradients across thylakoid membranes instead. Therefore, the orientation of the cell membrane proton gradient is reversed in *S. elongatus* relative to most heterotrophs (Fig 1B).

CscB exports cytoplasmic sucrose generated in response to osmotic shock when heterologously-expressed in *S. elongatus* (Fig. 2). We cloned *cscB* from *Eschericha coli* and integrated it into a genomic neutral site of *S. elongatus* under an IPTG-inducible promoter (*5*). When grown in constant light in the presence of IPTG and NaCl (100-200mM), sucrose is steadily exported to the culture supernatant (Fig. 2A). The rate of sucrose export can be modulated by the degree of osmotic pressure applied to the culture, and is inversely correlated with cyanobacterial growth (Fig. 2B). Sucrose export is undetectable in wild-type cultures and greatly reduced in *cscB*-containing cultures with only one inducer (salt OR IPTG; Fig. 2A, Supplemental Figure S1).

Sucrose export is light-dependent, accruing during the lighted periods of cultures grown under alternating periods of “day” and “night” (12/12 hours light:dark; Fig. 2C); no re-uptake of sucrose is observed in the dark. Sucrose production rates generally increase as cyanobacterial cultures age and become more dense (Fig. 2D), distinguishing sucrose production from other reported forms of photobiological production which exhibit feedback inhibition through product toxicity or backpressure (*6, 7*).

Since exported sucrose represents fixed carbon that is diverted from cell growth processes (Fig 2B), one measure of the productive capacity of engineered sucrose-exporting cyanobacteria is by comparison to the ‘potential’ cellular biomass that is accumulated in the absence of *cscB* expression. We monitored the production of cyanobacterial biomass (dry weight) and sucrose biomass 24 hours following *cscB*-induction and compared productivities to uninduced and wild-type cultures. *CscB*-induced cyanobacteria accumulated less cellular biomass than wild-type or uninduced cultures, and the biomass of sucrose exported was essentially equivalent to this difference (Fig. 3A). Thus, on short time scales, cellular biomass can be ‘exchanged’ for sucrose biomass with near 100% efficiency. Furthermore, the ratio of cellular biomass to sucrose can be tuned via the osmotic pressure imposed on the cyanobacterial culture, generating up to ~85% sucrose at higher salinities (200mM NaCl; Fig. **S2,** 2A,2B).

We followed the productivity of sucrose-exporting cells beyond the first day, diluting cultures to a constant volume and density (114mg/L ± 7mg) each day to equilibrate samples and minimize light-shading. We observed gradual increases in sucrose and cell biomass production in *cscB*-induced cultures over time, resulting in total biomass productivities that were up to 35% greater than biomass produced by wild-type cultures, and sucrose productivities up to ~200% of initial

cellular mass in 24h (Fig. 3B). These heightened levels of productivity were maintained for the length of our observations (2 weeks).

Since *S. elongatus* is a strictly autotrophic species of cyanobacteria, we asked if increased photosynthetic activity could partially explain the increased biomass fixed by *cscB*-expressing cells. We measured the oxygen evolution rates of Photosystem II (PSII) and observed gradual increases in activity of *cscB*-expressing cyanobacteria 48-96h following induction, to final oxygen evolution rates ~20% higher than wild-type or uninduced cultures (Fig. 3C). Chlorophyll concentration showed a similar increasing pattern in *cscB*-expressing cyanobacteria (~25%; Fig. 3D). These results indicate that photosynthetic activity is upregulated in *cscB*-expressing *S. elongatus,* which may partially account for the productivity gains realized by this strain.

We next sought to enhance sucrose production in *S. elongatus* by biasing glucose-1-phosphate flux towards sucrose, and away from storage as glycogen. Sucrose is produced from UDP-glucose and fructose via the combined actions of sucrose-phosphate synthase and sucrose-phosphate phosphatase (*8*); invertase (*invA*) hydrolyzes sucrose to glucose and fructose. Alternatively glucose can be polymerized into glycogen through the sequential activities of ADP-glucose pyrophosphorylase (*glgC*) and glycogen synthase (*glgA*; Fig. 4A). Glycogen breakdown is controlled by a number of activities, including glycogen phosphorylase (*glgP*). We generated *ΔinvA* and *ΔglgC* strains as well as strains overexpressing *glgP* and UDP-glucose pyrophosphorylase (*galU*; Fig 4A, S4). Heterologous expression of *galU*, or overexpression of *glgP* caused a reduction in the rate of sucrose export relative to cells expressing cscB alone (Fig. 4B). However, strains with deletions of the putative invertase (Synpcc7942_0397) or *glgC* exported sucrose at higher rates (~10-15%), and knockout of both *invA* and *glgC* produced additive effects (~25% increase; Fig. 4B).

The sucrose production rates displayed in Figure 4B (36.1 mg L^{-1} $hour^{-1}$ for *ΔinvAΔglgC*) were maintained for the duration of our observations (3 d) and, as they are derived from more mature cultures (~1.15g dry cell weight L^{-1}), may better represent the full productive capacity of *cscB*-expressing strains. This productivity is the highest photosynthetic rate of microbial target product formation reported in academic literature for cyanobacteria or algae (Fig. 4C). Targeted isobutyraldehyde production in *S. elongatus* has previously yielded the highest rates (6.13 mg L^{-1} $hour^{-1}$; week-long avg.)(*7*). Similarly, bulk lipids (for biodiesel generation) are naturally produced at high levels in the algae *Neochloris oleoabundans* (~6.83 mg lipid L^{-1} $hour^{-1}$)(*9, 10*), and cyanobacteria have been engineered to excrete a mix of fatty acids (*11*)(~8.2 mg fatty acid L^{-1} $hour^{-1}$; Fig. 4C).

