Vibrio sp. dhg as a platform for the biorefinery of brown macroalgae

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Although brown macroalgae holds potential as an alternative feedstock, its utilization by conventional microbial platforms has been limited due to the inability to metabolize one of the principal sugars, alginate. Here, we isolate Vibrio sp. dhg, a fast-growing bacterium that can efficiently assimilate alginate. Based on systematic characterization of the genomic information of Vibrio sp. dhg, we establish a genetic toolbox for its engineering. We also demonstrate its ability to rapidly produce ethanol, 2,3-butanediol, and lycopene from brown macroalgae sugar mixture with high productivities and yields. Collectively, Vibrio sp. dhg can be used as a platform for the efficient conversion of brown macroalgae sugars into diverse value-added biochemicals.
he global demand for bioproducts is increasing at a striking rate, with its market share expected to reach 22% of the chemical industry by 2025. To meet such a vast demand, stable supplementation and conversion of feedstocks have been critical over the past years. Although starch crops have been widely used up until now, many concerns exist regarding the consumption of food resources and limited cultivation capabilities. In this respect, brown macroalgae have been suggested as an alternative feedstock. Brown macroalgae are hugely abundant and have a high carbohydrate content (35–60% of dry weight). They can grow much faster than lignocellulosic biomasses and only require sunlight and seawater.

The most prominent sugars in brown macroalgae are alginate (a copolymer of α-L-guluronate and β-D-mannuronate) and mannitol. While a conventional microbial platform (e.g., *Escherichia coli*) can easily metabolize mannitol, its ability to assimilate alginate is hindered by the fact that it lacks certain related genes; it is known that alginate metabolism requires about 10–20 genes that encode transporters, lyases, and metabolic enzymes. Although recent studies have demonstrated that *E. coli* can be engineered to utilize alginate with introduction of a huge gene cluster for alginate utilization from naturally-occurring alginate-utilizing microorganism, seaweed sludge was collected as a microbial platform for producing diverse value-added biochemicals from the sugars of brown macroalgae.

In this study, we isolate a fast-growing microorganism capable of utilizing alginate efficiently. We name this microorganism as *Vibrio* sp. dhg and characterize it systematically to develop a genetic engineering toolbox. By exploitation as a microbial platform, we demonstrate diverse value-added biochemical production from brown macroalgae sugars with high productivities and yields. From these results, we propose *Vibrio* sp. dhg as a platform for the biorefinery of brown macroalgae.

**Results**

**Isolation of an alginate-utilizing microorganism.** To isolate alginate-utilizing microorganism, seaweed sludge was collected and inoculated in minimal medium supplemented with alginate as a sole carbon source (Supplementary Note 1). After a few rounds of sub-culturing at 30°C, a rod-shaped microorganism showing a rapid growth (maximum specific growth rate ($\mu = 0.98 h^{-1}$)) was successfully isolated (Fig. 1a). This microorganism is able to use not only alginate but also other biomass-derivable sugars (e.g., glucose, mannitol, sucrose, galactose, arabinose, and glycerol). Notably, with most sugars, the rates of growth and sugar uptake at 30°C were substantially higher or at least comparable with those of *E. coli* grown with glucose at 37°C (Fig. 1b, c).

In addition, this microorganism can grow even in a high concentration of salt (100 g L$^{-1}$ of sodium chloride) while other industrial hosts (*E. coli, Corynebacterium glutamicum, Saccharomyces cerevisiae*, Supplementary Data 1, and Supplementary Fig. 1a) showed severely reduced growth rates. Tolerance to other representative bioproducts (ethanol, 2,3-butanediol (2,3-BDO), and lactate) was also investigated (Supplementary Fig. 1b–d). In the case of ethanol, this microorganism was similar to *C. glutamicum* and maintained its growth with 50 g L$^{-1}$ of ethanol in the medium. For 2,3-BDO, it was similar to *E. coli* but more sensitive than *C. glutamicum* and *S. cerevisiae*; the growth was inhibited with 50 g L$^{-1}$ of 2,3-BDO. Finally, with lactate, its tolerance was slightly less than the tolerance of *S. cerevisiae* which showed the best growth in the presence of 50 g L$^{-1}$ of lactate. Overall, although its tolerance was not much superior to either *C. glutamicum* or *S. cerevisiae*, it was comparable to *E. coli*, suggesting this microorganism can be used as a host for biochemical production.

