Synthesis of three advanced biofuels from ionic liquid-pretreated switchgrass using engineered *Escherichia coli*

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One approach to reducing the costs of advanced biofuel production from cellulosic biomass is to engineer a single microorganism to both digest plant biomass and produce hydrocarbons that have the properties of petrochemical fuels. Such an organism would require pathways for hydrocarbon production and the capacity to secrete sufficient enzymes to efficiently hydrolyze cellulose and hemicellulose. To demonstrate how one might engineer and coordinate all of the necessary components for a biomass-degrading, hydrocarbon-producing microorganism, we engineered a microorganism naïve to both processes, *Escherichia coli*, to grow using both the cellulose and hemicellulose fractions of several types of plant biomass pretreated with ionic liquids. Our engineered strains express cellulase, xylanase, beta-glucosidase, and xylobiosidase enzymes under control of native *E. coli* promoters selected to optimize growth on model cellulosic and hemicellulosic substrates. Furthermore, our strains grow using either the cellulose or hemicellulose components of ionic liquid-pretreated biomass or on both components when combined as a coculture. Both cellulolytic and hemicellulolytic strains were further engineered with three biofuel synthesis pathways to demonstrate the production of fuel substitutes or precursors suitable for gasoline, diesel, and jet engines directly from ionic liquid-treated switchgrass without externally supplied hydrolase enzymes. This demonstration represents a major advance toward realizing a consolidated bioprocess. With improvements in both biofuel synthesis pathways and biomass digestion capabilities, our approach could provide an economical route to production of advanced biofuels.

consolidated bioprocessing | ionic liquid pretreatment

The microbial conversion of sustainable lignocellulosic biomass into biofuels could provide a source of fully renewable transportation fuels (1). Generating these fuels from abundant feedstocks such as lignocellulose and cellulosic waste avoids many of the problems associated with current grain-based biofuels, provided the feedstock is responsibly grown and harvested (2). While early efforts toward achieving economical biofuel production have typically focused on improving yields of ethanol made from fermentation of plant sugars (3), recent advances in metabolic engineering have enabled microbial production of fuels that are compatible with existing engines and fuel distribution infrastructure (4, 5). Many of these advances have been made possible by the unparalleled genetic and metabolic tractability of the model bacterium *Escherichia coli* (6, 7). *E. coli* has been engineered to biosynthesize perhaps the most chemically diverse range of chemicals of any organism, including hydrogen (8), higher alcohols (9, 10), fatty-acid based chemicals (11), and terpenes (12, 13). Extensive knowledge of *E. coli* physiology will continue to aid improvements in titers beyond those achieved in proof-of-concept stages toward levels required for a commercial-scale biofuel production process.

Unfortunately, several challenges must be overcome before lignocellulose can be considered an economically competitive feedstock for biofuel production. One of the more significant challenges is the need for large quantities of glycoside hydrolase (GH) enzymes to efficiently convert lignocellulose into fermentable sugars. These enzymes are typically generated in a dedicated process that incurs substantial capital and material expense and represent the second highest contribution to raw material cost after the feedstock itself (1, 14). An alternative approach, known as consolidated bioprocessing, could potentially avoid the costs of a dedicated enzyme generation step by performing it in a combined process that includes biomass hydrolysis and fuel production (Fig. 1*A*) (15, 16). This can be achieved by incorporating both biomass-degrading and biofuel-producing capabilities into a single organism through genetic engineering. Several microorganisms have been engineered to ferment model cellulosic and hemicellulosic substrates directly into ethanol or other fuels (reviewed in refs. 15 and 17). For example, the yeast *Saccharomyces cerevisiae* (18) and the bacterium *Klebsiella oxytoca* (19) have been modified to convert phosphoric acid swollen cellulose (PASC) directly to ethanol without the addition of exogenous cellulase. However, PASC and similar model substrates are typically prepared using techniques that are neither suitable for actual plant biomass nor feasible on a large scale (20). Furthermore, no biofuel with the combustion properties of petrochemical fuels, which could be used directly in existing infrastructure, has been generated directly from unrefined lignocellulosic biomass.

A cellulolytic strain of *E. coli* capable of growth on plant biomass would be a first step toward producing many varieties of advanced biofuels at lowered cost. One obstacle to engineering *E. coli* for consumption of lignocellulose is the organism's inferior capacity for protein export, which renders it unable to secrete cellulases in quantities required for industrial-scale lignocellulose hydrolysis. Various techniques, developed over decades of research, can be applied to generate secreted yields from *E. coli* of 0.5–0.8 g protein/L (21). Unfortunately, these concentrations are still too low for an industrial process, which are most efficient around levels of 20 mg cellulase/g solids and 200 g/L solids loading (22) [although recent work (23) has demonstrated that