Significantly, sucrose production in engineered *S. elongatus* compares favorably with sugarcane and other agricultural crops. Sugarcane yields range from 30-70 metric tons $hectare^{-1}$ $year^{-1}$ (dry weight), depending on location (*12, 13*). Cyanobacteria or algae cultivated in closed bioreactors generate ~40-120 t ha^{-1} y^{-1} in small-scale reactors (*14-17*), whereas "open pond" bioreactors yield ~25-50 t ha^{-1} y^{-1} (*18*). As the areal productivities of sugarcane and cyanobacteria are similar, percent of total biomass generated as sucrose is the simplest comparison between sugarcane (15%) and S*. elongatus* (up to 80%; Fig. **S2**, 4D, 2). Although speculative, we also calculated more detailed estimations of cyanobacterial sucrose productivity using median values from meta-analyses of microalgal productivity (*18-20*), which illustrate *S. elongatus* could hypothetically produce sucrose at rates up to several-fold higher than sugarcane (Fig. S3).

Cyanobacterially-derived sucrose could support production of other compounds, especially commodity products (e.g. biofuels) where carbohydrate feedstocks represent significant portions of input costs (*21*). Like algal-derived biodiesel, cyanobacterial cultivation can utilize

land and water resources not suitable for plant-based feedstocks and reduce land-use conflicts (*1*). Cyanobacterial sucrose achieves high %-yields and is exported from the cell, which may prove advantageous relative to algal biodiesel, where cell harvesting, lipid extraction, and transesterifaction represent significant economic barriers (*22*).

The enhancement of productivity, PSII activity, and chlorophyll concentration is a striking feature of engineered, sucrose-exporting cyanobacteria (Fig. 3). In a natural context, sucrose and other compatible solutes are hypothesized to affect cellular physiology through multiple mechanisms. For example, under stress conditions sucrose may have directly protective qualities (e.g. enhancing membrane integrity), provide an easily-accessible energy source, have anti-oxidant properties, act as a balancing osmolyte, and/or have signaling functions (*4, 23*). Finally, production of sugars consumes excess reducing equivalents and triose phosphate intermediates, which can alleviate inhibition of photosynthetic activity (photoinhibition) resulting from energy imbalances between light absorption (source) and metabolic capacity (sink)(*23-25*). Consistent with this function, transient increases in photosynthetic activity of other freshwater cyanobacterial species exposed to osmotic stress have been reported, but are restricted to a period of cytosolic sucrose accumulation (~2 days)(*26, 27*). Relaxation of photoinhibition by fatty-acid production and excretion may also promote enhanced cyanobacterial viability in specific, photobioreactor-like environments (high-light, high-CO_2)(*11*).

We propose that *cscB*-supported sucrose export acts as an expanded photosynthetic sink, helping maintain a relatively oxidized electron transport chain and suppressing photoinhibition; analogous to plants with large carbon sinks (e.g. potatoes)(*24*). We note that while any concentration-dependent protective effects of sucrose would be diluted in *cscB*-expressing cells, the continual replacement-level synthesis of sucrose could maintain an altered metabolic state

associated with sucrose production. The increased PSII activity and chlorophyll content of sucrose-exporting cyanobacteria (Fig. 3B, C) is consistent with a relaxation of photoinhibition, a common feature of cyanobacterial growth under photobioreactor-like conditions (*24, 28*).

As photosynthetic organisms have evolved light-gathering ability in excess of metabolic processing capacity under full illumination, optimization of source/sink balance is necessary to minimize photosynthetic inefficiencies (*2*). Limitation of light capture capacity has received considerable attention following reports of algae with reduced chlorophyll antennae and improved productivity (*17*). However, there are no previous reports of metabolite production and export that have led to enhancements in photosynthetic yield from microbes; although some plants exhibit complex phenotypes including increased productivity upon expansion of carbohydrate sinks (*24, 29*). The enhancement of cellular metabolic sinks represents an alternative, complementary balancing strategy for ultimately bringing photosynthetic rates closer to maximal theoretical yields (*2*).

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Figure Legends:

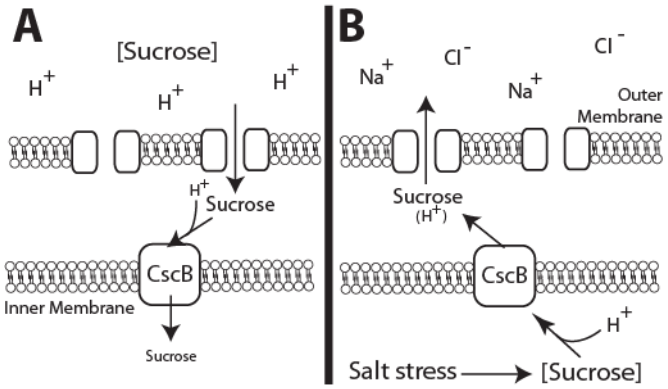
Figure 1 – Schematic of sucrose production and sucrose permease activity in *S. elongatus*. A) Typically, CscB supports sucrose import from relatively acidic environments through proton symport. **B)** *S. elongatus* naturally basifies its environment, leading to a reversed proton gradient and CscB directionality. Under hyperosmotic conditions, *S. elongatus* produces cytosolic sucrose for osmotic balance and which can be exported through the action of CscB.

Figure 2 – CscB-dependent export of sucrose in growing cyanobacterial cultures. A) Concentration of sucrose in the supernatant of *S. elongatus* cultures with/without *cscB*-expression when grown with 0-200mM NaCl one week in constant light. **B)** Cell growth of cultures in (A) as measured by optical density. **C)** Sucrose produced by *cscB*-expressing *S. elongatus* under alternating periods of light (yellow) and dark (grey). **D)** Daily rate of sucrose production for cultures shown in (A). Data are from representative experiments where error bars represent standard deviation from ≥3 replicates.

