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Research Article

Production of substituted styrene bioproducts from lignin and lignocellulose using engineered *Pseudomonas putida* KT2440

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Abbreviations: LB, Luria Bertani broth; 4VG, 4-vinylguaiacol; PCR, polymerase chain reaction; GVPL, Green Value Protobind lignin.

Abstract

Ferulic acid is a renewable chemical found in lignocellulose from grasses such as wheat straw and sugarcane. *Pseudomonas putida* is able to liberate and metabolise ferulic acid from plant biomass. Deletion of the hydroxycinnamoyl-CoA hydratase-lyase gene (*ech*) produced a strain of *P. putida* unable to utilise ferulic and p-coumaric acid, which was able to accumulate ferulic acid and p-coumaric acid from wheat straw or sugar cane bagasse. Further engineering of this strain saw the replacement of *ech* with the phenolic acid decarboxylase *padC*, which converts p-coumaric and ferulic acid into 4-vinylphenol and the flavour agent 4-vinylguaiacol, respectively. The engineered strain containing *padC* was able to generate 4-vinylguaiacol and 4-vinylphenol from media containing lignocellulose, or Green Value Protobind lignin as feedstock, and did not require the addition of an exogenous inducer molecule. Biopolymerisation of 4-vinylguaiacol and 4-vinylcatechol styrene products was also carried out, using *Trametes versicolor* laccase, to generate "biopolystyrene" materials on small scale.

1 Introduction

The hydroxycinnamic acids ferulic acid and p-coumaric acid are components of the lignocellulose structure in grasses such as wheat, rice, rye, barley, maize and miscanthus [1-3]. Some of the hydroxycinnamic acid content is attached via ester linkages to the lignin polymer, while the remainder is attached via ether linkages to the benzylic position of the aryl-C_3 lignin unit, thereby providing a linkage between lignin and hemi-cellulose, as shown in Figure 1. The amount of hydroxycinnamic acid and the ratio of ferulic acid to p-coumaric acid varies between different grasses, but typically consists of 0.5-1% of the lignocellulose dry weight [1,4]. Ester-linked ferulic acid can be released by treatment with aqueous alkali, or treatment with a ferulate esterase enzyme [5].

We have previously observed that, as a by-product of the generation of vanillin from lignocellulose in an engineered *Rhodococcus jostii* lignin-degrading bacterium, ferulic acid was also generated as a bio-product at 23-86 mg/L concentration [6]. Ferulic acid is an interesting renewable building block that could potentially be converted to high value chemicals, such as coniferyl alcohol by enzyme-catalysed reduction [7]. In this work we investigate whether ferulic acid can be accumulated via gene deletion, and converted into substituted styrene aroma chemicals via decarboxylation.

Pseudomonas putida KT2440 is a well-studied bacterial aromatic-degrading strain for which genetic tools are available, generation of gene knockout strains can be generated reliably, and the strain has been shown to be a robust micro-organism for metabolic engineering [8]. *P. putida* KT2440 is also known to degrade lignin [9], and has been used for metabolic engineering of lignin degradation to produce polyhydroxyalkoates [10] and muconic acid [11] bioproducts. *P. putida* KT2440 has also been engineered to utilize levoglucosan and cellobiosan [12], and has been engineered to produce ethanol by fermentation [13], therefore is an attractive host for bioconversion of lignocellulosic feedstocks.

Ferulic acid is metabolised in *P. putida* KT2440 via the hydroxycinnamic degradation operon, containing *fcs*, *ech* and *vdh* genes, encoding feruloyl CoA synthetase, enoyl CoA hydratase/aldolase, and vanillin dehydrogenase respectively

[14], as shown in Figure 1. This operon is regulated by *ferR* which represses expression, and derepression occurs when feruloyl CoA is bound to FerR, making *fcs* essential for the regulation of this operon [15]. In order to generate ferulic acid as a bioproduct from lignocellulosic biomass, *Δfcs* and *Δech* gene deletion strains were generated, as described below.

Phenolic acid decarboxylase, a cofactor-independent enzyme which catalyses the decarboxylation of ferulic acid and p-coumaric acid to the corresponding styrene, has been found in several Bacilli such as *Bacillus subtilis* [16], *Bacillus pumilus* [17,18], *Lactobacillus plantarum* [19,20]. 4-Vinylguaiacol is a valuable aroma chemical of interest to the food & flavour industry [21,22], and could potentially be polymerised to form bio-polystyrene derivatives, hence new bio-based routes to 4-vinylguaiacol would be of interest for industrial biotechnology. In order to generate substituted styrenes as bioproducts, the *padC* gene was inserted onto the *P. putida* genome.