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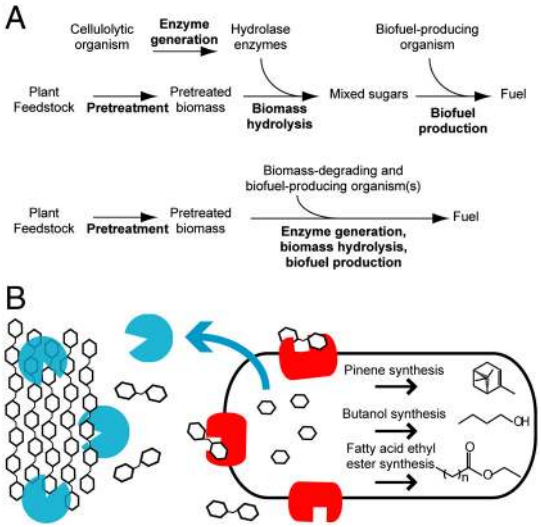

Fig. 1. Consolidated bioprocessing of plant biomass into biofuels by *E. coli*. (*A*) Two processes for biofuel production. Typically, cellulase and hemicellulase enzymes are produced in a process step separate from biomass hydrolysis and biofuel production (top). Consolidated bioprocessing (bottom) combines enzyme generation, biomass hydrolysis, and biofuel production into a single stage. (*B*) Engineering *E. coli* for use in consolidated bioprocessing. Cellulose and hemicellulose are hydrolyzed by secreted cellulase and hemicellulose enzymes (cyan) into soluble oligosaccharides. β-glucosidase enzymes (red) further hydrolyze the oligosaccharides into monosaccharides, which are metabolized into biofuels via heterologous pathways.

removal of soluble hydrolase inhibitors may substantially reduce the enzyme loading required]. To further engineer a cellulolytic *E. coli* strain for use in consolidated bioprocessing, biofuel production pathways must also be introduced and expressed at levels that yield high titers while not overburdening the cell. The integration of engineered cellulolytic capabilities together with pathways for advanced biofuel production into a single organism may present an insurmountable metabolic burden for *E. coli*, or indeed any microbe, without appropriate regulation.

We engineered *E. coli* to convert plant biomass into three advanced biofuels without the addition of exogenous GH enzymes (Fig. 1*B*). The carefully regulated expression of heterologous GH enzymes made suitable for export by *E. coli* allows rapid and efficient growth on model cellulosic and hemicellulosic substrates, as well as on the cellulose and hemicellulose components of raw plant biomass pretreated with ionic liquids (IL). IL pretreatment of plant biomass is a promising approach for enabling efficient biomass conversion (20). While the price of IL is currently a substantial barrier to commercialization, recent work has identified performance targets that could eventually enable adoption of this highly effective pretreatment technology (24). Unlike other pretreatment techniques, dissolution of plant biomass in IL nearly eliminates cellulose crystallinity and significantly decreases lignin content, thereby significantly decreasing the enzyme load required for hydrolysis (25). Our *E. coli* is capable of growing on the cellulose and hemicellulose fractions of several types of IL-pretreated plant biomass, even with low yields of secreted protein (<0.1 mg enzymes/g solids). Furthermore, we show that cellulolytic and hemicellulolytic capabilities can be expressed with any of three distinct biofuel synthesis pathways in the same organism. By using cocultures of fuel-producing cellulolytic and hemicellulolytic strains, we demonstrate the production of fuel substitutes or precursors suitable for three engine types (gasoline, diesel, jet) directly from both the cellulose and hemicellulose components of IL-treated switchgrass. This represents a major advance toward combining the extensive biosynthetic capabilities of *E. coli* with lignocellulose utilization, while avoiding a dedicated process for enzyme generation, a substantial cost barrier to advanced biofuel production. Our results are a proof-of-concept that provides the foundation to further developments in both *E. coli* engineering and IL pretreatment that could eventually realize the cost savings achievable by consolidated bioprocessing. The modifications described here could likely be transplanted into other industrial microorganisms.

Results

The first step of lignocellulose metabolism is hydrolysis of cellulose and hemicellulose by secreted cellulase and hemicellulase enzymes, respectively (Fig. 1*B*). We found previously that the *Clostridium stercorarium* endoxylanase Xyn10B can be produced extracellularly by *E. coli* when fused with the protein OsmY (11), a fusion shown to enable protein export (26). To find a cellulase exportable by *E. coli*, we expressed a library of 10 family 5 endocellulases as fusions with OsmY (Table S1). Expression of two of the OsmY-cellulase fusions generated endocellulase activity in