Figure 3 – Increased biomass accumulation and photosynthetic activity in *S. elongatus* exporting sucrose. A) Measurement of cyanobacterially-produced cellular biomass (dry weight; green) and sucrose biomass (blue) produced by wild-type (WT), uninduced(-), and *cscB*-expressing(+) strains in 24 hours following induction with 150mM NaCl and with (+) or without (-) 1mM IPTG. **B)** Extended time course of (A) depicting daily biomass generation from cultures backdiluted to constant density (114 ± 7 mg cell mass L^{-1}). **C)** Oxygen evolution rates of wild-type, uninduced and induced cyanobacterial cultures. **D)** Chlorophyll a content of indicated cyanobacterial cultures. Data for (A/B) is a representative experiment where similar results were obtained in at least 5 independent replicates. Data for (C/D) represents averages of ≥4 independent experiments. Error bars: standard deviation from ≥3 replicates.

Figure 4 – Comparison of cyanobacterial sucrose production rates. A) Schematic of sucrose metabolism in *S. elongatus*. **B)** Comparative sucrose production rates for strains of *S. elongatus* with modifications in sucrose biosynthesis genes by knockout (KO) or gene expression (+). **C)** Summary of photobiological product formation rates from existing literature on cyanobacteria (Cy) and algae (Al). **D)** Percent of total biomass directed to named metabolite for example species. References as indicated: (*6, 7, 9-11, 30*).

Figure 1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[Sucrose]
H+
H+
H+
H+
Sucrose
CscB
Inner Membrane
Sucrose
B
Na+
Cl-
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Outer
Membrane
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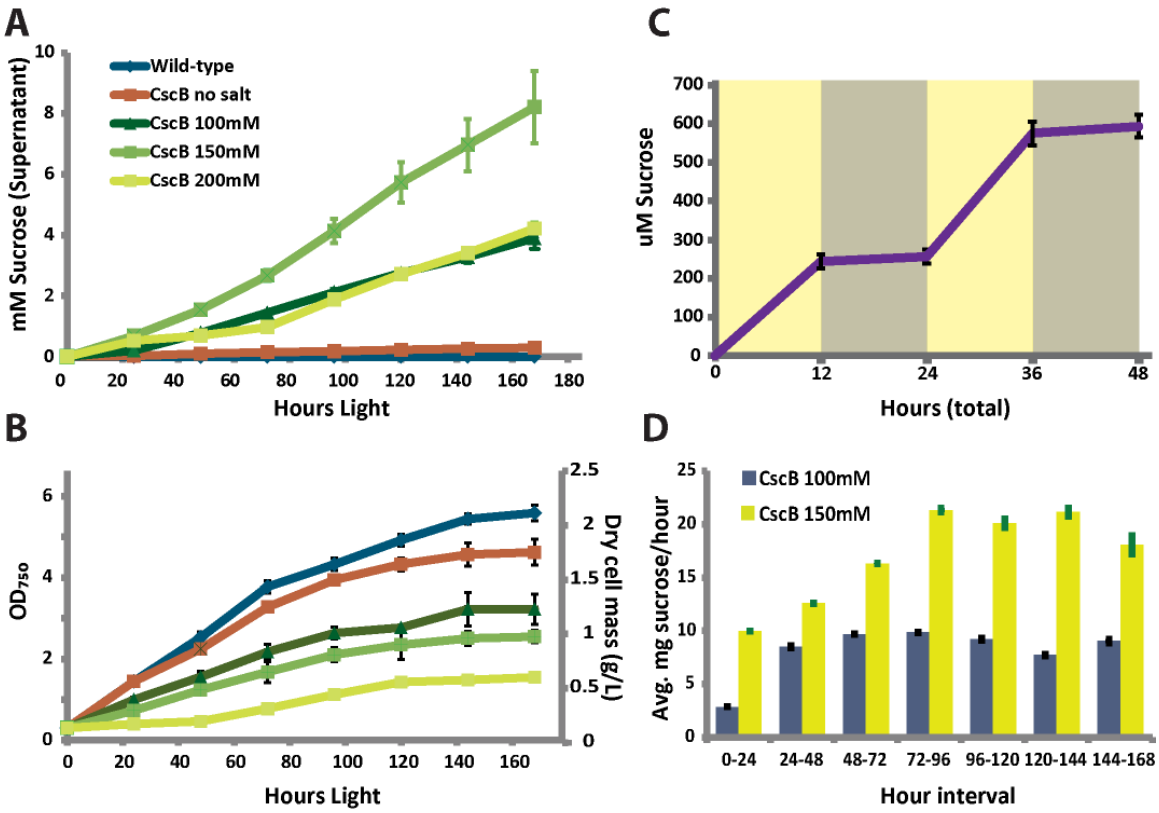
(H+)
CscB
H+
Salt stress
[Sucrose]

Figure 2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Wild-type
CscB no salt
CscB 100mM
CscB 150mM
CscB 200mM
Hours Light
C
uM Sucrose
Hours (total)
B
OD750
Dry cell mass (g/L)
Hours Light
D
CscB 100mM
CscB 150mM
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0-24
24-48
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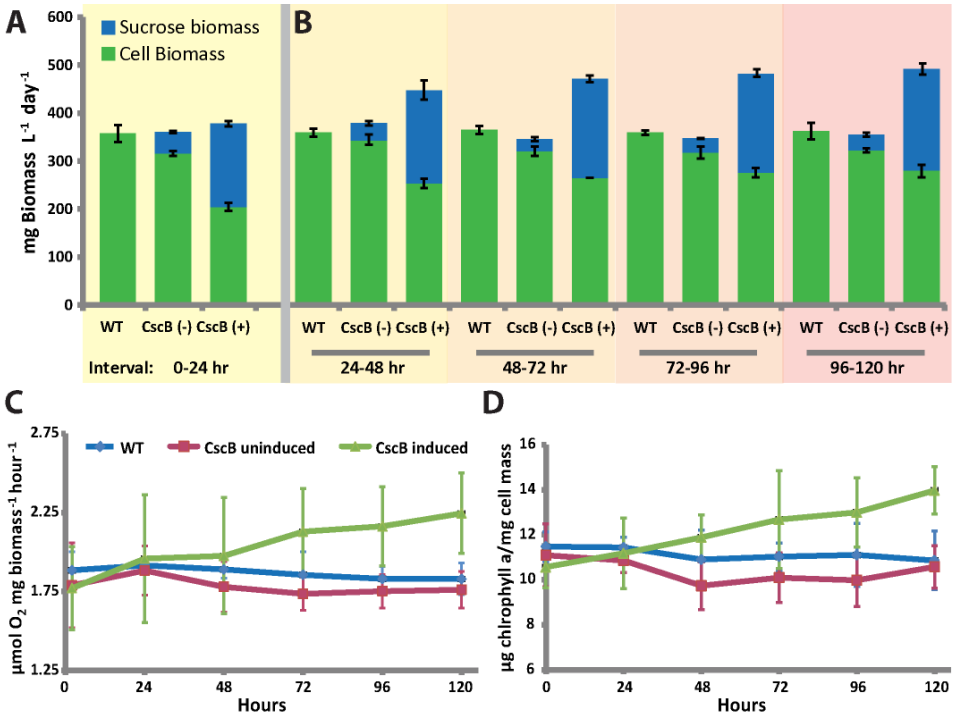
72-96
96-120
120-144
144-168
Hour interval