2 Materials and methods

Materials. Samples of sugarcane bagasse and straw were provided by Dr. Fabio Squina (University of Sorocaba, Brazil). Protobind 1000 soda lignin was purchased from Green Value SA (Granit, Switzerland), and was manufactured from wheat straw. Biochemicals were purchased from Sigma-Aldrich except where otherwise indicated.

Pseudomonas putida gene deletion and insertion

DNA was amplified using Phusion polymerase (New England Biolabs) following manufacturer's instructions, and using the primers listed in Supporting Information (Table S1). Deletion of *ech* (PP_3358) and *fcs* (PP_3356) was achieved by amplifying 1 Kb of the upstream and downstream regions of the respective genes and ligating them between *Sbf*I and *Xba*I of pK18mobsacB [23] with the two fragments ligated with an additional *Nde*I site. A *Not*I and *Xho*I site were introduced between the *Nde*I site by amplifying the ampicillin resistance marker *bla* from p15FerCA [24].

The *pad*C gene was amplified from pET200_*padC* and ligated into the *Not*I and *Xho*I site of pK18mobsacBΔech, plasmids were transformed into *P. putida* KT2440 by electroporation and integrants were selected on LB agar + kanamycin 50 µg/ mL at 30^0C. Colonies were restreaked and incubated overnight at 30^0C on LB agar + kanamycin 50 µg/ mL, then LB agar + 10% sucrose. Replicative plating was then carried out on to LB agar containing 10% sucrose and LB agar + kanamycin 50 µg/ ml. Colonies able to grow on LB agar + 10% sucrose and not on LB agar + kanamycin 50 µg/ ml were then simultaneously plated onto M9 agar + 0.1% glucose and M9 agar +0.1% ferulic acid. Colonies unable to grow on M9 agar + 0.1% ferulic acid were confirmed as mutants by PCR.

Media and culture

Cultures with lignin feedstocks

50 ml cultures were prepared by autoclaving 40 ml of distilled water with the appropriate percentage of lignin feedstock (weight by final volume 50 ml), in a 250 ml flask. M9 media salts were autoclaved at 5x working concentration; once cooled, $CaCl_2$ 0.5 mM, $MgCl_2$ 2 mM and trace elements solution ((1 in 1000 dilution of citric acid 5 g/L, $ZnSO_4 \bullet 7H_2O$ 5 g/L, $Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$ 1 g/L, $CuSO_4 \bullet 5H_2O$ 0.25 g, H_3BO_3 0.05 g, $Na_2MoO_4 \bullet 2H_2O$ 0.05 g/L) were added.

P. putida was grown overnight in Luria Bertani broth at 30 °C. The OD_{600} was measured, and the culture was then centrifuged (4,000 *g*, 10 min) and resuspended into M9 salts plus feedstocks in order to achieve a final OD_{600} of ~1.0 in 50 ml of M9 minimal media.

HPLC analysis of lignin feedstock trials

Samples of culture were first centrifuged in a benchtop microcentrifuge for 10 minutes and then filtered using 0.2 µm pore sized Mini-Uniprep™ vials (Whatman™ GE Healthcare life sciences). HPLC analysis was then carried out using Phenomenex Hyperclone 5u bds C_{18} reverse phase column (5 µ bds, 250 x 4.60 mm). Buffer A: Water +0.1% TFA; buffer B: Methanol +0.1% TFA. Gradient: 10%-40% B for 5 mins; 40% B for 5 mins; 40-70% B for 10 min; 70-100% B for 3mins; 100% B for 3 mins.

Flow rate 0.5 ml/minute, UV absorbance measured at 270 nm. Concentrations of 4-vinylguaiacol, ferulic and p-coumaric acid were calculated based on peak height and a standard curve made using commercial standards.

