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Supplementary File

Multi-step Enzymatic Synthesis of 1,9-Nonanedioic Acid from a Renewable Fatty Acid and Its Application for the Enzymatic Production of Biopolyesters

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Figure S1. Map of pCES208H36GFP-ChnDE for the ChnDE expression in *Corynebacterium glutamicum* ATCC 13032. The alcohol/aldehyde dehydrogenase genes (chnDE) of *Acinetobacter* sp. NCIMB 9871 [1] were inserted into an *E. coli*/*C. glutamicum* shuttle vector, pCES208H36GFP [2, 3].

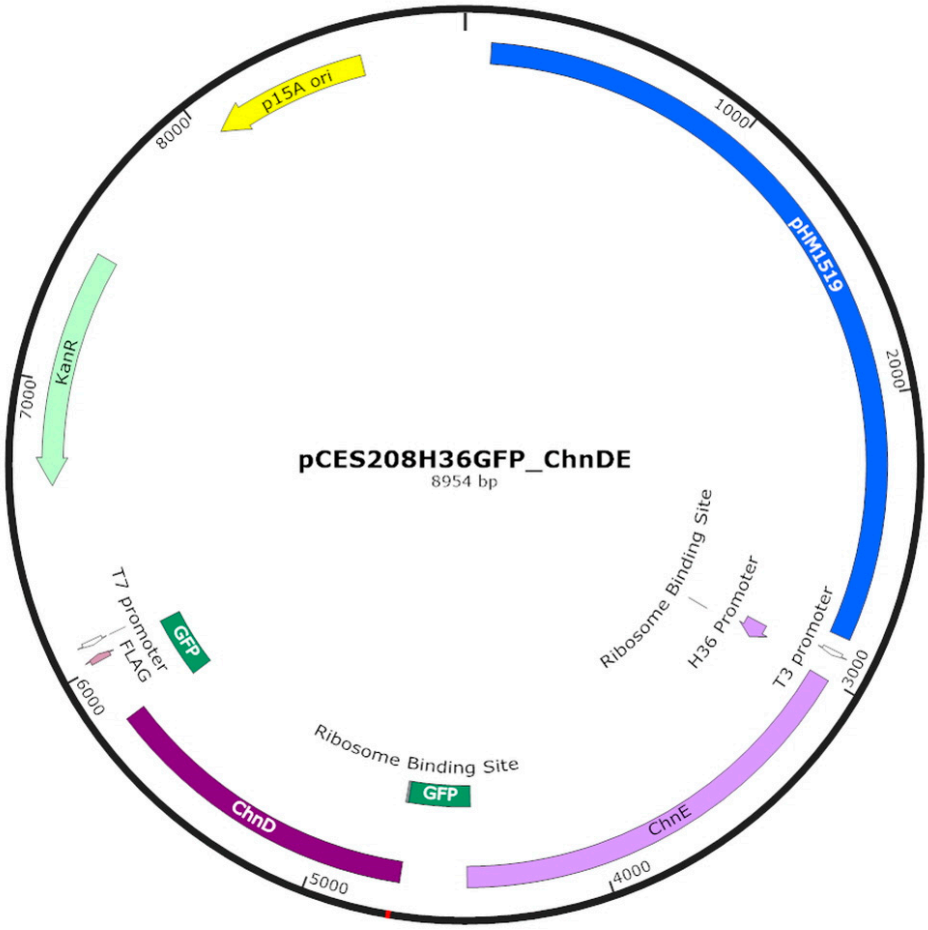


Figure S2. SDS-PAGE analysis of the protein extracts of *C. glutamicum* ATCC 13032 and the recombinant *C. glutamicum* ATCC 13032 pCES208H36GFP-ChnDE. The wild type (lanes 1,2,3) and recombinant cells (lanes 4,5,6) were harvested after 12 h of cultivation in CGXII medium and fractionated to total, soluble and insoluble fractions. Lane M: marker protein; lanes 1,4: total fraction; lanes 2,5: soluble fraction; lanes 3,6: insoluble fraction.