To identify this microorganism, we analyzed its 16S rDNA sequence using the universal primers (Supplementary Data 2) and found that it belongs to the family Vibrionaceae. In particular, it was highly similar to *Vibrio natriegens* ATCC14048 (99% identity, Supplementary Table 3), a gram-negative bacterium recently suggested to be a promising host for molecular biotechnology due to its rapid growth rate (doubling time < 10 min in rich medium). Upon sequencing the genome of our isolated microorganism (Supplementary Fig. 2, Supplementary Tables 1 and 2), three circular contigs representing a large chromosome, a small chromosome, and a plasmid were obtained. Although several *Vibrio* species are known to use alginate (e.g., *V. alginitolyticus* and *V. splendidus* 12B01), its growth rate is much higher than reported values of the known microorganisms (0.2–0.8 h$^{-1}$). Moreover, the genome context and phenotypic characteristics of our microorganism were closer to those of *V. natriegens* ATCC14048 (Supplementary Figs. 3 and 4). However, detailed physiological comparison (Supplementary Table 3) revealed that there is a difference between two microorganisms, probably due to a variation in more than 5% of their genomes. Particularly, *V. natriegens* does not possess the machinery needed to metabolize alginate. Therefore, we named our strain *Vibrio* sp. dhg and further investigated its potential as a platform for biorefinery from brown macroalgae.

Genome annotation revealed that many of the genes responsible for alginate assimilation in *Vibrio* sp. dhg were located in a 42-kb cluster within its small chromosome (chromosome 2, Supplementary Data 4). Sequence comparison with a well-studied gene cluster of *V. splendidus* indicated that our strain also assimilates alginate via a multi-step process (Fig. 1a). Specifically, endolytic alginate lyases are secreted to depolymerize alginate into oligo-alginates. The oligo-alginates are then transported into the cytosol through a transporter. Subsequently, alginate monomers (4-deoxy-L-erythro-5-hexose-luronate, DEHU) are produced by the action of exolytic oligo-alginic lyases. Finally, via a partial Entner–Doudoroff pathway, DEHU reductase (Dehr), 2-keto-3-deoxygluconate kinase (KdgK) and 2-keto-3-deoxyporylgluconate aldolase (Eda) convert DEHU into glyceraldehyde-3-phosphate and pyruvate, two metabolites that then enter glycolysis. On the other hand, the large chromosome contains two copies of the mannitol operon (chromosome 1). Mannitol is initially transported into the cell by a mannitol-specific phosphotransferase system (PTS, MtIA) and then converted into fructose-6-phosphate—an intermediate of glycolysis—by mannitol-1-phosphate dehydrogenase (MtID).

To confirm that the aforementioned genes are critical for alginate and mannitol assimilation, we analyzed their expression levels via RNA sequencing. As expected, these genes were highly induced by the addition of the cognate sugar (up to a 100-fold increase compared with the glucose condition).
Alginate Mannitol

uptake rates (g g⁻¹) in an oxygen-limited environment (see Supplementary Note 2)¹²,¹³. This is advantageous for biochemical production (e.g., ethanol) in an oxygen-limited environment, where the cells can simultaneously consume alginate and mannitol (Fig. 1e).

Gene expression control in Vibrio sp. dhg. To harness Vibrio sp. dhg as a microbial platform for brown macroalgal biorefinery, we first need to understand how gene expression is controlled. As shown in Fig. 1d, we observed that Vibrio sp. dhg could simultaneously consume alginate and mannitol (Fig. 1e). As these two sugars have different oxidation states, this property is highly advantageous for biochemical production (e.g., ethanol) in an oxygen-limited environment (see Supplementary Note 2)¹²,¹³. Thus, Vibrio sp. dhg is highly suited to using an alginate-mannitol mixture.

Furthermore, the promoter regions of ribosomal genes, which are highly expressed in Vibrio sp. dhg, also have similar sequences (TTGANN and TATAAT) to the bacterial −35 and −10 boxes (TTGACA and TATAAT, respectively) (Supplementary Fig. 7). These findings suggested that the common promoters used for E. coli would also be compatible with Vibrio sp. dhg. To test this idea, we prepared plasmids encoding the sgfp (super-folding green fluorescence protein) gene under the control of the conventional promoters P_1 (up to a maximum of 184-fold) upon the addition of cognate inducers (Isopropyl β-D-1-thiogalactopyranoside (IPTG), anhydrotetracycline (aTc) or arabinose).