Expression & purification of B. subtilis PadC

The *padC* phenolic acid decarboxylase gene was cloned from *Bacillus subtilis* genomic DNA by polymerase chain reaction, and cloned into expression vector pET200/D-TOPO (Invitrogen), containing an N-terminal His_6 fusion tag. Production of the recombinant enzyme from *E. coli* BL21 pET200_*padC* was carried out in 1 L Luria Bertani broth at 37°C in the presence of 35 μg/mL kanamycin. At A_{600} = 0.8, the culture was induced by the addition of IPTG (0.8 mM final concentration), and shaken at 16°C overnight at 160 rpm. Cells were harvested by centrifugation for 20 min at 5000 *g*. Cell pellets were re-suspended in lysis buffer containing 50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl and 10 mM imidazole. Cells were broken by passage through a OneShot cell disruptor (Constant Systems Ltd, UK) at 20 Psi, centrifuged again at 15000 *g* for 30 minutes to remove cell debris, and the supernatant decanted.

The supernatant containing PadC was filtered through a 0.2 μm syringe-filter to further remove particulate matter, and was applied to nickel-nitriloacetate agarose (Ni-NTA) affinity chromatography resin (10 mL, Qiagen) via slow mixing at 4 ^oC. The loaded resin was packed into a 10 mL column, then the unbound fraction was collected by elution with 20 mL wash buffer (50 mM NaH_2PO_4, 300 mM NaCl and 20 mM imidazole) at pH 8.0. Then, the His-tagged PadC was eluted 3 x 5 mL of elution buffer containing 50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl and 250 mM imidazole. All the active fractions were transferred into a 50 mL Millipore 10 kDa molecular-weight cut-off diafiltration tube and then centrifuged at 5000 *g* for 15 minutes. Fresh 50 mM sodium phosphate buffer (pH 7.4) was added to dialyse the bound fractions of the purified enzyme.

Assays and kinetic analysis of PadC

Substrate conversion was studied by UV-Vis scanning at 200-400nm for 15 minutes in 50 mM sodium acetate buffer pH 5.0 to which substrate (0.1 mM final concentration) and 8 μg enzyme was added to initiate the reaction. Extinction

coefficient of the substrate was determined from a standard curve of substrate dissolved in the assay buffer at 0.006-0.15 mM concentration, and was used to calculate the rate of reaction. Optimum pH was determined in a series of sodium acetate and sodium phosphate buffers at pH range 3.0–8.0. Kinetic parameters were obtained using the Michaelis–Menten equation and double reciprocal Lineweaver-Burk plot, using Microsoft Excel 2010 software.

Polymerisation of 4-vinylguiaiacol and 4-vinylcatechol by Trametes versicolor laccase

A 50 mL reaction mixture (in a stirred round-bottomed flask) contained 10 mM final concentration of ferulic acid (or caffeic acid) in 50 mM, pH 5.0 sodium acetate buffer, to which was added 1.1 mg purified phenolic acid decarboxylase, and incubated overnight at room temperature. Then 1.1 units *Trametes versicolor* laccase (Sigma Aldrich) was added to initiate polymerization, and the mixture was left for a further 48 hr at room temperature. Laccase transformations were found to proceed optimally under a balloon of oxygen gas. For co-polymerisation, 10 mM ferulic acid containing various molar concentrations of caffeic acid was pre-incubated with PAD at room temperature overnight, followed by addition of laccase. The precipitate formed in each reaction was recovered by centrifugation at 5000 g for 30 min, and the solid pellet retained for analysis.

Gel permeation chromatography (GPC). Each polymer sample was dissolved at a final concentration of 1 mg/mL in THF (HPLC grade, contains 2% TEA and 0.01% BHT (Topanol)) and filtered prior to injection into the GPC analyser. Gel permeation chromatography samples were run on an Agilent 390-LC system equipped with a PL-AS RT autosampler, a 100 µL injection loop, a 5 µm PLgel guard column (50 mm x 7.5 mm), two 5µm PLgel Mixed D columns (50 mm x 300 mm) in series and a differential refractive index (DRI) detector. The system was eluted with THF at a rate of 1 mL/min and the detector was calibrated with PL narrow polystyrene (157 to 300 000 g/mol) standard easy vials. Molecular weights of the samples were calculated using the polystyrene calibration.

Scanning electron microscopy (SEM). The morphology of the polymer was observed by the field emission scanning electron microscope (Zeiss SUPRA 55-VP).

The freeze-dried sample was mounted on a conducting double-sided carbon tape and inserted into a vacuumed chamber inside the scanning electron microscope (SEM), at an acceleration voltage of 5 kV.

