Bioremediation of complex organic pollutants by engineered Vibrio natriegens

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Industrial wastewater, petroleum pollution and plastic contamination are significant threats to global marine biosecurity because of their toxic, mutagenic and persistent nature1. The use of microorganisms in bioremediation has been constrained by the complexity of organic pollutants and limited tolerance to saline stress2. In this study, we used synthetic biology to engineer Vibrio natriegens into a strain capable of bioremediating complex organic pollutants in saline wastewater and soils. The competence master regulator gene tfoX was inserted into chromosome 1 of the V. natriegens strain Vmax and overexpressed to enhance DNA uptake and integration. Degradation gene clusters were chemically synthesized and assembled in yeast. We developed a genome engineering method (iterative natural transformation based on Vmax with amplified tfoX effect) to transfer five gene clusters (43 kb total) into Vmax. The engineered strain has the ability to bioremediate five organic pollutants (biphenyl, phenol, naphthalene, dibenzofuran and toluene)

covering a broad substrate range, from monocyclic to multicyclic compounds, in industrial wastewater samples from a chlor–alkali plant and a petroleum refinery.

The genome assembly generated in this study was deposited in NCBI under the BioProject PRJNA1240198. All other data are presented in the paper and Supplementary Information. The public data used in this study included function annotations of non-essential genes in the genome of Vmax and degradation gene clusters from

the NCBI database (https://www.ncbi.nlm.nih.gov). The accession numbers of the genes are listed in Supplementary Tables 4 and 5.

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H.T. and J.D. designed and supervised the overall research framework

and provided acquired funding. C.S., Y. Liu, C.W. and L.Q. conducted molecular biology experiments, including bacterial strain construction, transcriptomic analyses and qPCR gene expression analysis. C.S., H.C. and P.Z. performed bacterial growth characterization and pollutant degradation assays, including optimization of culture conditions, growth curve measurement and degradation testing. H.C., W.W., Z.C., M.Y., Y. Li and S.H. collected and processed industrial wastewater and soil samples and conducted pollutant degradation experiments under practical environmental conditions. C.S., H.C., W.W., Z.C., M.Y., Y. Li, P.X. and H.T. performed chromatographic and mass spectrometric analyses (HPLC, gas chromatography, UPLC–QTOF-MS and HRGC–MS) and conducted data analysis. H.C., W.W., Z.C., M.Y. and Y. Li conducted microbial diversity analyses of environmental samples and performed statistical analyses, data organization and significance testing. C.S., H.C., W.W., Z.C., M.Y., Y. Li, Y.C., S.H., J.Z., P.X., J.D. and H.T. wrote the paper. All authors contributed to reviewing the draft of the paper and approving the final paper.

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a, Heatmap of differentially expressed genes related to aromatic

compound resistance, detected based on RNA-seq of V. natriegens Vmax in the presence or absence of the indicated mixture of complex organic pollutants. Transcription factors are in green; energy metabolism genes are in black; multidrug-resistance-related genes are in purple; ABC transporter genes are in blue. b, qPCR analysis of expression of the indicated genes. Data are represented as the mean of three biological triplicates ± SD.

a, NT efficiency testing between VCOD-1 and the original strain with a linear fragment (xxxx) as the donor. b, NT efficiency testing between VCOD-1 and the original strain with the p15A plasmid. c. NT efficiency of VCOD-1 with the indicated quantity of the 9Gv&dc£¤6Õ donor DNA fragment containing the indicated length (in kbp) of homology arms on each side of the mutation. Statistical analysis: a-c, data are represented as the mean ± SD. n = 3 independent experiments. Statistical significance was assessed using one-way ANOVA with Tukey’s multiple comparisons tests.

a, NT efficiency of VCOD-2 with the indicated quantities of the 9Gv&dc£¤6Õ donor DNA fragment (with 0.5 kbp/0.5 kbp homology arms). b, NT efficiency of VCOD-2 in the indicated bacterial growth states (measured by OD600) with 200 ng 9Gv&dc£¤6Õ (with 2 kbp/2 kbp homology arms) of the donor DNA fragment. c, NT efficiency of VCOD-2 induced by the indicated concentrations of IPTG with 200 ng of the 9Gv&dc£¤6Õ (2 kbp/2 kbp) donor DNA fragment. d, NT efficiency of VCOD-2 with 200 ng of the 9Gv&dc£¤6Õ donor DNA fragment (containing the indicated lengths for homology arms). e, NT efficiency of VCOD-2 with different incubation times after adding 200 ng of the 9Gv&dc£¤6Õ (2 kbp/2 kbp) donor DNA fragment. Statistical analysis: a-e, data are represented as the mean ± SD. n = 3

independent experiments. Statistical significance was assessed using one-way ANOVA with Tukey's multiple comparisons tests.