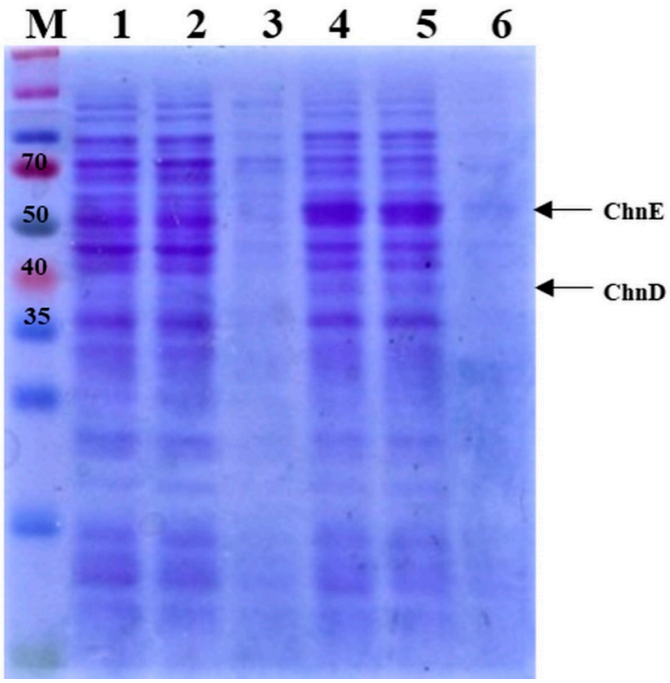


Figure S3. The specific oxidation rates of the recombinant *C. glutamicum* for the C9 to C12 ω-hydroxycarboxylic acids. The whole-cell bioconversion was initiated by adding 20 mM substrate (e.g., 9-hydroxynonanoic acid, 10-hydroxydecanoic acid, 11-hydroxyundecanoic acid, 12-hydroxydodecanoic acid), which were prepared in dimethyl sulfoxide (DMSO). The reaction was conducted in 50 mM Tris-HCl buffer (pH 8.0) containing 8 g dry cells/L and 0.5 g/L Tween 80 at 35°C, 200 rpm. The specific oxidation rates were determined based on the product concentrations at 10 or 30 min.

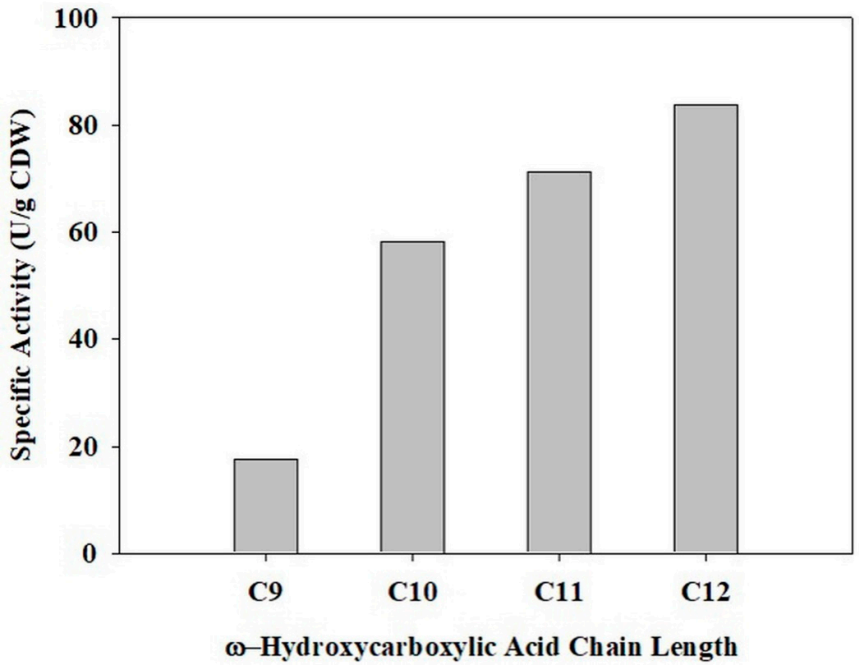
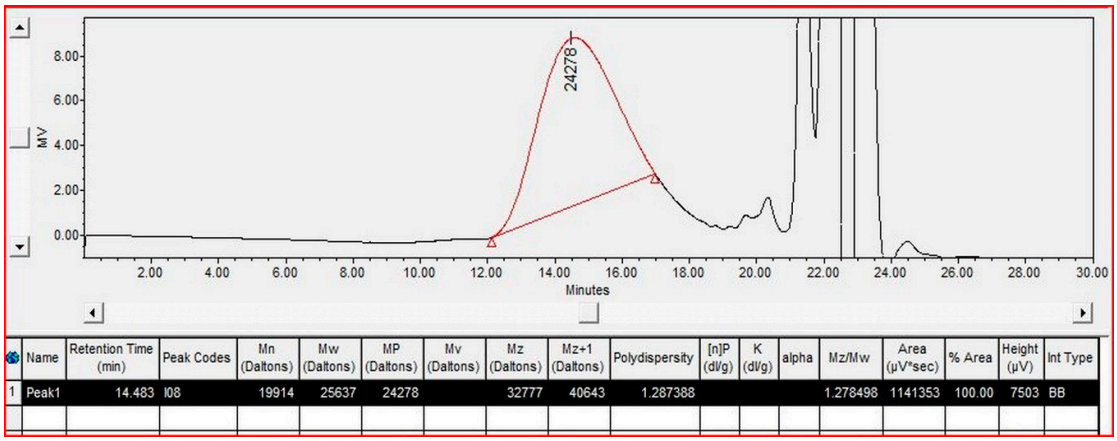


Figure S4. HPLC chromatogram of the biopolyester, which had been produced from azelaic acid and 1,8-octanediol by the immobilized lipase B from *Candida antarctica* (i.e., GF CalB-IM (GenoFocus (Korea))) (**A**). The biopolyester, which had been isolated from the reaction medium (**B**).