To achieve precise control of gene expression at the transcriptional level, we constructed a synthetic promoter library. This library was prepared by modifying the −35 and −10 boxes of the P_1 promoter with degenerate sequences (i.e., YTKAYR...
and KAYWRT, respectively) to cover the Anderson promoter library. By altering the promoter sequence, we successfully obtained 15 different promoters (named promoters VP1 to 15) of varied strengths, with a maximum fold difference of 41 (Fig. 2b, Supplementary Table 4). Based on our data, we were able to construct a predictive model that can estimate promoter strengths (Supplementary Table 5) with a strong predictive power ($R^2 = 0.79$) between the predicted promoter strength and the measured fluorescence values. The inset graph indicates a high correlation ($R^2 = 0.79$) between the predicted folding energies ($\Delta G_{\text{UTR}}$) and the measured fluorescence values. Values were adjusted for background fluorescence (fluorescence of the VDHG001 strain). Error bars indicate the standard deviations of three independent cultures ($n=3$). White dot indicates actual data point. Source data of Figs. 2a, 2b, and 2c are provided as a Source Data file.

**Genome editing in Vibrio sp. dhg.** As genome editing is necessary to develop an efficient cell factory, the possibility to engineer the genome of Vibrio sp. dhg was explored. To engineer its genome, the SXT recombination system naturally found in Vibrioaceae was chosen. It has been reported that the expression of $exo$ (a gene encoding alkaline exonuclease) and $beta$ (a gene encoding single-strand annealing protein) permits recombination in *V. natriegens* and *E. coli*. For its genome engineering, endogenous $exo$ and $beta$ in Vibrio sp. dhg, were expressed under the control of the Ptac promoter and a synthetic 5′-UTR (Supplementary Table 7). Additionally, $gamma$ (a gene encoding nuclease inhibitor) from lambda phage was expressed to facilitate recombination. As a template for allelic exchange, we introduced a chloramphenicol resistance (cat) cassette flanked with two identical FRT sequences and homology arms (1–3 kb) of the target locus. After electroporation, we confirmed the engineered locus by checking for colonies that could grow on a chloramphenicol agar plate. Indeed, we found colonies with target gene successfully replaced by the cat gene (Supplementary Fig. 8a). Subsequently, the integrated cat gene was easily removed by expressing an FRT-specific flipase from *S. cerevisiae*.

**Biochemical production from alginate and mannitol mixture.** After establishing a genetic toolbox for Vibrio sp. dhg, we attempted to produce value-added biochemicals using engineered Vibrio sp. strains. Although sugar composition of brown macroalgae is known to be influenced by geographical and seasonal changes, a mixture of alginate and mannitol in a 1:2 ratio was chosen to prepare a mimetic sugar medium to fully evaluate its performance. At first, production of ethanol, one of biofuels, was attempted in a microaerobic condition (Fig. 3a). Since the wildtype strain possesses both an aldehyde dehydrogenase and an alcohol dehydrogenase, ethanol can be produced by cultivating the wildtype strain via reduction of acetyl-CoA. However, only 1.6 g L$^{-1}$ of ethanol was produced from 30 g L$^{-1}$ of mixed sugars (Supplementary Fig. 9). In addition,
other by-products such as acetate, lactate, and succinate were produced in considerable amounts (a total of 4.7 g L\(^{-1}\)). To produce more ethanol, the pyruvate decarboxylase (pdc) and aldB (aldehyde dehydrogenase) from Z. mobilis were expressed under the P\(_{VP15}\) promoter and a synthetic 5'-UTR. To improve yield, ldhA, frdABC, and pflB were removed from the chromosome. A fermentation profile of the VDHG411 strain during 24 h. Closed black circle, OD\(_{600}\); closed inverted blue triangle, alginate; open blue triangle, mannitol; closed purple hexagon, acetoin; open orange diamond, acetate; closed red square, 2,3-BDO. 