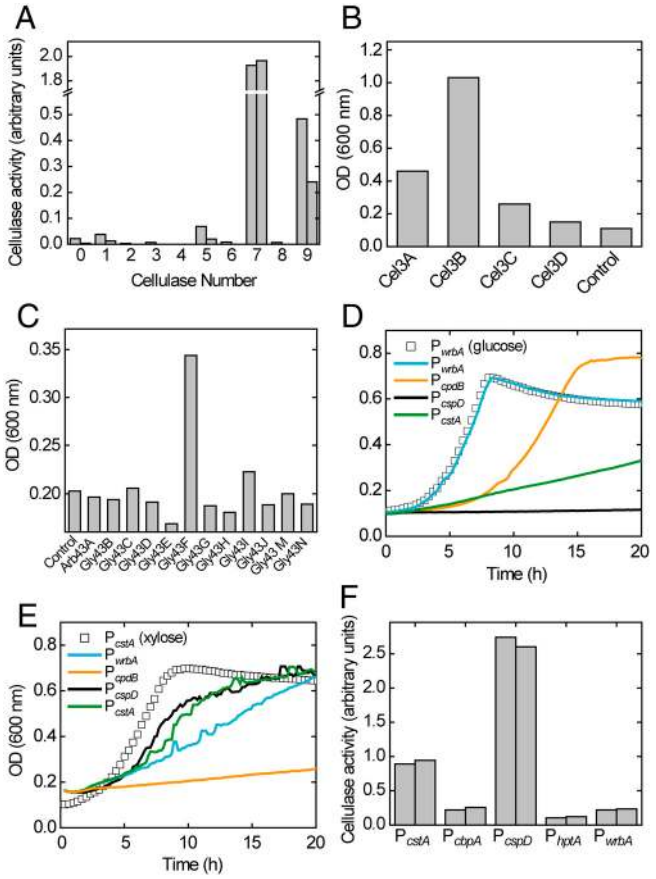

Fig. 2. Assembling biological parts required for lignocellulose hydrolysis and consumption by *E. coli*. (*A*) Secretion of cellulases. Cellulases were expressed as fusions with the OsmY protein, and extracellular cellulase activity measured using an azo-CMC assay. Cellulase identities that correspond to the numbers used can be found in Table S1. Two measurements from each cellulase are shown. (*B*) Growth after 18 h in M9/0.2% cellobiose medium of *E. coli* expressing four β-glucosidases from *Cellvibrio japonicus* under control of the lacUV5 promoter. (*C*) Growth of *E. coli* in MOPS-M9/0.2% xylodextrins after 15 h, enabled by expression of xylobiosidases. (*D*) Growth curves on MOPS-M9/0.5% cellobiose medium when expressing β-glucosidase Cel3A under control of *E. coli* promoters. A growth curve on glucose is shown for comparison. (*E*) Growth curves on enzymatically hydrolyzed xylan of *E. coli* expressing the xylobiosidase Gly43F under control of *E. coli* promoters, with a growth curve on xylose for comparison. Each curve is an average of two separate experiments. For growth curves on glucose and xylose, half of the data points are omitted for clarity. (*F*) Extracellular endocellulase activity levels of cellulase #7 (Cel from *Bacillus sp.*D04) when expressed under the control of several native *E. coli* promoters after 20 h of growth in LB medium. Measurements from biological duplicates are shown.

the growth medium (Fig. 2*A* and Fig. S1), with the Cel enzyme from *Bacillus sp.* D04 (cellulase #7) (27) demonstrating the highest activity. Both Cel and Xyn10B demonstrated activity against IL-treated switchgrass, indicating that enzymes expressed extracellularly by *E. coli* could potentially reduce or eliminate the need for exogenously added cellulolytic enzymes. Extracellular OsmY-Cel released glucose equivalent to 5% of the cellulose, producing cellotriose and cellobiose, while OsmY-Xyn10B hydrolyzed 11% of the xylan, mostly into xylotriose and xylobiose (Fig. S2). The combined biomass hydrolysis yield represents 8% of the total sugars available in the IL-treated switchgrass (28).

The soluble oligosaccharides that are produced by enzymatic hydrolysis of cellulose and xylan (cellodextrins and xylodextrins, respectively) cannot be metabolized by *E. coli* MG1655. To further hydrolyze cellodextrins into glucose, we screened four β-glucosidases cloned from *Cellvibrio japonicus*, a Gram-negative cellulolytic bacterium, and determined if their expression in *E. coli* could permit growth on cellobiose (Fig. 1*B*) (29, 30). *E. coli* grew best on cellobiose when expressing either *cel3A* or *cel3B* (Fig. 2*B*). To enable growth of *E. coli* on xylodextrins, the oligosaccharide products of xylan hydrolysis, we screened 12 xylobiosidase genes from *C. japonicus* (29). Expression of *gly43F* enabled growth on enzymatically hydrolyzed beechwood xylan (Fig. 2*C*).

