A Vibrio-based microbial platform for accelerated lignocellulosic sugar conversion

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Abstract

Background: Owing to increasing concerns about climate change and the depletion of fossil fuels, the development of efficient microbial processes for biochemical production from lignocellulosic biomass has been a key issue. Because process efficiency is greatly affected by the inherent metabolic activities of host microorganisms, it is essential to utilize a microorganism that can rapidly convert biomass-derived sugars. Here, we report a novel Vibrio-based microbial platform that can rapidly and simultaneously consume three major lignocellulosic sugars (i.e., glucose, xylose, and arabinose) faster than any previously reported microorganisms.

Results: The xylose isomerase pathway was constructed in Vibrio sp. dhg, which naturally displays high metabolic activities on glucose and arabinose but lacks xylose catabolism. Subsequent adaptive laboratory evolution significantly improved xylose catabolism of initial strain and led to unprecedentedly high growth and sugar uptake rate (0.67 h⁻¹ and 2.15 g dry cell weight⁻¹ h⁻¹, respectively). Furthermore, we achieved co-consumption of the three sugars by deletion of PtsG and introduction of GalP. We validated its superior performance and applicability by demonstrating efficient lactate production with high productivity (1.15 g/L/h) and titer (83 g/L).

Conclusions: In this study, we developed a Vibrio-based microbial platform with rapid and simultaneous utilization of the three major sugars from lignocellulosic biomass by applying an integrated approach of rational and evolutionary engineering. We believe that the developed strain can be broadly utilized to accelerate the production of diverse biochemicals from lignocellulosic biomass.

Keywords: Vibrio, Lignocellulosic biomass, Xylose, Adaptive laboratory evolution, Carbon catabolite repression, Lactate

Background

In recent decades, efficient conversion of lignocellulosic biomass (e.g., switchgrass, sorghum) into chemicals has been extensively studied owing to its high sugar content and abundance [1–4]. Various microorganisms such as Escherichia coli and Saccharomyces cerevisiae have been utilized and engineered to improve their catabolic activities for major sugars (i.e., glucose, xylose, and arabinose) [5–7]. These efforts have successfully demonstrated the potential of microbial processes for the sustainable production of diverse value-added chemicals.

Because process efficiencies are greatly affected by the innate metabolic activities of host microorganisms, it is essential to exploit a host that can efficiently and rapidly utilize sugars from lignocellulosic biomass (e.g., glucose, xylose, and arabinose) [8]. In this regard, Vibrio species have been recently suggested as a new powerful platform for biochemical production.
owing to their superior growth on various sugars over conventional host platforms [9–12]. In addition, its high tolerance to osmotic stress is expected to help improve biochemical production [13]. Indeed, a few pioneering studies have shown that a broad spectrum of biochemi-
cals (e.g., ethanol, 2,3-butanediol, lycopene) can be pro-
duced from biomass sugars at high rates [9, 14–16] with
the development of genetic toolboxes for controllable
gene expression and genome editing [17]. These studies
suggest that the use of Vibrio species would greatly expe-
dite biochemical production from biomass.

To expand its application for lignocellulosic biomass
conversion, several issues need to be addressed. A few
Vibrio species (e.g., Vibrio sp. dhg and Vibrio natrie-
gens) show no detectable growth or sugar consumption
when grown on xylose, the second most abundant sugar
in lignocellosic [10] (Additional file 1: Table S1), likely
owing to their aquatic habitat [18]. Indeed, only 0.43%
(61 out of 14,153) of Vibrio genomes deposited at the
National Center for Biotechnology Information (NCBI)
have complete sets of xylose catabolic genes. In con-
trast, more than 10% (338 out of 3,366) of E. coli
strains have the genes (Additional file 1: Fig. S1). Furthermore,
similar to many other microorganisms, the preferential
utilization of sugars by carbon catabolite repression
(CCR) [19] would lower the efficiency of bioprocesses
[20]. Therefore, further studies to construct efficient
catabolism of lignocellulose-derived sugars in Vibrio
species are warranted to leverage its huge innate meta-
bolic activity in bioprocessing.