Figure 3


A
B
Sucrose biomass
Cell Biomass
mg Biomass L-1 day-1
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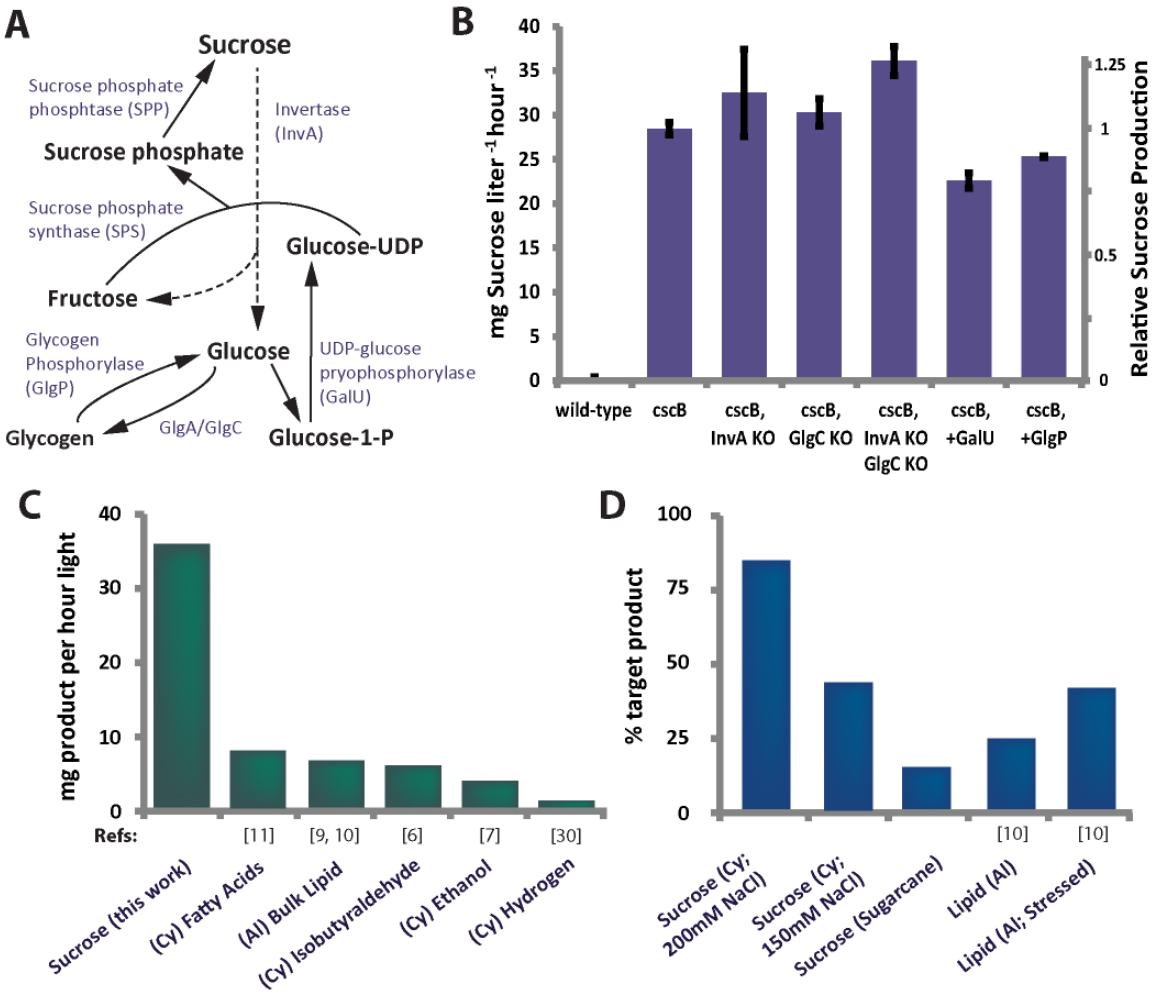
500
400
300
200
100
0
WT
CscB (-)
CscB (+)
Interval: 0-24 hr
24-48 hr
48-72 hr
72-96 hr
96-120 hr
C
D
WT
CscB uninduced
CscB induced
µmol O2 mg biomass-1 hour-1
2.75
2.25
1.75
1.25
0
24
48
72
96
120
Hours
µg chlrophyll a/mg cell mass
16
14
12
10
8
6

Figure 4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Sucrose
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Invertase (InvA)
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Glucose
UDP-glucose pryophosphorylase (GalU)
Glycogen
GlgA/GlgC
Glucose-1-P
B
mg Sucrose liter-1 hour-1
Relative Sucrose Production
wild-type
cscB
cscB, InvA KO
cscB, GlgC KO
cscB, InvA KO GlgC KO
cscB, +GalU
cscB, +GlgP
C
mg product per hour light
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Sucrose (this work)
(Cy) Fatty Acids
(Al) Bulk Lipid
(Cy) Isobutyraldehyde
(Cy) Ethanol
(Cy) Hydrogen
D
% target product
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[10]
Sucrose (Cy; 200mM NaCl)
Sucrose (Cy; 150mM NaCl)
Sucrose (Sugarcane)
Lipid (Al)
Lipid (Al; Stressed)