The need for exogenously added chemicals to activate expression of biomass-consumption pathways might require extensive optimization of both the timing of induction and the induction strength, complicating engineering of biofuel generation from biomass. Therefore, we used native *E. coli* promoters to control expression of the selected β-glucosidase and xylobiosidase genes. This places expression of the biomass-consumption pathways under control of environmentally responsive promoters and avoids the costs of expensive chemical inducers for activation of the biomass-consumption pathways. We sought to achieve growth rates of oligosaccharide-utilizing *E. coli* that matched rates observed on the corresponding monosaccharide. Reasoning that expression of biomass-consumption pathways should be limited to periods when *E. coli* is starved of carbon (for instance, when the cells are freshly inoculated into biomass-containing medium from a glucose-based seed culture), we screened several promoters that have been shown to increase in transcriptional activity prior to stationary phase (31) or known to be activated by the gene regulator CRP (32). We expressed *cel3A* and *cel3B* using several native *E. coli* promoters to determine which promoter-enzyme combination would permit the fastest growth on cellobiose. Remarkably, a strain expressing *cel3A* under the control of the *wrbA* promoter (P_{wrbA}) grew on cellobiose as fast as on glucose (Fig. 2*D* and Fig. S3). We screened the same set of promoters to optimize expression of *gly43F* as determined by growth on xylodextrins. We found that expression of *gly43F* using the promoters P_{cstA} or P_{cspD} enabled a growth rate on xylodextrins nearly as high as on xylose (Fig. 2*E*). Surprisingly, the use of native promoters to drive expression of appropriate β-glucosidase and xylobiosidase genes enables *E. coli* to grow on oligosaccharides at a rate limited only by the consumption rate of the monosaccharides and perhaps as fast as native cellulolytic organisms.

To express the complete biomass conversion pathways under native promoters rather than the chemically inducible promoter used to screen the cellulase library, we placed expression of the *osmY-cel* fusion under control of the members of our promoter library to determine which promoter generated the maximum extracellular cellulase yield. Expression of *osmY-cel* using the promoter for the *cspD* gene (P_{cspD}) resulted in the highest cellulase activity of the promoters tested (Fig. 2*F*). We combined P_{cspD}-*osmY-cel* with P_{wrbA}-*cel3A* into a single plasmid designated pCellulose (Fig. 3*A*) to enable growth on cellulose. *E. coli* bearing pCellulose grew on the model substrate PASC as the sole carbon source (Fig. 3*B*), though growth on cellobiose was slowed relative to plasmids bearing P_{wrbA}-*cel3A* alone (Fig. S4). In the same manner, we combined P_{cspD}-*osmY-xyn10B* with P_{cstA}-*gly43F* into a single plasmid, designated pXylan (Fig. 3*A*). Impressively, *E. coli* bearing pXylan grew on beechwood xylan, a model hemicellulosic substrate, at nearly the limit set by the consumption rate of xylose (Fig. 3*C*).