In this study, we reported an engineered Vibrio sp.
dhg that can rapidly and simultaneously utilize three
major sugars (glucose, xylose, and arabinose) of ligno-
cellulosic biomass (Fig. 1). First, based on the genome
analysis of Vibrio sp. dhg, we heterologously expressed
xylose isomerase from E. coli W, which was absent in
Vibrio sp. dhg, to complete the xylose utilization path-
way. This engineered strain was evolutionarily opti-
mized by continuous growth in a xylose-supplemented
minimal medium to achieve high xylose catabolic effi-
ciency. Furthermore, we enabled the simultaneous
utilization of glucose, xylose, and arabinose by deregul-
ating its native glucose-induced CCR. Finally, we dem-
onstrated the huge potential of the generated platform
by achieving high lactate production (83 g/L in 72 h)
with rapid co-consumption of sugars. Collectively, we
believe that the developed strain will be widely utilized

**Fig. 1** Generation of Vibrio-based microbial platform for efficient utilization of lignocellulosic sugars
and greatly accelerate the production of diverse bio-
chemicals from lignocellulosic biomass.

Results

**Construction of the xylose isomerase pathway in Vibrio sp. dhg**

We analyzed the genome of Vibrio sp. dhg to identify its endogenous pathways for sugar catabolism and to determine the genes required for xylose utilization. To this end, we queried the names of essential enzymes in each sugar catabolic pathway from the annotated reference genome of Vibrio sp. dhg [9]. If there was no matched enzyme name, we queried the amino acid sequence of an enzyme from a representative microorganism using Protein BLAST [21]. Genomic analysis revealed that Vibrio sp. dhg can catabolize glucose and arabinose via the Embden–Meyerhof–Parnas (EMP) pathway and pentose phosphate pathway (PPP) (Fig. 2A, Additional file 1: Table S2). Still, it does not have any complete gene sets of four known xylose utilization pathways (i.e., the isomerase pathway, oxidoreductase pathway, Weinberg pathway, and Dahms pathway, Additional file 1: Fig. S2). Considering that the xylose isomerase pathway provides the highest carbon yield and energy generation [22] (Additional file 1: Tables S3, 4), we decided to construct the xylose isomerase pathway in Vibrio sp. dhg.

To achieve this goal, we expressed heterologous *xylA* from *E. coli* W in Vibrio sp. dhg, which was the only required gene to construct the xylose isomerase pathway; all other genes including *xylB* encoding xylulokinase, another key enzyme for xylose isomerase pathway, were identified in Vibrio sp. dhg (Additional file 1: Table S2). To ensure the stable and constitutive expression of *xylA*, we used a synthetic promoter (VP13, equivalent to P_{123100} and an optimized 5’-UTR generated by UTR Designer [23]. Furthermore, the expression cassette was integrated into the chromosome by replacing *dns* encoding an extracellular nuclease; this gene was known to be non-essential for cell viability and its deletion enhances transformation efficiency [24]. Notably, the resulting VXA0 strain showed growth on xylose as the sole carbon source by successfully activating the xylose isomerase pathway. However, the strain showed a long lag phase (up to 4 days) before its growth and a low growth rate (0.01 h^{-1}), indicating a necessity for further optimization.

**Evolutionary optimization of xylose catabolism in Vibrio sp. dhg**

We applied an adaptive laboratory evolution (ALE) strategy [25–27] to further improve xylose utilization (Fig. 2B). Given that the VXA0 strain grew on xylose as a sole carbon source, we grew and iteratively passaged this strain in a xylose-supplemented minimal medium (see Methods for detail). Surprisingly, the population in the second flask displayed no lag phase time and a significantly increased growth rate (0.1 h^{-1}). Thereafter, the growth rates of the populations gradually increased over time (Fig. 2B). Within 2 months, a dramatically improved growth rate of 0.58 h^{-1} was achieved in the 38th flask. This observation implied the generation and accumulation of novel mutations augmenting xylose catabolism. The evolved population underwent 164 generations, equivalent to $1.4 \times 10^{12}$ cumulative cell divisions (CCD).

For a detailed characterization, we isolated evolved strains from multiple timepoints. We evaluated their maximum specific growth rates and specific sugar consumption rates in the xylose minimal medium (Fig. 2B). Specifically, three clones were isolated from four timepoints (a total of 12 clones): three intermediate timepoints (the 1\textsuperscript{st}, 3\textsuperscript{rd}, and 15\textsuperscript{th} flasks), where clear jumps in growth rates were observed, and the endpoint of the ALE experiment (the 38\textsuperscript{th} flask). The growth rates of the isolated clones generally showed a similar trend to the population growth rates; higher growth rates were observed with clones isolated from later flasks. In addition to increases in growth rates, xylose uptake rates were greatly improved. Resultantly, the three endpoint isolates showed the growth rates and specific xylose uptake rates of 0.67 h^{-1} and 2.15 g g_{dry\textsuperscript{-}weight}^{-1} h^{-1}, respectively, as a maximum. It should be noted that these values were superior to those of any other reported microbial platforms [5, 22, 28–31] that consume xylose (Additional file 1: Table S5).