Supplemental Materials and Methods, Figures, and Calculations:

Supplementary Methods and Calculations:

Growth Conditions:

Synechococcus elongatus PCC 7942 was grown in temperature controlled (35^{o}C) and CO_2 controlled (2%) Multitron (Infors HT) incubators outfitted with twelve 18-inch fluorescent bulbs (Gro-Lux, 15W; Sylvania), approximately 35 cm from the culture surface. Light intensity at the growth surface was measured at 3000 lux (~80 µE m^2 s^{-1}). Flasks were shaken at 150rpm. Cultures were grown in BG11 media buffered with 1g/L HEPES (pH 8.9; SIGMA) to improve consistency during culture dilutions (but which was not necessary for sucrose export).

Strain Construction:

All cloning was conducted using classic restriction/ligation approaches with a BioBrick format (Bgl Brick standard 21(*31*)), or with isothermal assembly methods (*32*) in *E. coli* before transfer of the relevant genetic material to *S. elongatus* through suicide vectors. Sucrose permease (*cscB*) was cloned from *E. coli* genomic DNA (ATCC #700927; primers detailed below) and cloned into the neutral site 3 vector under an IPTG-inducible promoter (*5*). *S. elongatus* were transformed overnight in the presence of ~200ng of this construct and selected on BG11 plates with 12.5µg/mL chloramphenocol, and verified through PCR/sequencing. Knockout strains were generated through the use of a novel, modular suicide vector containing an antibiotic resistance (corresponding to kanamycin, spectinomycin, chloramphenicol, or hygromycin) flanked by loxP sites, and regions of homology to the genomic site containing the target gene (Supplemental Figure 4A). Invertase (*invA*) mutants were generated through the targeted insertion of a hygromycin resistant-cassette (Supplemental Figure 4B; 100µg/mL for selection), ADP-glucose pyrophosphorylase (*glgC*) utilized a spectinomycin cassette (Supplemental Figure 4C; 20µg/mL

for selection). Overexpression studies of glycogen phosphorylase (*glgP*) or UDP-glucose pyrophosphorylase (galU) involved cloning the relevant gene (cloned from *S. elongatus* or *E. coli*, respectively) into the neutral site 2, Isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible vector(*33*), and selecting for double recombinants on BG11 plates supplemented with 20μg/mL kanamycin.

Sucrose Assays:

CscB-expressing cells were grown as described above, sampling from cultures as indicated, with replacement of removed volume by fresh BG11 of the appropriate osmolarity (0, 50, 100, 150, or 200mM NaCl). For growth curve experiments, flasks were weighed at the setup of time course experiments, and water was added prior to each sampling to correct for evaporation rates. CscB-expressing cells were induced with 1mM IPTG and indicated salt concentrations. After pelleting cyanobacterial cells, sucrose was measured from the supernatant fraction using Sucrose/D-Glucose assay kits (Megazyme).

Oxygen evolution/chlorophyll measurements:

For time course analysis of sucrose production, oxygen evolution, and chlorophyll content, the relevant cyanobacterial cultures were grown in BG11 + 150mM NaCl media and backdiluted to an OD_{750} of 0.3 (SpectraMax M5; Molecular Devices) every 24h to minimize cell shading and standardize cell density. For analysis of dry cell mass as shown in Figure 3 or S2, 6-10 mL of cyanobacterial culture was pelleted for 30 min at 20,000g, desiccated in a GeneVac E2-2 Plus evaporator (SP Scientific), and weighed. Dry weight values displayed in Figure 2B are estimated from OD_{750} based on a standardized curve. Dissolved oxygen and chlorophyll content was measured on 2mL of backdiluted (OD_{750}= 0.3) cultures with the use of Clark electrodes (model 1302; Strathkelvin) outfitted with polypropylene membranes, and monitored with a 782 dual

channel oxygen meter (Strathkelvin). Cyanobacterial cultures were contained in transparent, water-jacketed respiration cells (RC350; Strathkelvin), illuminated with constant light (3000 lux; white fluorescent), and continuously stirred. Constant temperature (22^{o}C) was maintained throughout the length of each oxygen measurement (10 min) through water exchange in the repiration cell jacket, and an external fan. Chlorophyll was extracted from cyanobacterial cells using 85% methanol with 1mM sodium dithionite and concentration was determined as previously described(*34*). Wild-type *S. elongatus* cultures in normal media (i.e. not exposed to salt stress) exhibited similar oxygen-evolution capacity and growth rates to wild-type strains acclimatized to 150mM NaCl.

Calculations for scaled sucrose production (Fig S3):