a-e, Catabolic pathways and LC-MS spectra for degradation intermediates produced by the VCOD-3 (a), VCOD-4 (b), VCOD-5 (c), VCOD-6 (d), and VCOD-7 (e) strains. The organic pollutants were added to resting cell suspensions in nine-salt solution (see Supplementary Information Table 2 for the detailed composition); metabolites were extracted by ethyl acetate after six-hour incubation of cultures with the pollutants (see Methods and Materials for details). Detected biphenyl degradation intermediates included: biphenyl-2,3-diol (2), 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate (3), and benzoic acid (4). Catechol was detected as a phenol degradation intermediate (6). Detected naphthalene degradation intermediates included naphthalene-1,2-diol (8) and salicylic acid (9). Detected dibenzofuran degradation intermediates included 2,2',3-trihydroxybiphenyl (11) and salicylic acid (9). Detected toluene degradation intermediates included benzyl alcohol (13) and benzoic acid (4).

a-e, Cultures of all strains were induced using 1 mM IPTG. The analyte genes (as indicated) were assessed with qPCR for strains VCOD-3 (a), VCOD-4 (b), VCOD-5 (c), VCOD-6 (d), and VCOD-7 (e) strains. Data are represented as the mean ± SD. n = 3 independent experiments.

a, Schematic for the organization of gene clusters in VCOD-12. Two gene clusters were inserted into the neutral site chr2_297. The screening marker kanamycin resistance gene KanR was present at the end of the dmp gene cluster. b, qPCR analysis of the indicated genes from the complex pollutant degrading gene cluster in

VCOD-12, induced with 1 mM IPTG. c-d, Complex organic pollutant remediation efficiency of VCOD-12 in nine-salt solution. e, Growth of the VCOD-2 and VCOD-12 strains in LB3 medium. Statistical analysis: b-e, data are represented as the mean ± SD. n = 3 independent experiments. Statistical significance was assessed using unpaired t-tests with Welch's correction.

a, Schematic for the organization of gene clusters in VCOD-13. Three gene clusters were inserted into the neutral site chr2_297. The screening marker chloramphenicol resistance gene CmR was present at the end of the nah gene cluster. b, qPCR analysis of the indicated genes from the complex pollutant degrading gene cluster in VCOD-13, induced with 1 mM IPTG. c-e, Complex organic pollutant remediation efficiency of VCOD-13 in nine-salt solution. f, Growth of the VCOD-2 and VCOD-13 strains in LB3 medium. Statistical analysis: b-f, data are represented as the mean ± SD. n = 3 independent experiments. Statistical significance was assessed using unpaired t-tests with Welch's correction.

a, Schematic for the organization of gene clusters in VCOD-14. Four gene clusters were inserted into the neutral site chr2_297. The screening marker kanamycin resistance gene KanR was present at the end of the nah gene cluster. b, qPCR analysis of the indicated genes from the complex pollutant degrading gene cluster in transformed V. natriegens cultures, induced with 1 mM IPTG. c-f, Complex organic pollutant remediation efficiency of VCOD-14 in nine-salt solution. g, Bacterial growth of strains VCOD-2 and VCOD-14 in LB3 medium. Statistical analysis: b-g, data are represented as the mean ± SD. n = 3 independent experiments. Statistical significance was assessed using unpaired t-test with Welch's correction.

a-e, Catabolic pathways and LC-MS spectra for degradation intermediates of biphenyl (1) (a), phenol (6) (b), naphthalene (8) (c), dibenzofuran (15) (d), and toluene (18) (e). The organic pollutants were added to resting cell suspensions in nine-salt solution; metabolites were extracted by ethyl acetate after six hours (see Methods and Materials for details). Detected biphenyl degradation intermediates included biphenyl-2,3-diol (3), 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate (4), and benzoic acid (5). Catechol was a detected degradation intermediate from phenol (7). Detected naphthalene degradation intermediates included naphthalene-1,2-diol (10) and salicylic acid (14). Detected dibenzofuran degradation intermediates included 2,2',3-trihydroxybiphenyl (16) and salicylic acid (14). Detected toluene degradation intermediates included benzyl alcohol (19) and benzoic acid (5).

a, Photograph of the multi-parallel bioreactors. Industrial wastewater samples were treated with VCOD-15. b-f, Complex organic pollutant bioremediation efficiency of VCOD-15 in industrial wastewater samples. g, The relative abundance of microbial genera in the wastewater samples' microbial communities was measured at 0, 24, and 48 h during the bioremediation process (n = 3). b-f, Data are represented as the mean of three biological triplicates ± SD.

This file contains Supplementary Figs. 1–21 and Tables 1–10. Supplementary figures: schematic of the workflow of gene cluster assembly and insertion, as well as growth curve, promoter strength, transformation/recombination efficiency, pollutant remediation efficiency and metabolite analysis in this study. Supplementary tables: strains, broths, genes, primers, wastewater samples and selected genome insertion targets used in this study.

Raw data for Figs. 1–5, Extended Data Figs. 1–10 and Supplementary Figs. 1, 6, 8, 9, 12 and 14–21.

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