**Identification and validation of beneficial mutations improving xylose utilization**

Whole-genome sequencing was performed for the VXA0 strain and the evolved isolates to identify mutations that improved xylose utilization (Fig. 2C). We identified five mutations in four regions in the genomes of the evolved isolates by the comparison with the genome of the starting strain (Additional file 1: Table S6, 7): (i) an deletion mutation substituting 1423 bases (Chr2; 1,846,276–1,847,699) into T of two neighboring genes (*yrKL* encoding an NADH oxidoreductase and *deoR* encoding a transcription factor for mannitol utilization family proteins), (ii) a single nucleotide variation (SNV) mutation in *scrC* encoding bifunctional diguanylate cyclase/phosphodiesterase (Chr2; 1,409,023, C to T resulting T32I), (iii and iv) SNV mutations in the promoter (Chr1; 2,768,285) and coding sequence (Chr1; 2,768,325, C to A resulting A3D) of the *xylA* gene, and (v) a SNV mutation in *cafA* encoding a cytoplasmic axial filament protein (Chr1; 2,875,221, T to A resulting early termination). Among these mutations, three mutations (i, iii, and iv) appear to substantially improve the maximum specific
Fig. 2 Improved xylose catabolism via ALE and elucidation of mutational mechanisms. A Predicted metabolic pathway of glucose, xylose, and arabinose in the VXA38 strain and genetic context of the region with major effective mutations. Key pathways are colored in yellow (glycolysis), gray (pentose phosphate pathway), blue (arabinose utilization pathway), and green (xylose isomerase pathway). Light and dark green indicate xylose isomerase (xylA) and xylulokinase (xylB), respectively. Light brown indicates the transcription factor for mannitol utilization family proteins (deoR), a putative repressor for attA, xylB, and attT. yrkL, attA, and attT encode NADH oxidoreductase, D-arabitol 4-dehydrogenase, and MFS superfamily transporters, respectively. Xylulose 5-phosphate is abbreviated as XSP. B Specific growth rates and xylose uptake rates of the isolates. Blue indicates the maximum specific growth rate, and red indicates the specific xylose consumption rate. The subset graph indicates the specific growth rates in each flask during the ALE experiment. Red arrows indicate flasks selected for evolved clone isolation. C Mutation analysis of the starting and evolved strains. The red boxes indicate the presence of mutations. V55Hfs2X means that the 55th amino acid was changed from valine to histidine (V55H) and the stop codon was generated after 2 amino acids (57th) owing to the frameshift (fs2X). X indicates the generation of a stop codon. D Cell growth (optical density at 600 nm, OD₆₀₀) over time with xylose minimal medium. Symbols: black circle, VXA38C (VXA38-1 with empty vector); green square, VXA38Y (VXA38-1 with additional yrkL expression); purple diamond, VXA38D (VXA38-1 with additional deoR expression). E Relative amounts of xylB transcripts in the VXA38C and VXA38D strains. F Catalytic efficiencies (kcat/Km in min⁻¹ mM⁻¹) of wild-type (VXW) and mutant (VXM) xylose isomerase G Normalized specific fluorescence of strains expressing the xylA-sgfp fused protein under the original P₁₃₁₀₀ (VXPW) and mutant promoter (VXPM).
growth rate on xylose. While the mutation in scrC did not affect growth during the exponential phase, it significantly reduced the lag time (Additional file 1: Fig. S3). It is likely that the cafA mutation does not affect xylose catabolism, given that no significant difference was observed between the VXA15-1 and VXA15-3 strains (Additional file 1: Fig. S3) and the mutation did not persist.