To further minimize by-product formation, we deleted the endogenous ldhA (lactate dehydrogenase), frdABC (fumarate reductase) and pflB (pyruvate-formate lyase) genes, creating the VDHG411 strain (Supplementary Fig. 8b). This deletion successfully minimized the loss of carbon (a total of only 1.1 g L\(^{-1}\)) of by-products while slightly improving ethanol production (8.4 g L\(^{-1}\), or a 10% increase). Upon providing a total of 80 g L\(^{-1}\) of mixed sugars with a 1:2 ratio by fed-batch fermentation, VDHG411 strain produced 25.7 g L\(^{-1}\) (3.3% v/v) of ethanol in 24 h (Fig. 3b). Surprisingly, this 1.1 g L\(^{-1}\) h\(^{-1}\) average ethanol productivity (maximum 1.8 g L\(^{-1}\) h\(^{-1}\)) was dramatically higher than that of other microbial platforms for alginate utilization (Supplementary Table 8). Furthermore, the yield (64% of the theoretical maximum) of our VDHG411 strain was comparable to the yield of other alginate-consuming microbial platforms.

We next attempted to produce 2,3-BDO, a chemical that can be used industrially as an antifreeze reagent or solvent\(^{21}\) (Fig. 3c). Although the production of 2,3-BDO from the sugars of brown macroalgae has previously been attempted\(^{21}\), this was carried out using E. coli, which is only able to slowly assimilate mannitol. In contrast, alginate has not yet been considered as a carbon source in such a bioprocess. Thus, to produce 2,3-BDO using our alginate-assimilating Vibrio sp. dhg, we expressed the budABC operon (encoding for acetoacetate decarboxylase, acetoacetate synthase, and acetoin reductase) from L. purpurea was expressed under the P\(_{VP15}\) promoter and a synthetic 5'-UTR. To improve the titer, dcs (DXS synthase), idi (IPP isomerase) and ispA (FPP synthase) from E. coli W3110 were additionally expressed. Lycopene production by engineered Vibrio sp. dhg strains (VDHG131, VDHG132, and VDHG133) after 9 h. Abbreviations: DXP deoxyxylulose 5-phosphate, HMBPP 4-hydroxy-3-methylbut-2-enyl pyrophosphate, IPP isopentenyl pyrophosphate, DMAPP dimethylallyl pyrophosphate, FPP farnesyl diphosphate, GGPP geranylgeranyl pyrophosphate. Some reactions of glycolysis have been omitted for simplicity. Error bar indicates the standard deviations of three independent cultures (n = 3). White dot indicates actual data point.
compared with the *E. coli* platform of the previously mentioned study, Vibrio sp. *dhg* showed both superior productivity (1.3 g of 2,3-BDO and acetoin L⁻¹ h⁻¹) and yield (0.40 g product g⁻¹ sugar or 81% of the theoretical maximum), along with an efficient co-utilization of alginate and mannitol.

We lastly demonstrated the ability of our *Vibrio* sp. *dhg* platform to produce lycopene, which is a C-40 phytochemical used as a nutraceutical antioxidant[22,23] or as a feedstock[24] for chemical production with advantages of recent advances in synthetic biology[26–29]. Using our developed toolbox could enhance lycopene production even further.

Brown macroalgae fermentation for ethanol production.

Finally, we tested ethanol production using brown macroalgae as feedstock (Fig. 4). The sugar content of used Kelp powder was quantified to 0.16 g alginate and 0.3 g mannitol per g powder (see “Methods”), consistent with the composition of the mimetic sugar mixture. Therefore, the Kelp powder can be directly used as a feedstock. When the VDHG411 strain was cultivated in a bioreactor containing a 1 L medium, total 120 g L⁻¹ of the Kelp powder was provided during 24 h. Subsequently, even without any enzymatic pre-treatment or hydrolysis, 19.2 g L⁻¹ of ethanol (2.4% v/v) was successfully produced with a similar yield (63% of theoretical maximum) compared to the yield from mimetic sugar fermentation. Although the productivity (0.8 g L⁻¹ h⁻¹) was reduced, suggesting a requirement of further optimization including genetic engineering and parameter study, the obtained result clearly indicates that *Vibrio* sp. *dhg* is also capable to produce a biochemical directly from brown macroalgae.

Discussion

The ultimate goal of the biorefinery process is the efficient conversion of abundant carbon sources into value-added biochemicals. While the use of brown macroalgae as a feedstock has been limited due to the difficulties of alginate assimilation, the use of *Vibrio* sp. *dhg* strain as a microbial platform could overcome this issue. Moreover, its outstanding growth rate and metabolic activity over conventional platforms allow envisioning accelerated biochemical production from brown macroalgae.