3 Results

3.1 Generation of ferulic acid in Δfcs, Δech gene knockouts in Pseudomonas putida

Ferulic acid is metabolised in *Pseudomonas putida* KT2440 via conversion to its CoA thioester by feruloyl CoA synthetase (*fcs* gene), followed by enoyl CoA hydratase/lyase (*ech* gene) to form vanillin [12]. In order to generate ferulic acid from lignocellulose, Δfcs and Δech gene deletions were generated using *sacB* mediated homologous recombination (see Materials and Methods) [23]. Mutants were identified by replicative plating on to M9 media with 0.1% ferulic acid, and were confirmed by PCR analysis of genomic DNA. Whereas wild-type *P. putida* KT2440 is able to grow on M9 minimal media containing 0.1% ferulic acid as carbon source, both Δfcs and Δech gene deletion strains were unable to grow on M9 +0.1% ferulic acid.

Growth of the Δfcs and Δech gene deletion strains was then carried out on M9 minimal media containing 2% chopped wheat straw. The release of ferulic acid was observed by HPLC analysis, giving a peak at retention time 16.6 min which matched a commercial standard. A second peak at retention time 15.9 min corresponded to p-coumaric acid, the other hydroxycinnamic acid component of wheat straw. Both Δfcs and Δech gene deletion strains were found to generate ferulic acid and p-coumaric acid, with no discernible difference, however the Δech mutant was used for further characterisation, since the *fcs* gene maintains the regulation of the hydroxycinnamic acid degradation operon, hence integrating into the *ech* locus gave the possibility of establishing an auto-induction system based on the release of ferulic acid.

3.2 Testing against different feedstocks

To explore the amount of hydroxycinnamic acids available to *P. putida* from different grass lignin feedstocks, *P. putida* Δ*ech* was inoculated into M9 minimal

media containing either wheat straw (WS), sugarcane bagasse (SB), sugarcane straw (SS), miscanthus (M) or Green Value Protobind lignin (GVPL), at 2% (w/v). After six days of incubation at 30^0C samples were taken and analysed by HPLC. The results are shown in Figure 2.

From the biomass-containing feedstocks, growth on M9/WS produced the most ferulic acid at 8.3 +/- 3.2 mg/ L, while M9/SB produced the most *p*-coumaric acid at 16.1 +/- 1.4 mg/ L, however, the starting concentration of *p*-coumaric acid was 13.9 mg/L, hence much of the *p*-coumaric acid product may have been released through autoclaving of the biomass. Control incubations of M9/WS containing no bacterial cells verified that *P. putida* Δ*ech* was responsible for release of 80-90% of the observed ferulic acid from WS, although substantial release of p-coumaric acid and ferulic acid was observed from GVPL in autoclaved M9 media (see Supporting Information Figure S1). Examination of the effect of autoclaving in either water or M9 media (see Supporting Information Figure S2) showed that autoclaving was responsible for the majority of ferulic acid release, although some ferulic acid could be released from GVPL by treatment with M9 media alone. The higher proportion of *p*-coumaric acid released from SB, SS and M is consistent with ratios of *p*-coumaric acid/ferulic acid of 2-4:1 reported for sugarcane [24] and miscanthus [25], whereas WS contains ferulic acid and *p*-coumaric acid in a ratio of 2:1 [1]. Growth of *P. putida* Δech on M9/GVPL generated higher titres of 48.3 +/- 7.7 mg/ L ferulic acid and 40.6 +/- 1.1 mg/ L p-coumaric acid, although the starting concentrations of each acid were higher than for the lignocellulosic feedstocks. GVPL gave the highest titre of ferulic acid in these experiments, and was therefore used for further optimization.

3.3 Formation of substituted styrene bioproducts by chromosomal integration of B. subtilis padC

The *B. subtilis padC* gene encoding phenolic acid decarboxylase was chromosomally integrated into the hydroxycinnamic acid induced *fer*R operon [26], replacing *ech,* using the same method used to create the original *ech* deletion mutant. Integration into this site produced an auto-induction system for *pad*C in the presence of its substrate, ferulic or p-coumaric acid, requiring no exogenous inducer in the fermentation. To test the autoinduction of *padC*, cultures of *P. putida*

Δ*ech*::*pad*C were grown to an OD_{600} = 1.00 in LB media, and then 0.05% ferulic acid or p-coumaric acid was added, and the cultures were incubated at 30^0C for 18 hours. Analysis by HPLC, as shown in Figure 3A, gave new peaks at retention times 22.3 and 22.8 min respectively, corresponding to 4-vinylguaiacol and 4-vinylphenol. The identity of these peaks was confirmed using commercial standards, and co-eluted with samples of 4-vinylguaiacol and 4-vinylphenol generated from decarboxylation of ferulic acid and p-coumaric acid respectively by recombinant PadC (see below).