To understand how xylose catabolism was improved during exponential growth, we characterized the effects of the three mutations (i, iii, and iv). Initially, the effect of the mutation in yrkL and deoR was studied, since it first occurred, and an operon, divergently expressed next to deoR, contained a putative xylulokinase gene (52% amino acid identity of XylB from E. coli, Fig. 2A and Additional file 1: Fig. S4). This operon additionally contains attA and attT which encode D-arabitol 4-dehydrogenase and MFS superfamily transporter, respectively; Xylulokinase has often been found from arabitol utilization operons in many microorganisms [32–34], suggesting the importance of the putative xylB gene in the xylose metabolism in Vibrio sp. dhg. Initially, we individually expressed the intact yrkL and deoR genes in the endpoint isolate, VXA38-1, using a plasmid. The resulting VXA38Y (VXA38-1 with yrkL expression), VXA38D (VXA38-1 with deoR expression), and VXA38C (VXA38-1 with an empty plasmid as a control) strains were cultivated in xylose minimal medium (Fig. 2D). Notably, DeoR complementation completely impaired growth on xylose, whereas YrkL expression did not affect growth. Next, we analyzed the expression level of xylB upon DeoR expression; xylB was barely expressed (up to 100-fold, Fig. 2E), confirming the importance of this xylB gene in xylose metabolism. Collectively, these observations showed that the insufficient activity of xylulokinase was one of the rate-limiting steps in xylose metabolism, and higher growth was achieved by the truncation of DeoR, which upregulated xylB expression.

Next, we investigated the roles of these two mutations in the xylA coding sequence and its promoter region. Since the coding sequence mutation is non-synonymous, it was expected that the activity of XylA would be affected. Thus, we compared the specific activities (k_cat/K_m) of the purified mutant and wild-type XylA. Notably, it was found that the A3D mutation resulted in a 1.3-fold higher catalytic efficiency (k_cat/K_m) (Fig. 2F and Additional file 1: Fig. S5). Given that the mutated residue is located at the N-terminus, far from known active sites of similar XylA in other microorganisms [35, 36], this mutation likely affects the assembled structure of its homotetramer; a further detailed study is warranted. Nevertheless, this analysis confirmed that the higher activity of XylA enhanced xylose catabolism.

Finally, we investigated the effect of mutations on the expression levels of xylA. Since they are located in either the -10 box of P_J23100 or the proximal region to the start codon, it was likely that xylA expression was affected. In particular, the mutated promoter sequence became more similar to the consensus sequence of bacterial promoters [37], suggesting that the mutation potentially led to a higher expression of xylA. For validation, we quantified the amount of XylA by generating a fusion protein of the wild-type or mutant XylA with a green fluorescent protein (sGFP) expressed under the wild-type and mutant promoters (Fig. 2G). It was found that the amount of the fusion protein was sixfold higher with the mutant promoter compared to that with the wild-type promoter, whereas the mutation in the coding sequence did not affect the expression level (Additional file 1: Fig. S6). Collectively, the low activity of XylA was a bottleneck for xylose catabolism, and its expression cassette was mutated to increase its transcription level and specific enzyme activity.

Enabling simultaneous utilization of glucose, xylose, and arabinose

We further engineered the VXA38-1 strain to simultaneously utilize the major sugars (glucose, xylose, and arabinose) obtained from lignocellulose (Fig. 3A). Cultivation of the wild-type and VXA38-1 strains with a mixture of the three sugars (Fig. 3B, C) showed that the xylose and arabinose catabolism is repressed in the presence of glucose, similar to many other bacteria [38–40]; after glucose was depleted, xylose and arabinose were consumed simultaneously. In many bacteria, including Vibrio species [41–44], it is known that glucose is preferentially utilized by suppressed gene expression of the non-favored sugar utilization pathway by the cAMP receptor protein (CRP) (Fig. 3A). Given that the activity of adenylate cyclase (AC), which controls intracellular cAMP levels, is regulated by the phosphotransferase system (PTS) [40, 45], it was shown that altered PTS by the deletion of PtsG (a key enzyme consisting of PTS, EIIBC) enabled co-consumption of multiple sugars in E. coli, Klebsiella oxytoca, Enterobacter aerogenes, and Corynebacterium glutamicum [38–40]. Similarly, we also tested whether the deletion of ptsG could enable the co-consumption of sugars. Consistent with previous studies, this strain successfully co-utilized the three sugars (Fig. 3D). However, the glucose utilization and byproduct (i.e., acetate) formation were severely reduced, indicating the necessity for further optimization [41].

To restore glucose transportation, we additionally expressed an alternative non-PTS galactose symporter, GalP, from E. coli in VXA38P (Fig. 3A). Although its primary substrate is galactose, it can also uptake glucose
without affecting the cAMP level [45, 46]. Surprisingly, the resulting VXA38PG strain showed a substantial increase in glucose uptake without affecting the simultaneous utilization of xylose and arabinose (Fig. 3E). Moreover, the total sugar consumption rate was significantly increased in VXA38PG (2.01 g g<sub>dcw</sub> −1 h<sup>−1</sup>) strain compared to the VXA38P strain (1.69 g g<sub>dcw</sub> −1 h<sup>−1</sup>). The recovered acetate production also confirmed its potential to serve as a production host. Considering the rapid and simultaneous utilization of all major sugars in lignocellulose, the resulting strain was further engineered for chemical production.