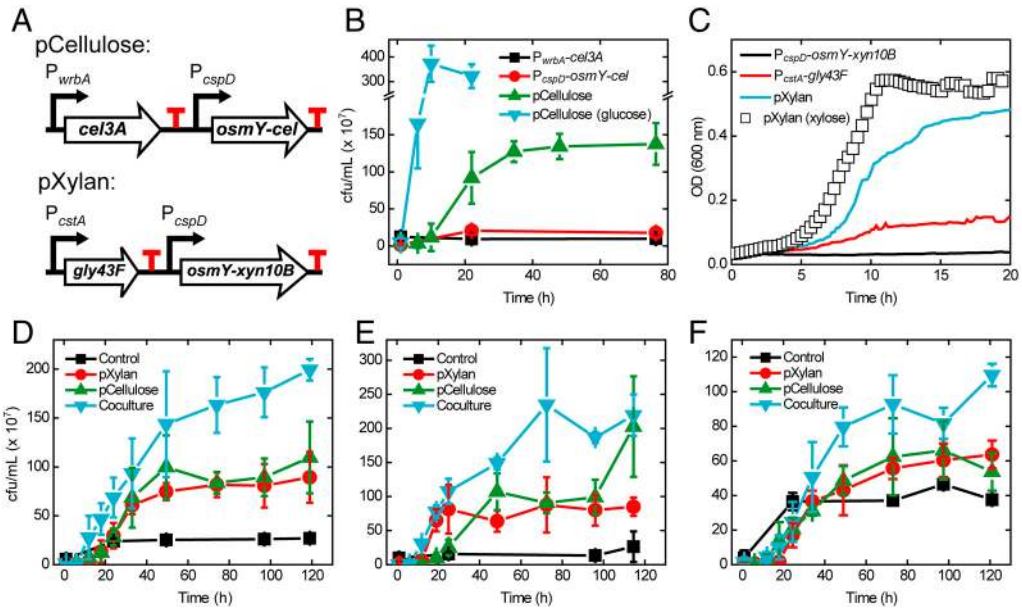

Fig. 3. Engineered *E. coli* grows on model cellulosic substrates and IL-treated plant biomass. (*A*) Gene schematics for the pCellulose and pXylan plasmids, designed to enable *E. coli* to metabolize cellulose and xylan, respectively. (*B*) Growth on phosphoric acid swollen cellulose (PASC) monitored by serial dilution, plating, and colony counting. Cells expressing either Cel3A or OsmY-Cel alone, or containing the pCellulose plasmid, were grown in MOPS-M9/0.7% PASC. Growth of the pCellulose-bearing strain in MOPS-M9/0.4% glucose is shown for comparison. (*C*) Growth of strains expressing either Gly43F or OsmY-XynB alone, or bearing pXylan in MOPS-M9/0.5% beechwood xylan. Growth of pXylan-bearing strain in 0.5% xylose is shown for comparison. Each curve is an average of three separate growth experiments. (*D–F*) Growth on the cellulose and hemicellulose fractions of IL-treated switchgrass, eucalyptus, and yard waste, respectively. Error bars represent standard deviation of biological triplicates, except for yard waste control strain (biological duplicates).

We next attempted to grow *E. coli* bearing either pXylan or pCellulose on plant biomass treated with the ionic liquid 1-ethyl-3-methylimidazolium acetate [C_2mim][OAc]. We inoculated *E. coli* MG1655 strains bearing pXylan, pCellulose, or a control plasmid into minimal medium containing 2.6% w/vol IL-treated switchgrass as the sole carbon source, without adding exogenous enzymes. The strains containing pXylan and pCellulose grew well, indicating that both the cellulose and hemicellulose components of the pretreated switchgrass can be used as carbon sources (Fig. 3*D*, red and green curves). The control strain showed minimal growth (Fig. 3*D*, black curve), indicating that most of the growth observed by the pCellulose and pXylan strains is enabled via enzymatic hydrolysis of cellulose and xylan, rather than any monosaccharides present in the switchgrass or released by pretreatment. The monocultures continued to produce up to 0.5 mg/L xylanase and cellulase enzymes during growth (Fig. S5). We also observed leakage of other cellular proteins into the growth medium, which may be a consequence of expressing fusions with a periplasmic protein (33) (Fig. S5). When the strains were combined and grown on switchgrass as a coculture, the cells grew to a cell density approximately equal to the sum of the individual monocultures (Fig. 3*D*, cyan curve), demonstrating growth on both fractions of switchgrass in one medium.

We tested growth on IL-pretreated *Eucalyptus globulus* to determine if IL pretreatment could render a range of lignocellulose types digestible by our engineered *E. coli.* Both pXylan and pCellulose monocultures and a coculture of the two strains grew well in minimal medium containing 4.0% w/vol IL-treated eucalyptus (Fig. 3*E*). Finally, we tested growth on IL-treated yard waste, a feedstock that could avoid the costs of growing dedicated energy crops while decreasing landfill usage (34). Once again, both monocultures and coculture grew in minimal medium containing 2.6% w/vol yard waste (Fig. 3*F*).

To demonstrate production of advanced biofuels from plant biomass without the use of exogenously added GH enzymes, we next engineered the biomass-consuming *E. coli* strains to generate three advanced biofuels directly from IL-treated switchgrass. We chose pathways that produce alcohols, linear hydrocarbons, or branched-chain hydrocarbons to test the integration of our biomass-consumption pathways with the extensive biosynthesis capabilities of *E. coli*. Biodiesel, typically made from plant oils that have been chemically esterified with methanol or ethanol, can also be made by *E. coli* in vivo in the form of fatty-acid ethyl esters (FAEE) (11). We encoded a six-gene FAEE production pathway on a single plasmid (pES120, Fig. 4*A*) and introduced the construct into a strain of *E. coli* MG1655 lacking the acyl-CoA dehydrogenase gene *fadE*. We found that this strain generates 405 ± 27 mg/L from MOPS-M9/1% glucose (10 g/L) (or 0.04 g FAEE/g glucose, 12% of the theoretical yield of 0.33 FAEE/g glucose) (11), and 0.022 g FAEE/g xylose from 10 g/L xylose. *E. coli* MG1655 Δ*fadE* pES120 bearing pXylan or pCellulose produced FAEE from xylan or cellobiose, respectively (Fig. S6*A*), indicating that both strains are capable of FAEE production from their substrates. In order to produce FAEE from plant biomass, a coculture of both strains was grown in minimal medium containing 5.5% w/vol IL-treated switchgrass. The coculture produced 71 ± 43 mg/L of FAEE, well above the no-carbon control (6.1 ± 0.5 mg/L, Fig. 4*B*) and the noncellulolytic *E. coli* control (4 ± 3 mg/L), indicating production of FAEE directly from pretreated switchgrass. This corresponds to 80% of the estimated yield obtainable with this pathway from the amount of sugars anticipated to be released from 5.5% switchgrass by the Cel and Xyn10B enzymes (0.14% glucose and 0.14% xylose).