Growth of the Δ*ech*::*pad*C mutant strain on M9 minimal media containing 5% GVPL gave rise to the same two peaks for 4-vinylguaiacol and 4-vinylphenol (see Supporting Information Figure S3), which were not present in incubations of *P. putida* Δ*ech* on 5% GVPL. Since the highest titre of ferulic acid observed with *P. putida* Δ*ech* had been obtained using GVPL as feedstock, the Δ*ech*::*pad*C mutant strain was tested on varying percentages of GVPL (5%, 10%, 20%, 30%), to investigate the concentration of 4VG produced (see Figure 3B). The highest observed titre of 4VG was 62.4 +/- 1.9 mg/ L, using 10% GVPL as feedstock. Given the titre of 48.3 mg/L ferulic acid obtained from *P. putida* Δech using 2% GVPL as feedstock, these data suggested some metabolism of 4VG, which was tested by measuring the time-course of 4VG production from *P. putida* Δ*ech*::*pad*C in M9 media containing 2% GVPL over the course of 168 hr. Under these conditions, 15-16 mg/L 4-vinylguaiacol was formed over 48-168 hr, but no significant decrease in 4VG concentration was observed (see Supporting Information Figure S4) from 48-168 hr. At 168 hr, 5 mg/L residual ferulic acid was still observed, so the conversion of ferulic acid to 4-vinylguaiacol is incomplete.

To investigate whether the *P. putida* Δ*ech*::*padC* culture is inactive after 48 hours of culture, two sets of *P. putida* Δ*ech*::*padC* were grown on 5% wheat straw, and after 48 hours, 250 mg/l of ferulic acid was spiked to one set of cultures. As shown in Supporting Information (Figure S5), ferulic acid was still converted to 4-VG in this experiment, indicating that PadC is still active after 48 hr, but only 20% conversion of ferulic acid was observed in the following 24 hr, lower conversion than observed for cultures grown on wheat straw as feedstock.

3.4 In vitro conversion using recombinant Bacillus subtilis phenolic acid decarboxylase

In order to verify the production of 4-vinylguaiacol, recombinant *Bacillus subtilis* phenolic acid decarboxylase was expressed in *E. coli* as a His_6 fusion protein, and the recombinant enzyme was purified by immobilized metal affinity chromatography (see Supporting Information Figure S6) in a titre of 170 mg protein per litre culture. Leisch *et al* have previously reported the use of phenolic acid decarboxylase *in vitro* to generate 4-hydroxystyrene and vinylguaiacol [27].

Incubation of purified PadC with 100 μM ferulic acid in 50 mM sodium acetate buffer pH 5.0 led to the disappearance of the UV-vis spectrum of ferulic acid (λ_{max} 280, 310 nm), and the appearance of a new species absorbing at 260 nm corresponding to 4-vinylguaiacol (see Figure 4A), which co-eluted with a commercial sample of 4-vinylguaiacol by reverse phase HPLC (Supporting Information Figure S7). PadC was also found to convert p-coumaric acid and caffeic acid, as observed by UV-visible spectroscopy (see Figure 4B, Supporting Information Figure S8), but did not convert sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) or 4-methoxycinnamic acid. Steady state kinetic parameters were measured for ferulic acid and caffeic acid (see Figure 4C,D): v_{max} for ferulic acid was 3.5-fold higher (39.9 μmol/min/mg, K_M 1.6 mM), than for caffeic acid (v_{max} 11.2 μmol/min/mg, K_M 1.1 mM), with substrate inhibition observed for caffeic acid at >2 mM concentration.

3.5 Biopolymerisation of 4-vinylguaiacol and 4-vinylcatechol products

We also investigated whether 4-vinylguaiacol could be polymerised enzymatically, to form a "biopolystyrene" product. Addition of *Trametes versicolor* (Tv) laccase at pH 5.0 to solutions of 4-vinylguaiacol generated using PadC gave rise to the rapid disappearance of 4-vinylguaiacol, and a new product peak was formed at 290 nm. In the case of 4-vinylcatechol, formed from caffeic acid, the broad absorbance at 260-300 nm decreased over 5 minutes, and a new, weaker absorbance was formed at 400-450 nm. In the case of 4-vinylguaiacol, a

yellow/orange precipitate was observed (see Supporting Information Figure S9), while a brown/black precipitate was observed in the case of 4-vinylcatechol, which could each be isolated by centrifugation at 5,000 x g, to give in each case a solid product, in approximately 60 wt% yield. The poly(4-vinylguaiacol) product was insoluble in water or methanol, but could be partially dissolved in tetrahydrofuran, whereas the poly(4-vinylcatechol) product was found to be insoluble in water, methanol and THF. Analysis of the poly(4-vinylguiaicol) product by gel permeation chromatography revealed that the major product showed M_W 515 & 1190 g/mol, with a minor peak at M_W 48,500 g/mol (see Supporting Information Figures S10, S11).