**Efficient lactate production from lignocellulose-derived sugars**

We applied the developed VXA38PG strain, which can rapidly and simultaneously utilize the three lignocellulosic sugars for biochemical production. As a model compound, we chose lactate, which has various industrial applications, such as an acidulant, a preservative, and a monomer for biodegradable plastics [47] (Fig. 4A). To efficiently produce lactate, byproduct-producing pathways were blocked by deleting fumarate reductase (>frd<sub>ABCD</sub>) and pyruvate-formate lyase (>pfB) in the genome. Furthermore, endogenous lactate dehydrogenase (>ldhA) was overexpressed in the plasmid.

We cultivated the engineered strain, VXA38PGL, in a medium (Fig. 4B) supplemented with a mixture of the three sugars (i.e., glucose, xylose, and arabinose) at a ratio of 6:3:1, mimicking the contents of these sugars in lignocellulose [48, 49]. Mimetic sugar (40 g/L) was periodically supplemented with a 12 h interval. As a result, 83 g/L of lactate was produced over 72 h, which corresponds to a productivity of 1.15 g/L/h. Notably, byproduct formation was minimized, resulting in a high yield (0.80 g/g, 133 g of lactate from 166 g of the total sugars), equivalent to 80% of the theoretical maximum yield (Fig. 4C). The titer was the highest, and the yield was comparable with those of other similar studies (Additional file 1: Table S8). Moreover, 1.4-fold higher productivity was achieved...
compared with that of *E. coli* in a medium with an identical sugar composition by leveraging the high metabolic efficiency of *Vibrio* sp. dhg. These results collectively support the potential of the strain as a platform for the lignocellulose-based fermentation process due to its high performance.

**Discussion**

This study supports the power of the ALE strategy for generating platform strains for strain. Although rational engineering approaches have been widely applied to engineer microorganisms, they are often limited due to insufficient comprehension of the complex and multilayered network in microorganisms. In this regard, the ALE strategy can efficiently complement the limitations of rational engineering, particularly for growth-associated phenotypes [50, 51]. Rate-limiting steps in a given microorganism can be identified and autonomously optimized by natural selection. Indeed, it was found that the low activities of XylA and XylB were bottlenecks, and they were evolutionarily optimized by ALE.

Although the effect of ScrC mutation was not studied in detail, further studies are needed to elucidate its role in *Vibrio* species. It has been reported that ScrC in *Vibrio* species controls the intracellular level of cyclic di-GMP (c-di-GMP) by converting two molecules of GTP into c-di-GMP followed by c-di-GMP into GpG via its diguanylate cyclase activity and phosphodiesterase activity [52–54]. C-di-GMP is known to be involved in bacterial global stress responses by regulating the expression of genes related to motility, biofilm formation, and virulence factors [55, 56]. Although mutations in a global stress response mechanism have been commonly observed in recent ALE studies [57–59], additional studies are needed to understand the clear mutational mechanism for removing lag in xylose conditions. Potentially, c-di-GMP might directly affect the expression of genes related to xylose catabolism or indirectly affect catabolism via reduced biofilm formation, which is important for overall planktonic cell growth and sugar utilization [60, 61].

To apply the developed platform for actual lignocellulose conversion, further fermentation studies with biomass hydrolysates are warranted. Potentially, its performance could change with actual hydrolysates, since they are known to contain diverse growth-inhibiting compounds, such as furfural, 5-hydroxymethylfurfural, and levulinic acid, which originated from the degradation of sugars during acid/heat treatments [62, 63]. This issue could be overcome by optimizing pretreatment to minimize the formation of toxic compounds and maximize sugar yields, which have been actively investigated [64–66]. Alternatively, the developed strain can be subjected to another round of ALE to tolerize it against toxic compounds.

Finally, *Vibrio* species have high potential as a novel microbial chassis for the bio-based industry. The high metabolic efficiency of *Vibrio* species can improve the productivity of any target compounds [9, 10, 17], which is greatly helpful in increasing the economic feasibility of microorganism-based biochemical production processes. Moreover, its remarkable growth shortens biological experiments, making it a suitable chassis for research purposes, such as molecular biology, evolutionary
biology, and protein engineering [11, 12, 67]. Therefore, further studies on the deployment of Vibrio species as microbial platforms are highly promising.