Butanol has been proposed as a gasoline replacement because it is fully compatible with existing internal combustion engines. Based in part on previous work (9), we constructed a heterologous butanol pathway encoded on a single plasmid (pButanol, Fig. 4*A*) and inserted into an *E. coli* DH1 strain lacking the alcohol dehydrogenase gene, *adhE*. When bearing either pXylan or pCellulose, *E. coli* DH1 Δ*adhE* pButanol produced butanol from either xylan or cellobiose, respectively (Fig. S6*B*). A coculture of both strains yielded 28 ± 5 mg/L butanol from defined rich medium containing 3.3% w/vol IL-treated switchgrass as the main carbon source (Fig. 4*C*). A control strain lacking pXylan or pCellulose produced 8 ± 2 mg/L butanol from pretreated switchgrass.

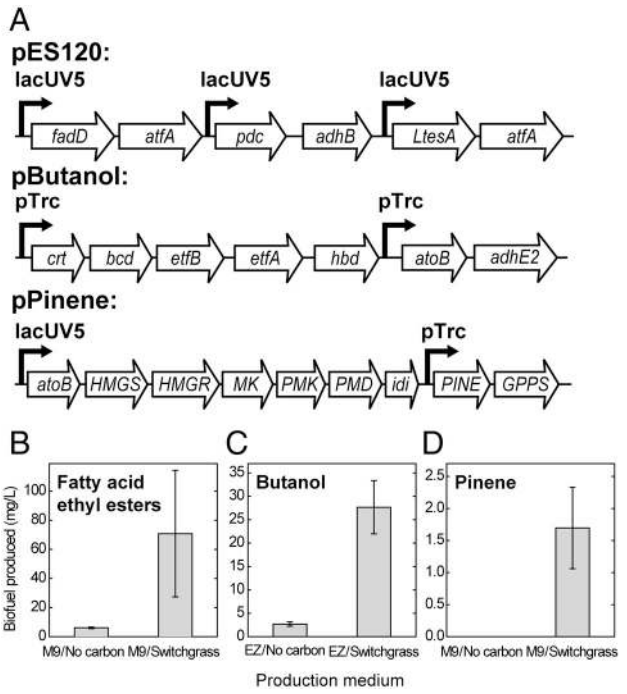

Fig. 4. Conversion of IL-treated switchgrass into advanced biofuels. (*A*) Gene schematics of plasmids encoding biofuel production pathways demonstrated in this work. Gene names listed in Table S2. Production of fatty-acid ethyl esters (*B*), butanol (*C*), and pinene (*D*) from IL-treated switchgrass by cocultures of cellulose- and xylan-consuming *E. coli*. Error bars represent standard deviation of biological triplicates.

Finally, we constructed a metabolic pathway to produce the monoterpene pinene, an immediate chemical precursor to a potential jet fuel (35), directly from switchgrass. The pinene synthesis pathway was encoded on a single plasmid (pPinene, Fig. 4*A*) and introduced into *E. coli* MG1655. We combined pXylan and pCellulose into separate strains of *E. coli* MG1655 pPinene and confirmed that each strain is capable of producing pinene from either xylan or cellobiose, respectively (Fig. S6*C*). We inoculated the strains as a coculture into MOPS-M9 medium containing either 3.9% IL-treated switchgrass or no carbon source. The pinene pathway yielded 1.7 ± 0.6 mg/L pinene from pretreated switchgrass (Fig. 4*D*). No pinene was produced from a culture grown in MOPS-M9 medium without a carbon source or from switchgrass medium inoculated with a strain lacking pXylan or pCellulose.

Discussion

We have demonstrated the engineering of *E. coli* to produce three advanced biofuels suitable for existing fuel infrastructure directly from lignocellulosic plant biomass without using externally supplied GH enzymes. While our engineering was greatly facilitated by the tractability of *E. coli*, the approach we have described here could be readily adapted for other microorganisms for use in a consolidated bioprocess to generate advanced biofuels from biomass. IL pretreatment using [C_2mim][OAc] rendered three types of lignocellulose suitable for use by our strains as sole carbon sources, indicating that our system is likely applicable to lignocellulose feedstocks that are ecologically and eco-

nomically appropriate to grow and harvest anywhere in the world. Overall, our results illustrate that the wide portfolio of compounds that can be synthesized by *E. coli*, or any other microorganism, can be produced directly from any IL-pretreated plant feedstock.