One risk in exploiting environmental isolates as a host is their potential virulence. Our genome analysis revealed that *Vibrio* sp. *dhg* strain does not have major toxins found from pathogenic *Vibrio* strains[30] (see “Methods”). However, one barely expressed open reading frame (fragments per kilobase per million (FPKM) < 1 based on RNA-seq analysis) showed a marginal identity (24%, Supplementary Fig. 11a and b) when compared with its amino acid sequence with an auxiliary toxin (Zot, zona occludens toxin). Although it has a low sequence identity (less than 30%)[31] and *Vibrio* sp. *dhg* strain does not have two major toxins (CtxA and CtxB) concurrently found with Zot[32], we tested that the potential toxin can be deleted to minimize the risk. We confirmed that this gene can be deleted and does not affect the growth of the strain (Supplementary Fig. 11c and d).

For the realization of the brown macroalgae biorefinery, there could still be many hurdles. As mentioned, one challenge is a potential fluctuation of bioprocess efficiency due to seasonal and geographical differences in biomass sugar composition. Indeed, we observed that the ratio of alginate and mannitol, which have different oxidation states, hugely affected ethanol production (up to 4.5-fold) (Supplementary Fig. 12 and Supplementary Note 2). To address this issue, microorganisms should be engineered for dynamic response to intracellular redox state for robust biochemical production with advantages of recent advances in synthetic biology[26–29].

Further studies aimed at unveiling the characteristics (e.g., rapid growth, halophilicity) and improving its tolerance[33,34] of *Vibrio* sp. *dhg* would be valuable for maximizing its potential as a microbial platform[35]. Additionally, the exploitation of fast-growing microorganisms as hosts can also expedite evolutionary processes that lead to the development of improved proteins or microorganisms themselves. The strain development repertoire that we demonstrate here, could also be applied to the construction of other microbial platforms that utilize non-traditional carbon sources[36].

Methods

Chemical reagents and oligonucleotides. Primers were synthesized by Cosmo genetech (Seoul, Korea) and are listed in Supplementary Data 2. Plasmid and genomic DNA were prepared using the GeneAll® Exprep™ Plasmid SV kit and the Exegen™ Cell SV kit (GeneAll, Seoul, Korea), respectively. Procedures for plasmid cloning are explained in Supplementary Note 3. For purification of
fragmented DNA, we used the ExpnSTM Gel SV and ExpnSTM PCR SV kits. Q5 polymerase, the NEBuilder® HiFi DNA Assembly Cloning Kit, restriction enzymes, and the Quick LigationTM kit were purchased from New England Biosciences (Ipswich, MA, United States). For routine colony PCR, EmeraldAmp® GT PCR Master Mix was used (Clontech, Mountain View, CA, USA). All reagents for cell cultures were purchased from BD Bioscience (Sparks, MD, USA). Chemical reagents were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated. Buffer and medium compositions were described in Supplementary Note 1.

**Isolation of Vibrio sp. dhg strain.** To isolate an alginate-metabolizing microorganism, we obtained seaweed sludge from the coastal area of Pohang, Korea. The sludge sample was inoculated into 20 mL of alginate minimal medium in a 350-mL flask. When growth was detected by a change in turbidity, the culture was transferred to the same fresh medium using a 1/10 dilution. After several sub-culture, the broth was streaked on alginate agar plate. We manually inspected the upstream sequences of ribosomal genes and identified a single colony named Vibrio sp. dhg.