(**A**)



	Name	Retention Time (min)	Peak Codes	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mv (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	[n]P (dl/g)	K (dl/g)	alpha	Mz/Mw	Area (µV*sec)	% Area	Height (µV)	Int Type
1	Peak1	14.483	I08	19914	25637	24278		32777	40643	1.287388				1.278498	1141353	100.00	7503	BB

(**B**)

Figure S5. FT-IR spectra of the reaction components, (A) 1,8-octanediol and (B) azelaic acid and (C) polyester product formed in the poly-esterification in toluene at 75°C.

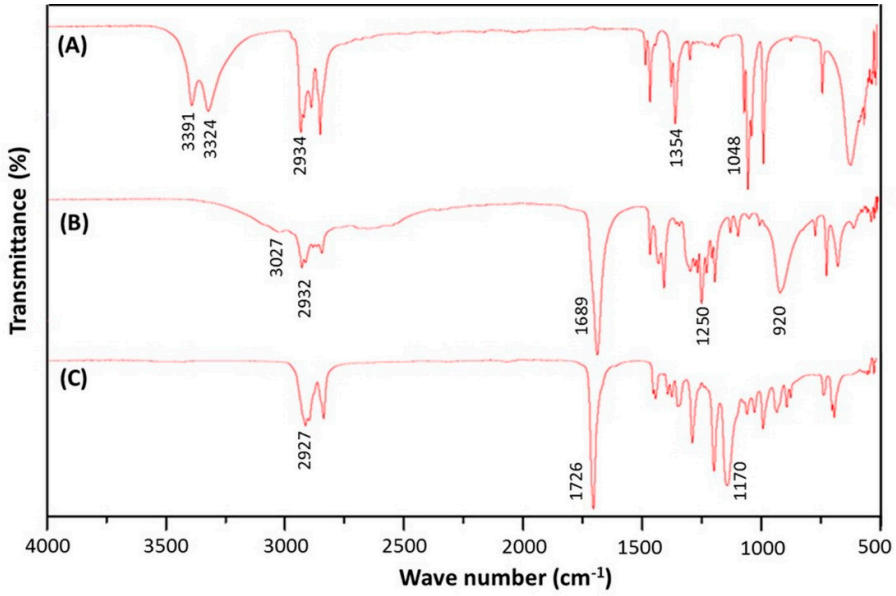


Table S1. Bacterial strains, plasmids, and oligonucleotides used in this study.

	Relevant characteristics	Reference or source
Strains		
E. coli DH5α	F- (80d lacZ M15) (lacZYA-argF) U169 hsdR17 (r - m +) recA1 endA1 relA1 deoR	RBC (Real Biotech)
C. glutamicum	Wild type	ATCC 13032
Plasmids		
pCES208H36GFP	6.7 kb, *E. coli-C. glutamicum* shuttle vector, Km^r, pCES208 derivative; P_{H36}, eGFP	[3]
pCES208H36GFP-ChnDE	8.9 kb, pCES208 derivative; P_{H36}, eGFP	This study
Primers		
ChnE-F	5′-GAGTAGCATG**GGATCC**ATGAACTATCCA AATATACCTTTATATATCAACGGTGAG-3′	*Bam*HI
ChnE-R	5′-TCATGCTGTTT**CATATG**CTAATTGAGTTG CGTAATAAATTTGGTTCTGAGGT-3′	*Nde*I
ChnD-F	5′- AATGGAATCAAA**GTT**AGAAAGGAGGAT GCACTGTTACTGCGTGACG-3′	*Hpa*I
ChnD-R	5′- TCTAATTTTGAA**GTT**TCAGTTTTCGTGCA TAAGCACAATACG-3′	*Hpa*I

Restriction sites are shown in bold. The underlined nucleotides represent ribosome binding site.

References

1. Iwaki, H.; Hasegawa, Y.; Teraoka, M.; Tokuyama, T.; Bergeron, H.; Lau, P.C. Identification of a Transcriptional Activator (ChnR) and a 6-Oxohexanoate Dehydrogenase (ChnE) in the Cyclohexanol Catabolic Pathway in *Acinetobacter* sp. Strain NCIMB 9871 and Localization of the Genes that Encode Them. *J. Appl. Environ. Microbiol.* **1999**, *65*, 5158-5162.
2. Park, J.-U.; Jo, J.-H.; Kim, Y.-J.; Chung, S.-S.; Lee, J.-H.; Lee, H.-H. Construction of Heat-Inducible Expression Vector of *Corynebacterium glutamicum* and *C. ammoniagenes*: Fusion of λ Operator with Promoters Isolated from *C. ammoniagenes*. *J. Microbiol. Biotechnol.* **2008**, *18*, 639-647.
3. Yim, S.S.; An, S.J.; Kang, M.; Lee, J.; Jeong, K.J. Isolation of Fully Synthetic Promoters for High-Level Gene Expression in *Corynebacterium glutamicum*. *Biotechnol. Bioeng.* **2013**, *110*, 2959-2969, doi:10.1002/bit.24954.