In order to make our *E. coli* strains suitable for use in an industrial bioprocess, both biofuel-producing and biomass-degrading capabilities require significant improvements. For instance, an optimal strain capable of producing FAEE at theoretical yield (0.33 g FAEE/ 1 g glucose) and achieving complete hydrolysis of IL-treated switchgrass, of which 78% is cellulose and xylan by weight (28), would obtain 0.26 g FAEE/ 1 g of IL-treated switchgrass, far higher than what could be achieved here. This requires an eightfold improvement in biofuel yield from glucose over what the current FAEE production pathway can achieve. More relevant to the engineered cellulolytic capabilities described in this work, the cellulose and hemicellulose fractions (95% and 89%, respectively) not digested by the enzymes we use here must be saccharified by the *E. coli* strain to achieve high biofuel yields or even to reach cell densities typical of an industrial fermentation process. A wide variety of enzymes found to have activity against IL-treated plant biomass was recently found in a cow rumen metagenome (36), and these enzymes could be screened to find either replacements for or supplements to the hydrolysis activity of Cel and Xyn10B enzymes. Furthermore, protein export pathways that do not compromise the cell membrane (as our OsmY fusions may be doing) should be used instead to avoid compromising cellular fitness and biofuel yields. Along with chromosomal integration of the biomass-consumption pathways (as opposed to encoding the pathways on plasmids), these steps should also improve the genetic stability of our modifications as well as their suitability for industrial-scale fermentations. In parallel with optimizations of the *E. coli* strain, the IL pretreatment could be modified to render the lignocellulose completely susceptible to hydrolysis by the GH enzymes we used here. For instance, acid catalysis during IL pretreatment of cellulose has been shown to dramatically increase the extent of subsequent enzymatic hydrolysis (37). These improvements will be required to fully realize the cost savings of a consolidated bioprocess that could provide a versatile platform for producing any advanced biofuel from any plant biomass at economical yields.

Materials and Methods

Selection, Optimization, and Screening of Cellulase Genes. The set of cellulases was chosen to maximize diversity within family 5 endocellulases. First, the CAZy database was used to collate all known family 5 enzymes (38). At the time this work was done, there were 689 such enzymes in the database. The enzymes were aligned using Muscle (39) and then 10 were selected to maximize diversity using HyperTree (40). The 10 genes were then optimized for expression in *E. coli* using GeneDesigner and synthesized by DNA 2.0 (41). Two cultures each of *E. coli* DH10B cells bearing pGB012 plasmids encoding each individual OsmY-cellulase fusion were grown overnight in LB medium supplemented with 100 μg/mL carbenicillin and inoculated 1/100 into fresh LB. Cultures bearing pGB012 were used as a cellulase-free control. Cultures were grown at 37 °C to an optical density at 600 nm (OD_{600}) of 0.4 and induced by addition of IPTG to 200 μM, and expression proceeded at 37 °C for 20 h. As described in *SI Text*, 200 μL of the supernatant was assayed for endocellulase activity.

Measurement of Native Promoter-Driven Cellulase Secretion. *E. coli* MG1655 bearing plasmids with *osmY-cel* under control of several *E. coli* promoters was grown in LB medium (100 μg/mL carbenicillin) for 20 h before endocellulase activity present in the supernatant was measured.

Beta-Glucosidase Screening and Native Promoter Selection. *E. coli* BL21 bearing beta-glucosidase genes was grown overnight in LB medium with 100 μg/mL carbenicillin, transferred 1/100 into M9/0.2% cellobiose medium with 100 μg/mL carbenicillin, and allowed to grow for 18 h at 37 °C before OD measurements were taken. A cell line bearing a plasmid with a beta-xylosidase was used as a control. For Cel3B-native promoter screening, plasmids bearing Cel3B under control of several *E. coli* promoters were introduced into *E. coli* BL21 cells, and transformants were grown in LB medium with 100 μg/mL carbenicillin overnight and inoculated 1/25 into a 96-well plate with 200 μL of M9/0.2% cellobiose medium or M9/0.2% glucose medium with 200 μg/mL carbenicillin. Growth was monitored with a microplate incubator and reader (TECAN). For Cel3A-native promoter screening, plasmids bearing Cel3A under control of one of several promoters were introduced into MG1655 cells, and overnight cultures were inoculated 1/40 into 800 μL of MOPS-M9/0.5% cellobiose or MOPS-M9/0.5% dextrose with 100 μg/mL carbenicillin in a 24-well plate. Growth was monitored with a microplate incubator and reader (TECAN).

Beta-Xylosidase Screening and Native Promoter Selection. *E. coli* DH10B carrying beta-xylosidase genes under control of P_{cspD} was grown overnight in LB medium with 100 μg/mL carbenicillin. The cultures did not grow at similar rates, likely due to the expression of proteins at toxic levels. Cultures were inoculated into MOPS-M9/0.2% xylan with 0.5 μg/mL thiamin and 100 μg/mL carbenicillin, into which sterile LB containing secreted OsmY-Xyn10B had been added (1/10 volume) to hydrolyze the xylan into xylodextrins. Growth was monitored on a 96-well plate with a microplate reader (TECAN). For Gly43F-native promoter screening, plasmids carrying gly43F under control of several *E. coli* promoters were introduced into *E. coli* MG1655 cells. Cells were grown overnight in LB medium with 100 μg/mL carbenicillin and inoculated 1/40 into 800 μL of MOPS-M9/0.5% beechwood xylan or xylose with 100 μg/mL carbenicillin supplemented with 5% of sterile LB containing secreted OsmY-Xyn10B on a 24-well plate. Growth was monitored with a microplate reader.