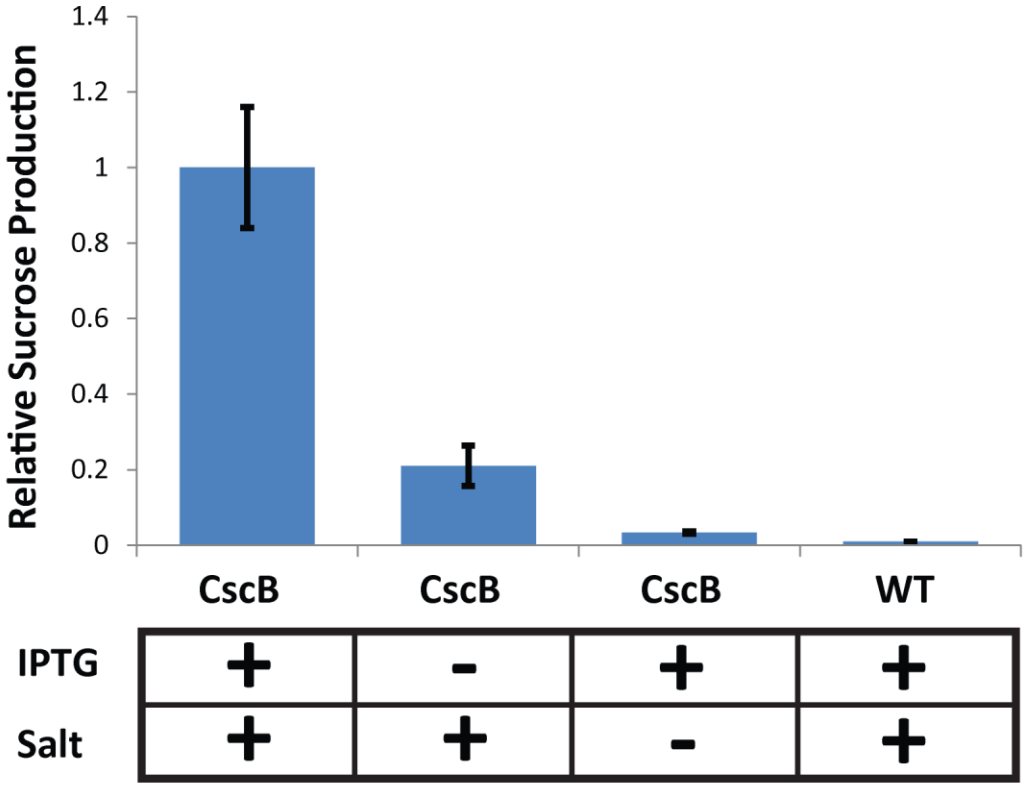
In surveying the literature, we came across substantial variation in the assumptions used to model potential biomass yield from cyanobacteria or algae. Since relatively limited information is available on the design or productivities of scaled, enclosed photobioreactors, we used numbers from meta-analyses of microalgal production models (for both open and closed reactors)(*18, 19*) to calculate a high (volumetric), and low (areal) theoretical upper yield of scaled sucrose production from *S. elongatus*. Using volumetric productivities we achieved under laboratory conditions (36.1mg L^{-1} h^{-1}) scaled to a one-hectare photobioreactor, with a volume of 640,000 L(*19*), a median growing season (300 days; (*18*)), and 8 hours of light per day, we calculate a theoretical yield of ~55 metric tons sucrose per hectare per year. Estimating yields from areal productivities achieved under laboratory conditions (50mL cultures in 150mL flasks, with a bottom surface area of ~.002826 m^2: 637 mg m^{-2} h^{-1}) and using median values for growing season (300 days), 8 hours light/day, and 86.7% coverage of photobioreactor(*18*), we estimate a yield of ~15 metric tons sucrose per hectare per year. We note that the ~4X fold difference

between these calculations is largely a function of underestimation of potential areal productivities from our small-scale cultivars (which are only grown at depths of ~2cm), under relatively low light intensities in (~10-fold less than full sunlight).

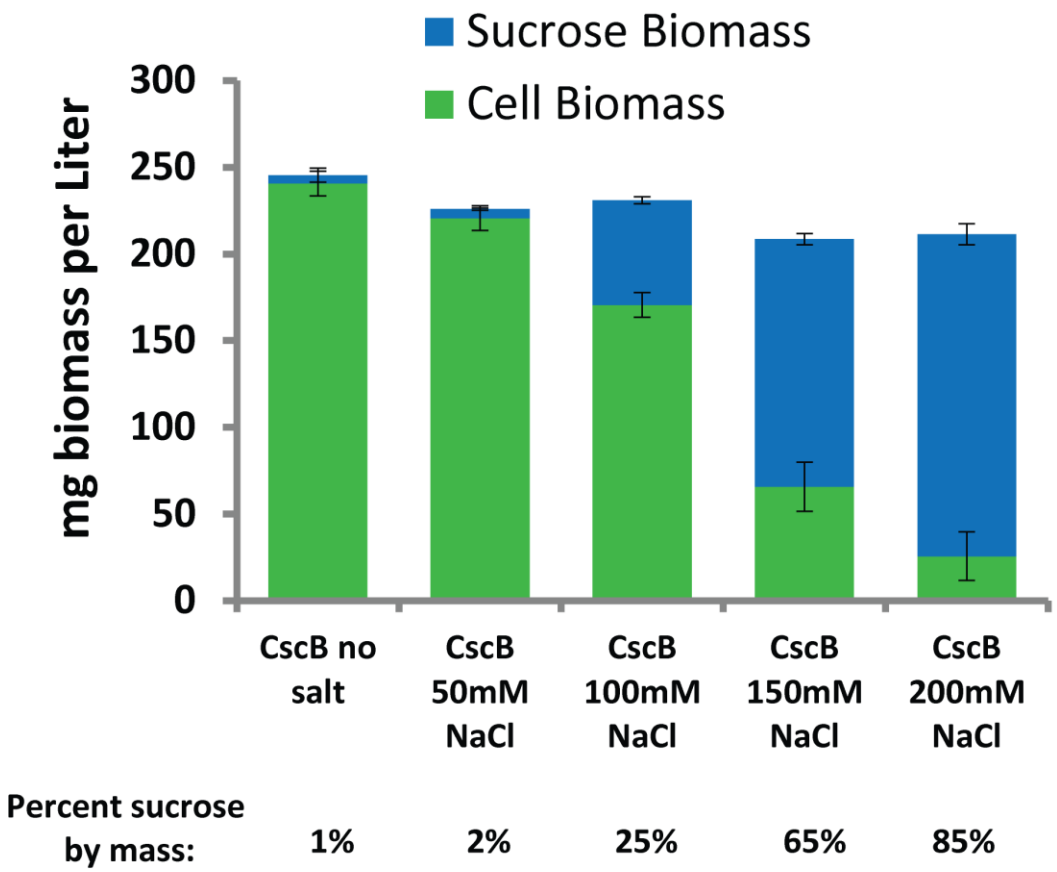
Supplementary Table 1: Primers used for strain generation:

Gene	**Orient:**	**Primer**
cscB	Fwd	GTTTCTTCGAATTCGCGGCCGCTCTAGAATGGCACTGAATATTCCATTCAG
cscB	Rev	ATTGCTGCAGCGGCCGCTACTAGTCTATATTGCTGAAGGTACAGG
invA (KO) upst.	Fwd	AGAGTAAGCTTGGCAAAGTCCTGCGGGAGAAC
invA (KO) upst.	Rev	CTCACGCGGCCGCGAGCAATTTGAAGCGACCAGCGATC
invA (KO) downst.	Fwd	AAGTGGAATTCCGATTGGGGCCGCTACAAC
invA (KO) downst.	Rev	GTTACACTAGTGATTTATGAAGACCTTTGCTGCCGACG
glgC (KO) upst.	Fwd	GGATCTCAAGAAGATCCTTTGATCTGGTACCTACCAGCGATCCGTGTCCCTACTCG
glgC (KO) upst.	Rev	GAGATTTTGAGACACAACGTGGCTTTCCAAGCGGCGCTGAGACCCAGTG
glgC (KO) downst.	Fwd	AGGCAGACCTCAGCGCCCTCTAACACCATGCGCCTCGGC
glgC (KO) downst.	Rev	GCAACGCGGGCGCGCCGGGGCGATTCTTTGTTTCCACTGAGTGG
glgP	Fwd	CACAAGGAGGAAAAAAAAAATGTCTAGAATGAGTGATTCCACCGCCCAACTC
glgP	Rev	CCGGCCAAGCTTGCTAGCGCGGCCGCCTAAAAGCTAGTGCTTAGGCTGACGGG
galU	Fwd	CATGCGGCCGCGTTACTTCTTAATGCCCATCTCTTCTTCAAGC
galU	Rev	GTATCTAGAATGGCTGCCATTAATACGAAAGTCAAAAAAGCCGTTATCC
HygR (for KO)	Fwd	ATCCGGGCCCACCGGTATAACTTCGTATAGCATACATTATACGAAGTTATACTAGTTCTGTCTATTTCGTTC
HygR (for KO)	Rev	ATCCGTCGACCCATGGATAACTTCGTATAATGTATGCTATACGAAGTTATAAGCTTGGGCTAGCGGCTTTAG
SpR (for KO)	Fwd	CACTGGGTCTCAGCGCCGCTT GAAGGCACGAACCCAGTGGAC
SpR (for KO)	Rev	GCCGAGGCGCATGGTGTTAGAGGAAGTCGACCCATGGATAACTTCG

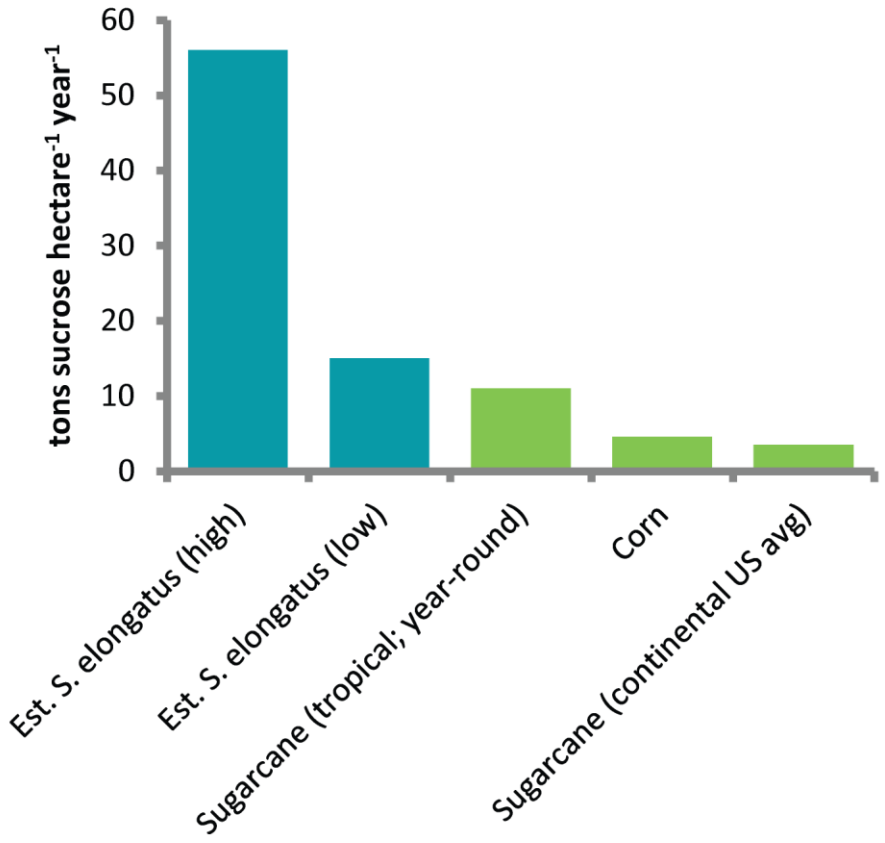
Supplementary Figures and Legends:



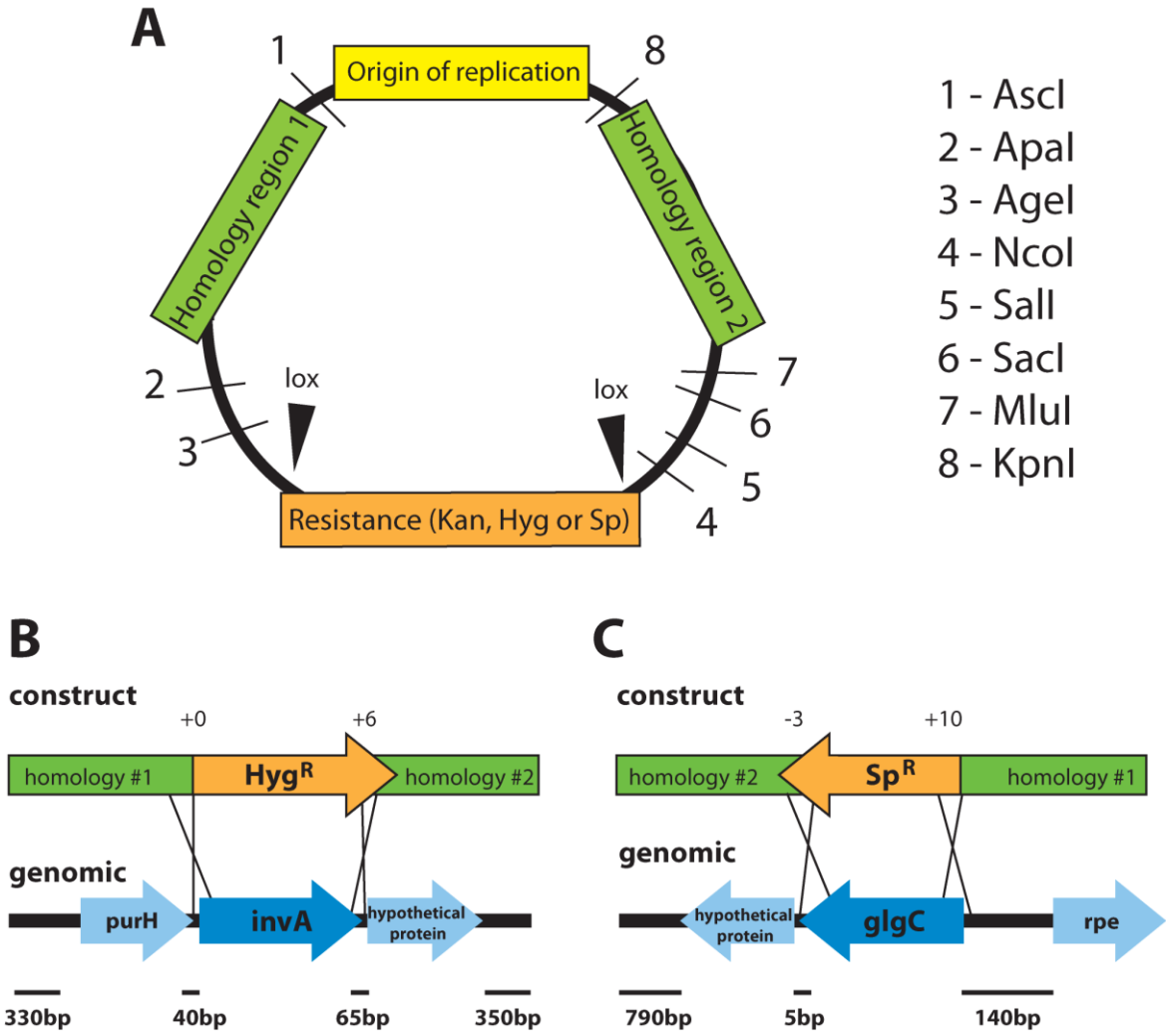
Supplemental Figure 1 - **Requirement of IPTG and osmotic stress for full sucrose export activity.** Relative sucrose concentration in the supernatant of indicated strains 24 hours following incubation with/without (+/-) 1mM IPTG and with/without 150mM NaCl. Representative results are displayed with error bars reporting standard deviation from 3 replicates.



Supplemental Figure 2 – Ratio Cell:Sucrose Biomass Production is Dependent Upon Osmotic Pressure. 24 hour production of cellular biomass and sucrose biomass in CscB-expressing *S. elongatus* following induction with salt (indicated) and IPTG (1mM). First-day biomass production is displayed in dry cell mass (green) and sucrose (blue). Total biomass production (cell + sucrose) is similar across different osmotic stresses, though the proportion (%) of sucrose biomass generated is increased at higher salinities. Beginning cyanobacterial density ~0.25 OD_{750}. Representative results are displayed with error bars reporting standard deviation from 3 replicates.



Supplemental Figure 3 – Comparison of existing carbohydrate feedstocks with theoretical yields of cyanobacterial sucrose. Scaled production of sucrose from cscB-expressing *S. elongatus* is estimated from volumetric (high) and areal (low) productivities obtained under laboratory environmental conditions (blue; see Supplemental Calculations for details). Known carbohydrate productivities for traditional terrestrial crops are plotted for comparison (green; (*12, 13, 35*)).



Supplemental Figure 4 – Novel *S. elongatus* suicide vector design and gene knockout description. A) Illustration of the backbone of a novel, modular suicide vector for the transformation of *S. elongatus* designed for the generation of targeted knockouts. Unique restriction sites of interest are displayed. Regions of homology (~1000bp) were amplified from genomic DNA and cloned into the relevant "homology regions" using the appropriate restriction sites (see above for primer design). Different constructs were made for co-integration of resistance markers for kanamycin (Kan), hygromycin (Hyg), or spectinomycin (Sp), flanked by loxP sites for potential flip-out of resistance cassettes. The origin of replication is cloned from a pUC57-containing plasmid allowing propagation in *E. coli*, without replication in *S. elongatus*. The backbone from (A) was used to create knockout constructs for the putative invertase (*invA* **(B)**) and ADP-glucose pyrophosphorylase (*glgC* **(C)**). The position of the target genes in the *S. elongatus* genome is depicted, including the position, and orientation of neighboring genes. The space between coding regions of DNA is displayed. Full-gene replacements with the indicated antibiotic resistance cassettes were designed in both cases; any additional/fewer base pairs recombined relative to the start or stop codons of the target gene are indicated above the "construct" depiction.

Supplemental References:

31. Registry of Standard Biological Parts - Assembly standard 21. (2009). <http://partsregistry.org/Assembly_standard_21>.
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