**Strain characterization.** For routine cell cultures, colonies were picked from rich medium agar plates (LBv2 medium for Vibrio sp. dhg, LB medium for E. coli, BHI medium for C. glutamicum, and YPD medium for S. cerevisiae) and inoculated in 3 mL of minimal medium (buffered medium for Vibrio sp. dhg, M9 medium for E. coli, CG medium for C. glutamicum, and SC medium for S. cerevisiae) supplemented with 4 g L−1 of a single sugar (glucose, mannitol, galactose, sucrose, arabinose, galactose, or glycerol). After growing overnight, the culture was refreshed by inoculating into fresh medium at an OD600 of 0.05–0.1. When the OD600 reached 1.0, the culture broth was transferred into 350 mL of 10-cm culture plate (aerobic culture) containing 20 mL of medium or 175 mL serum bottle (anaerobic culture) containing 50 mL of medium. The cultures were conducted in a rotary shaker (Hanil Scientific, Gimp, Korea) at 30 °C and 200 rpm (rotation per minute) unless otherwise mentioned. Especially, cells were handled in the anaerobic chamber (Coy, Wilmington, MA, USA). This short gDNA library was sequenced using the Min-Seq system (Illumina, San Diego, CA, USA). The quality of the rRNA-free samples was also checked using the Bioanalyzer with the RNA 6000 Pico kit from Agilent Technologies (Santa Clara, CA, USA). This short gDNA library was sequenced using the Ribozero Zero RNA Removal Kit for Gram-negative bacteria (Illunina, San Diego, USA). The quality of the RNA-free samples was also checked using the Bioanalyzer with the RNA 6000 Pico kit. The strand-specific RNA-seq library was constructed using the KAPA Stranded RNA-Seq Library Preparation Kit for Ilumina platforms according to the manufacturer’s instructions. The size distribution of the complementary DNA library was assessed using the Bioanalyzer with a High Sensitivity DNA kit from Agilent Technologies. All samples were sequenced using the MinSeq 75-cycle High-output Kit (Illunina, San Diego, USA) according to the manufacturer’s instructions. The quality of the sequence reads obtained from RNA-seq was checked by FastQC[19], and then aligned to the modified genome using bowtie[20]. The Cufflink package (http://cufflinks.cbcb.umd.edu/) was run to obtain transcriptomic data.

**Genetic engineering of Vibrio sp. dhg.** Purified plasmids were transformed into Vibrio sp. dhg strain via a previously established electroporation method[10]. Briefly, a single colony was inoculated into 3 mL of LBv2 medium. When the medium became turbid, the culture was transferred into 10 mL of fresh medium using a 1/100 dilution. When the OD600 of 0.6 was reached, the culture was incubated in an icebox for 10 min. Subsequently, a cell pellet was prepared by centrifugation at 13,000 × g for 1 min at 4 °C. This pellet was washed twice with electroporation buffer and resuspended to achieve an OD600 of 16. Then, more than 100 mg of plasmid DNA was added to 90 μL of cell suspension and 0.8 kV was applied for 2.5 ms using a microplasmid-electroporator (Bio-Rad Laboratories, Richmond, CA, USA). The cells were recovered by addition of 1 mL of BHI recovery medium and incubated at 37 °C. Finally, the cells were spread on antibiotic-containing LBv2 agar plates. Plasmid introduction was confirmed by agarose gel or colony PCR using primers that specifically anneal to the plasmid DNA.

For genome engineering, we first made electro-competent cells using the cells expressing SXT recombinases (following the same protocol used for plasmid transformation). During sub-culture, 1 mM of IPTG was included. For single nucleotide exchange, more than 10 μg of the dsDNA fragment was added to the cell suspension. The cells were recovered by incubating in BHI recovery medium for 3 h at 37 °C. Then, cells were spread on an agar plate containing 10 g L−1 of chloramphenicol. The integrated selection marker was removed via the activity of FLP (flippase)[19]. The change of target locus was confirmed by colony PCR and Sanger sequencing.

**Fluorescence measurements.** To measure the fluorescence of each strain, colonies were inoculated into 180 μL of buffered minimal medium containing 4 g L−1 of glucose using BioscreenC (Growth Curves, Helsinki, Finland). After two rounds of sub-culture, the fluorescence of 100-μL samples were analyzed using the VICTOR2 1420 Multimode Plate Reader (Perkin Elmer, MA, USA). Fluorescence was calculated by dividing the fluorescence by the OD800. For inducible promoters, different concentrations of inducers were included at time zero (1 mM IPTG, 100 μg L−1 of tetracycline, or 10 g L−1 of arabinose).

**Synthetic promoter strength prediction.** To construct a predictive model from the synthetic promoters, we first converted the promoter sequence into a set of binary vectors. Each nucleotide symbol was converted into one of the following symbols: 0 (0, 1, 0, 0), 1 (0, 0, 0, 0), 0 (0, 0, 0, 1), 0 (0, 0, 1, 0), or 0 (0, 1, 0, 1) for ‘A’, ‘C’, ‘G’, ‘T’, respectively. Then, we used the Ridge regression model to determine the coefficients and predict promoter strengths. The Ridge regression model addresses the ordinary least squares problem to determine the coefficients that minimize a residual sum of squares and the size of coefficients as shown below:

\[
\min \left[ \sum_{i} \left( y_i - \hat{y}_i \right)^2 + \alpha \sum_{j} \hat{x}_j^2 \right]
\]

where \(y_i\) is the observed value, \(\hat{y}_i\) is the predicted value, \(\hat{x}_j\) is the coefficient, and \(\alpha\) is the ridge parameter.
where X denotes the vector-transformed promoter sequences, y denotes the promoter strengths, and w is a coefficient vector. a is a complexity parameter that balances the weight of the bias term given to minimize the residual sum of squares and minimizing the sum of the square of coefficients. We performed leave-one-out cross-validation (LOOVC) in order to estimate how accurately the predictive model performs in practice. For each synthetic promoter sequence, we made a predictive model with the other sequences as a training set (i.e., the target sequence was excluded). For the implementation, we used the linear_model module from the sklearn package of python.