Growth Measurement on Beechwood Xylan. Biological triplicates of *E. coli* MG1655 cells carrying either pXylan, pP_{cstA}-*gly43F*/*p15A*, or pP_{cspD}-*osmY-xyn10B*/*SC101*** was inoculated into LB with 100 μg/mL carbenicillin and grown at 37 °C for 16 h. Overnight cultures were inoculated 1/20 into 800 μL MOPS-M9/0.5% xylan or 0.5% xylose medium with 100 μg/mL carbenicillin and grown with shaking in a microplate reader (TECAN) at 37 °C. Curves shown are averages of the triplicates, and median-averaged over a five-point window.

Growth Curves on Biomass and PASC. For biomass medium, IL-treated biomass (prepared as described in *SI Text*) was washed with water to remove any growth inhibitors present (such as residual IL). A full description of washing procedure can be found in *SI Text*. We used 10 mL of MOPS-M9 with biomass and 100 μg/mL carbenicillin as growth medium. For growth on PASC, triplicates of *E. coli* MG1655 bearing plasmids pCellulose, pP_{wrbA}-*cel3A*/*p15A*, or p(P_{cspD}-*RBS3-osmY-cel*/*SC101*** were grown overnight in LB with 100 μg/mL carbenicillin. For growth on plant biomass, *E. coli* MG1655 bearing either plasmid pXylan, pCellulose, or a control plasmid were grown for 18 h at 37 °C in LB medium containing 100 μg/mL carbenicillin. For growth of monocultures on biomass, the biomass medium was inoculated 1/20 (0.5 mL) with either the control, pXylan, or pCellulose cultures. For growth of pXylan/pCellulose cocultures, the biomass medium was inoculated with 0.25 mL pXylan and pCellulose cultures. All growth curves were performed with biological triplicates (three different colonies), with the exception of the yard waste control culture, which was performed in duplicate. Growth was measured by serially diluting a sample 10^{-6} (2 μL in 200 μL three times) in sterile phosphate-buffered saline, and 100 μL of the 10^{-6} dilution was spread on an LB-agar plate. Colonies were counted the next day.

Conversion of Switchgrass to FAEE. Three aliquots of 5 mL MOPS-M9 medium containing either 5.5% sterilized washed switchgrass or no carbon source were prepared and inoculated 1/20 with cultures of *E. coli* MG1655 Δ*fadE* pES120 with either pXylan or pCellulose (or 1/10 with control culture) grown for 24 h in LB medium. Cultures were grown at 37 °C for 92 h, at which point FAEE production was induced by addition of 50 μM IPTG. The production cultures were left at room temperature for 4 h after induction and returned to 37 °C for 96 h of production time. Free fatty-acid ethyl esters, and free fatty acids, were measured largely as described in ref. 11.

Conversion of Switchgrass to Butanol. Twelve cultures of 5-mL EZ-Rich medium (Teknova) were prepared as described by the manufacturer except without glucose. Six of the cultures contained 3.3% (w/vol) washed, IL-treated switchgrass. *E. coli* DH1 Δ*adhE* pButanol carrying pCelluose or pXylan was grown in LB medium for 38 or 25 h, respectively, at 37 °C. Biomass and null media were inoculated with 0.25 mL of each culture (0.5 mL total inoculum size) and cultures moved to 37 °C for 6.5 h, after which 2 mL of EZ-Rich salts (to final concentration, including original formulation, of 2X) was added to the cultures. Cultures were grown at 30 °C and induced after 30 min by addition of

200 μM IPTG, sealed with parafilm (creating a microaerobic environment), and returned to 30 °C for 96 h. Butanol was extracted and quantified as described in *SI Text*.

Conversion of Switchgrass to Pinene. Twelve aliquots of 5 mL MOPS-M9 medium, six of which contained 3.9% (w/vol) washed switchgrass, were prepared. Three overnight cultures each of *E. coli* MG1655/pPinene carrying a control plasmid, pXylan or pCellulose were grown for 24 h in LB medium. 0.5 mL of control culture, or 0.25 mL of both pXylan and pCellulose, was added to the switchgrass and null media and the cultures grown at 37 °C. After 22 h, pinene production was induced by addition of 200 μM IPTG, 0.55 mL dodecane was added to trap the pinene, and the cultures were incubated to 30 °C for 72 h. Extraction and quantification of pinene was performed as described in *SI Text*.

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