**Conclusions**

In this study, we developed a Vibrio-based microbial platform with rapid and simultaneous utilization of the three major sugars from lignocellulosic biomass by applying an integrated approach of rational and evolutionary engineering. We constructed a xylose isomerase pathway by heterologous chromosomal expression of the xylose isomerase gene based on genomic analysis. Furthermore, we obtained an efficient strain displaying a growth rate of 0.67 h\(^{-1}\) and a sugar uptake rate of 2.15 g g\(_{\text{dcw}}\)\(^{-1}\) h\(^{-1}\) on xylose, via ALE. It was confirmed that the evolved strain catalyzed xylose at a faster rate than any reported microorganism (Additional file 1: Table S5). Subsequent mutation analysis and reverse engineering revealed that the improved phenotype was achieved by autonomous optimization in a multi-level process for catalyzing xylose. We then achieved simultaneous utilization of glucose, xylose, and arabinose by removing CCR in the strain and demonstrated efficient lactate production with remarkable productivity.

**Methods**

**Microbes and culture media**

*Escherichia coli* strains were cultured in LB medium with appropriate antibiotics at 37 °C. Vibrio sp. dhg and its derivative strains were cultivated in the buffered minimal medium (5 g/L (NH\(_4\))\(_2\)SO\(_4\), 30 g/L NaCl, 10.7 g/L K\(_2\)HPO\(_4\), 5.2 g/L KH\(_2\)PO\(_4\), 0.5 g/L MgSO\(_4\)·7H\(_2\)O, and 2 mL L\(^{-1}\) trace metal solution (ATCC MD-TMS), supplemented with various concentrations of carbon sources and yeast extract) and LBv2 medium (10 g/L yeast, 5 g/L yeast extract, 21.92 g/L NaCl, 0.3 g/L KCl, and 2.2 g/L MgCl\(_2\)) with appropriate antibiotics at 37 °C [9]. Agar plates were prepared by including 15 g/L of agar into the media. The pH of all media and a buffer was adjusted to 7.

**Culture conditions**

For routine cell cultures at the flask scale, colonies were picked from LB or LBv2 medium agar plates, inoculated in 3 mL of LBv2, and cultured overnight. Subsequently, the culture was refreshed by inoculating into several flasks containing 50 mL of LBv2 supplemented with 20 g/L of a sugar mixture (glucose:xylose:arabinose = 6:3:1) in 350 mL Erlenmeyer flasks at an OD\(_{600}\) of 0.1. When the OD\(_{600}\) reached 1–2, cells were harvested and transferred into a 7 L bioreactor (Biotron Limited, State of New South Wales, Australia) containing 1 L of the medium at an OD\(_{600}\) of 0.5. Cultures were incubated at 37 °C and 800 rpm. Appropriate antibiotics were supplemented. All cell cultures were conducted in triplicates. OD\(_{600}\) was measured using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). OD\(_{600}\) of 1.0 corresponds to 0.31 g\(_{\text{dcw}}\) /L and 0.27 g\(_{\text{dcw}}\) /L for E. coli and Vibrio sp. dhg, respectively. The maximum specific growth rate (\(\mu, \text{h}^{-1}\)) was calculated by linear regression of ln(OD\(_{600}\)) and time (h) during the exponential growth phase. Maximum specific sugar uptake rates (g g\(_{\text{dcw}}\)\(^{-1}\) L\(^{-1}\)) were calculated by dividing the maximum specific growth rates by the biomass yields. The cumulative cell division number was calculated by summation of division events calculated from the initial cell number and the total number of generations in a flask [68]. An OD\(_{600}\) of 1 was regarded as an 8 × 10^8 cell number [69].