Freeze-dried samples of poly(4-vinylguaiacol) and a 2:1 copolymer poly[(4-vinylguaiacol)-co-(4-vinylcatechol) were analysed by scanning electron microscopy, as shown in Figure 5. In each case, particles were mostly spherical in shape, the poly(4-vinylguaiacol) particles were 0.2-0.3 μM in diameter, whereas the copolymer particles were more variable in size, with diameter range 0.2-1 μM.

4 Discussion

Hydroxycinnamic acids ferulic acid and p-coumaric acid can be generated from grasses such as wheat straw, miscanthus, and sugarcane, and therefore represent a feedstock for conversion to high value chemicals or materials. In this study we have shown that ferulic acid and p-coumaric acid can be generated as bioproducts in a *P. putida* Δech gene deletion strain, and can be converted to 4-vinylguaiacol and 4-vinylphenol products by chromosomal insertion of the *padC* gene encoding phenolic acid decarboxylase into a operon which requires no exogenous addition of inducer molecule. 4-Vinylguaiacol has been produced enzymatically from ferulic acid using recombinant ferulic acid decarboxylase [27], but in this work we demonstrate that it can be generated directly from plant biomass or commercial lignin, using an engineered microbe. The substituted styrene products can be polymerised enzymatically to form “biopolystyrene” products, or could be used as building blocks for other metabolic engineering applications. Li and co-workers have developed biocatalytic routes from styrene intermediates to chiral

diol, amino-alcohol, α-hydroxy-acid and α-amino acid bioproducts [28,29], which could potentially be applied to extend this work.

The titre of substituted styrene products obtained using *P. putida* Δech::padC was somewhat lower than that of ferulic acid from *P. putida* Δech. Monisha *et al*. have suggested that 4-vinylguaiacol can be converted to vanillin via oxidative cleavage by an unknown oxidase enzyme [30], hence one possible explanation is that 4VG is metabolised by *P. putida*, however, a time-course experiment showed no significant decrease in 4VG concentration from 2-7 days. Moreover, residual ferulic acid was also present in this experiment after 7 days, corresponding to 15-20% of the original concentration. As shown in Supporting Information Figure S5, PadC is still active after 48 hr, but lower activity was observed in a culture spiked with ferulic acid. Possible explanations could be reduced uptake of ferulic acid at later stages of growth, toxicity of 4-vinylguaiacol [31], or less efficient conversion by PadC at low concentrations of ferulic acid due to the relatively high K_M value (1.6 mM).

The use of a gene operon which is autoinducible by the release of the starting material is also potentially useful for industrial biotechnology, as this system avoids the use of an exogenous inducer molecule, which could be expensive for industrial scale fermentation.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Figure legends

Figure 1. A. p-Hydroxycinnamic acids present in wheat straw lignin. B. Scheme for generation of ferulic acid from wheat straw lignocellulose, and conversion to substituted styrenes. C. Genetic constructs used in this work.

Figure 2. Titres of ferulic acid and p-coumaric acid generated by *P. putida* Δech grown on M9 minimal media containing 2% (w/v) of different lignocellulose biomass, or containing Green Value Protobind lignin.

Figure 3. Production of substituted styrene products. A. HPLC traces showing the conversion by *P. putida* Δech::padC of p-coumaric and ferulic acid to 4-vinylphenol and 4-vinylguaiacol respectively. B. Titre of 4-vinylguaiacol formed at different loadings of Green Value Protobind lignin.

Figure 4. UV-visible assay of PadC against ferulic acid (panels A,C) and caffeic acid (panels B,D).

Figure 5. Formation (panel A) and scanning electron microscopy images of samples of poly(4-vinylguaiacol) (panel B) and copolymer 2:1 poly[(4-vinylguaiacol)-co-(4-vinylcatechol) (panel C)