**Biochemical production.** Biochemical production (ethanol, 2,3-BDO, and lycopene) from mimetic sugar mixture was conducted using the buffered minimal medium, however, the concentration of NaCl was reduced to 10 g L⁻¹. In the case of 2,3-BDO, an additional 5 g L⁻¹ of yeast extract was included in the medium. The medium was supplemented with 30 g L⁻¹ of an alginate-mannitol mixture (1:2 ratio) as carbon source. When 80 g L⁻¹ of sugar was provided, 30 g L⁻¹ and 20 g L⁻¹ of the sugar mixture were additionally added at 6 h and 12 h. Cells were cultivated in either 50 ml (for ethanol production) or 25 ml (for 2,3-BDO and lycopene production) of medium in a 350-ml flask at 200 rpm. 

For ethanol production from brown macroalga, a powder of kelp (60 mesh) harvested in Korea was purchased from a local market. Its sugar composition was quantified using 10 g L⁻¹ of the kelp solution with water. Culture was conducted in the bioreactor containing the 1 L buffered medium with 10 g L⁻¹ of alginate and mannitol (1:2) as initial carbon sources. Especially, phosphate buffer concentration was adjusted to 20 mM and 5 g L⁻¹ of yeast extract were additionally provided. Sterile air at 2 L min⁻¹ was provided during initial 6 h. Then, the cells were cultured anaerobically and 20 g L⁻¹ of the kelp powder was added with a 3 h interval. The culture was continuously stirred at 300 rpm.

**Metabolite quantification.** Sugars and metabolites (including ethanol and 2,3-BDO) were quantified using the 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 0.125% carbazole in absolute ethanol was added and the mixture gently was shaken. The absorbance at 530 nm was measured using the Hidex Sense microplate reader. 40 μL of sulfuric acid at a concentration of 5 mM of was added and the mixture gently was shaken. The absorbance of the samples was then measured at 475 nm. 

**Pathogenicity analysis.** Potential pathogenicity of Vibrio sp.ogh was evaluated by searching known Vibrio toxins (Ace, CT, MARTX, TDH, TRH, VCC, and Zot) using the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/BLAST.cgi?PROGRAM=blastn)[10,42,43] Amino acid sequence of the toxins and genome sequence of Vibrio sp. dhg were used as inputs.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Data supporting the findings of this work are available within the paper and its Supplementary Information files. A reporting summary for this Article is available as a Supplementary Information file. The genome sequence of Vibrio sp. dhg has been uploaded to Genbank under accession number CP028843, CP028844, and CP028854. The transcriptomic data was deposited in Gene Expression Omnibus under accession number GSE119357. A reporting summary for this article is available as a Supplementary Information file. The source data underlying Figs. 1b–d, 2a–c are provided as a Source Data file. The other datasets generated and analyzed during the current study are available from the corresponding authors upon request.

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Author contributions

H.G.L., D.H.K. and S.H.W. isolated, characterized, and engineered Vibrio sp. dhg. S.P. prepared samples for high-throughput sequencing and analyzed the omics-data with J.S. Y. C.W.K. carried out biochemical production and metabolite quantification. B.H.K. and M.H.N. contributed to genome sequencing and annotation of the assembled genome. H.G.L., D.H.K., S.H.W., S.W.S. and G.Y.J. wrote the manuscript together with the other authors. S.W.S. and G.Y.J. supervised the overall project. All authors approved the final version of the manuscript.

Additional information

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Competing interests: H.G.L., D.H.K., S.W.S., and G.Y.J. are inventors on Korean patent application 10-2018-0054287 and PCT patent application PCT/KR2018/005437, filed by Pohang University of Science and Technology Research and Business Development Foundation and Seoul National University Research and Development Foundation. These patents are based on this work. All other authors declare no competing interests.

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