For a bioreactor scale culture with a mimetic sugar medium, colonies were picked from LBv2 medium agar plates, inoculated in 3 mL of LBv2, and cultured overnight. Subsequently, the culture was refreshed by inoculating into several flasks containing 50 mL of LBv2 supplemented with 20 g/L of a sugar mixture (glucose:xylose:arabinose = 6:3:1) in 350 mL Erlenmeyer flasks at an OD\(_{600}\) of 0.1. When the OD\(_{600}\) reached 1–2, cells were harvested and transferred into a 7 L bioreactor (Biotron Limited, State of New South Wales, Australia) containing 1 L of the medium at an OD\(_{600}\) of 0.5. Cultures were incubated at 37 °C and 800 rpm. The pH was maintained at 6.5–7.0, using a pH controller (Biotron Limited). During the aerobic culture phase, oxygen gas was supplemented at 4 L/min until an OD\(_{600}\) of 12–15 was achieved. Thereafter, the cells were grown anaerobically by providing nitrogen gas at a rate of 2 L/min. The sugar feeding stock solution (300 g/L glucose, 150 g/L xylose, 50 g/L arabinose, 5 g/L yeast extract, 10 g/L tryptone, and 10 µg/mL chloramphenicol) was intermittently added when the total sugar concentration was below 10 g/L; each feeding increases the total sugar concentration by approximately 20 g/L. At least three identical cultures were independently performed to confirm the reproducibility.

**Construction of strains and plasmids**

Bacterial strains and plasmids are listed in Additional file 1: Table S9 and the primers, synthesized by Cosmogenetech (Seoul, Korea), are listed in Additional file 1: Table S10. Detailed plasmid construction methods are organized in Additional file 1: Table S11. Plasmid and genomic DNA were prepared using a GeneAll® Exprep™ Plasmid SV kit and Exgene™ Cell SV kit (GeneAll, Seoul, Korea, respectively). For purification of fragmented DNA, we used an Expin™ Gel SV kit (GeneAll, Seoul, Korea). For cloning, Q5 polymerase, a NEBuilder® HiFi DNA Assembly Cloning Kit, restriction enzymes, and Quick Ligation™ kit were
purchased from New England Biolabs (Ipswich, MA, United States). For routine colony PCR, EmeraldAmp® GT PCR Master Mix was used (Takara Bio Inc., Kusatsu, Japan).

The recombination was performed as the previous study [9] using pCDF_xylA_ins, pCDF_ptsG_del, pCDF_frdABCDEL_del, pCDF_pflB_del plasmids. dsDNA fragment for the integration of xylA gene, which contains homology adjacent to dns gene, FRT_cat_FRT, and xylA gene overexpression cassette was amplified using xylA_1K_F and xylA_1K_R primers with pCDF_xylA_ins as a template. dsDNA fragments for deletion of ptsG, frdABCD, and pflB gene, which contain homology adjacent to the target gene and FRT_cat_FRT was amplified using [gene name]_1K_F and [gene name]_1K_R primers with pCDF_[gene name]_del as a template. Recombination was confirmed by PCR with [gene name]_ch_F/R primers and sanger sequencing.

xylA gene was integrated into Vibrio sp. dhg wild-type strain to construct VX0 strain. After ALE, VXA38C, VX38Y, and VX38D strains were constructed by transforming pACYC_Duet, pACYC_yrkL, and pACYC_deoR plasmids into VX38 strain, respectively. VXW, VXMP1, VXMP1, VXWP2, and VXMP2 strains were constructed by transforming pACYC_xylAWT_Histag, pACYC_xylAMUT_Histag, pACYC_PWT_xylAWT_sgfp, pACYC_PWT_xylAMUT_sgfp, pACYC_PMUT_xylAWT_sgfp, and pACYC_PMUT_xylAMUT_sgfp plasmids into wild-type Vibrio sp. dhg strain, respectively. VX38P strain was constructed by knockout of ptsG gene in VX38 strain and VX38PG strain was constructed by transformation of pACYC_galP plasmid into VX38P strain. VX38PGL strain was constructed by knockout of frdABCD and pflB gene and transformation of pACYC_galP_ldhA plasmid in VX38P strain.

Adaptive laboratory evolution in xylose sole carbon source condition

ALE was performed at the flask scale by growing cells in a xylose-supplemented minimal medium. A single colony of the VX0 strain from an LBv2 agar plate was inoculated into 3 mL of LBv2 medium. After overnight incubation, the culture was washed twice with a minimal buffered medium without any carbon sources. Then, cells were inoculated in 25 mL of the medium supplemented with 4 g/L xylose in 350 mL Erlenmeyer flasks at an OD_{600} of 0.1. When the OD_{600} was higher than 2, the cultures were passaged to the next flask at an OD_{600} of 0.1. The ALE was completed once a growth rate of 0.6 h^{-1} was achieved.

Whole-genome sequencing and mutation identification

Genomic DNA was extracted from cells grown in LB medium using a GeneAll Exgene™ Cell SV kit (GeneAll Biotechnology, Seoul, Korea). Pair-end libraries were prepared using the KAPA HyperPlus Kit (KAPA Biosystems, Wilmington, MA, USA). Raw reads were obtained using a MiSeq 300-cycle Mid-Output kit on the MiSeq system (Illumina, San Diego, CA, USA). Mutations were identified using the Bresseq analysis software (version 0.33.2) [70]. A new reference genome of the VX0 strain was generated based on Vibrio sp. dhg (NCBI accession number: CP028943.1, CP028944.1, and CP028945.1) to include the introduced xylA and to identify mutations in this strain. All genomes were sequenced with at least 25 × sequencing coverage. Raw sequencing files were deposited at SRA (Bioproject number: PRJNA720008). All discovered mutations were validated by Sanger sequencing.

Quantification of the xylB transcript levels

XylB transcripts were measured by quantitative PCR (qPCR) using rpoA as a reference. The total RNA of the VX38D and VX38C strains in the mid-log phase were extracted using a Ribospin II kit (GeneAll, Seoul, Korea). Complementary DNA of rpoA and xylB mRNA for each sample was synthesized using M-MLV Reverse Transcriptase (Elpis-Biotech, Daejeon, Korea) and rpoA_RT_F/R and xylB_RT_F/R primer sets, designed to amplify 200 bp regions of each gene (Additional file 1: Table S10). A StepOnePlus Real-time PCR system (Applied Biosystems, Foster City, CA, USA) was used for amplification and signal detection. To determine the relative transcript amount, the comparative CT method (2^{−ΔΔCT}) was utilized [71].

Measurement of the specific activity of xylose isomerase

Specific activities of the purified xylose isomerases were determined using an enzymatic assay. Cells harboring 6His-tagged XylA were grown in LBv2 medium and lysed at an OD_{600} of 0.8−1, by mixing with BugBuster Master Mix (Merck, Darmstadt, Germany). XylA was purified using a MagListo™ His-tagged protein purification kit (Bioneer, Daejeon, Korea) and concentrated using an Amicon Ultra Centrifugal Filter (Merck, Darmstadt, Germany). The purified protein amount was quantified by following the Bradford assay with bovine serum albumin (BSA) as a reference [72]. The concentration was adjusted to 0.3 mg/mL by the addition of the buffered medium. Various xylose concentrations (0.5, 1, 2, and 5 g/L) were
used, and the reactions were performed at 37 °C in triplicate. The initial reaction rate of xylose isomerase was calculated as the slope of the linear regression line of the change in the amount of xylulose over time. \( K_m \) and \( k_{cat} \) were calculated using the Lineweaver–Burk equation [73].

Measurement of synthetic promoter strengths

Cells grown in the LBv2 medium were inoculated into 3 mL of fresh medium in a 15 mL test tube at an OD\(_{600}\) of 0.1. When the OD\(_{600}\) reached 0.8–1, the culture was transferred to 3 mL of fresh medium at an OD\(_{600}\) of 0.1. After 16 h, cells were harvested, and their fluorescence was measured using a Hidex Sense microplate reader (Hidex, Turku, Finland). Specific fluorescence was determined by dividing the measured fluorescence by the OD\(_{600}\). Each specific fluorescence was normalized to the value of the VXWP1 strain as a control.

Sugar and metabolite quantification

Sugars (glucose, xylose, xylitol, and arabinose) and metabolites (lactate, pyruvate, and acetate) were quantified using an UltiMate™ 3000 analytical high-performance liquid chromatography system (Dionex, Sunnyvale, CA, USA) equipped with an Aminex HPX-87H column (Bio-Rad Laboratories). As a mobile phase, we used 5 mM sulfuric acid at a flow rate of 0.6 mL/min and 30 °C. The refractive index signal was monitored using an UltiMate™ Performance liquid chromatography system (Dionex, Sunnyvale, CA, USA) equipped with an Aminex HPX-87H column (Bio-Rad Laboratories).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13068-022-02157-3.

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Author contributions

SW, HGL, YHH, SWS, and GYJ conceived the project. SW and HGL engineered Vibrio sp. dhg. YHH and SP analyzed and prepared next-generation sequencing data. MHN, DB, and JHM contributed to strain engineering and biochemical production. SW, HGL, YHH, SWS, and GYJ wrote the manuscript together with the other authors. SWS and GYJ supervised the project. All authors approved the final version of the manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

SW, H.G.L., Y.H.H., S.W.S., and G.Y.J. are inventors on provisional patents based on this study, filled by Pohang University of Science and Technology Research and Business Development Foundation and Seoul National University Research and Development Foundation.

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