SUPPORTING INFORMATION

Streptomyces virginiae PPDC is a New Type of Phenylpyruvate Decarboxylase Composed of Two Subunits

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Inventory of Supporting Items:

Supporting Methods

Supporting Tables and Figures

Reagents

The enzymes for DNA manipulation were from Fermentas (Thermo Fisher Scientific Inc.) or Vazyme (Vazyme Biotech Co., Ltd.). The other main chemicals used, including sodium phenylpyruvate, sodium pyruvate, thiamine pyrophosphate, $MgCl_2$•$6H_2O$, and phenylacetaldehyde were purchased from Sigma-Aldrich.

Protein expression and preparation of *E.coli* crude lysate

Recombinant proteins were overexpressed in *E.coli* BL21 (DE3). The cells harboring different plasmids were grown in Luria-Bertani (LB) medium supplemented with kanamycin (50 μg/mL) or ampicillin (100 μg/mL) overnight at 37 °C. Fresh LB medium (100 mL) containing the relevant antibiotic was inoculated with a 1 mL overnight preculture. Cells were grown at 37 °C until the cultures reached an A_{600} of 0.6–0.8 and then IPTG (0.5 mM final concentration) was added to induce protein expression. After incubation for 5 h at 30 °C while shaking, cells were harvested at 5000 rpm for 20 min.

Cells were suspended in 7 mL buffer A (50 mM Tris-HCl, 200 mM NaCl, 2 mM DTT, pH8.0). The suspension was subjected to a French press (Constant Systems Limited) followed by centrifugation at 12000 rpm for 1 h at 4 °C to remove insoluble debris. The resultant insoluble pellets and soluble proteins were analyzed by SDS-PAGE. *E.coli* BL21 (DE3) transformed with empty vector (Ev) was treated in the same way, and was used as the negative controls.

Qualitative detection by GC-MS

We used an Agilent 7890 A GC system equipped with a G4567 A auto-sampler and a quadrupole time-of-flight Agilent 7200 B mass spectrometer (Agilent Technologies). An apolar HP-5MS (5% phenyl-polymethyl siloxane) capillary column (30-m length, 250 μm i.d., and 0.25 μm film thickness) (Agilent Technologies) was used. The gas carrier was helium with a flow rate of 1.5 mL/min. The temperature of the injector and detector were set as 220 °C and 300 °C, respectively. The column temperature was maintained at 50 °C for 2 min, then increased to 210 °C at a rate of 10 °C/min. Split ratio was kept at 5:1 and mass spectra were acquired in the range 30–500 *m*/*z*. For qualitative analysis of the products, 1 μL isooctane extracts was injected.

Table S1 Plasmids and primers used in this study

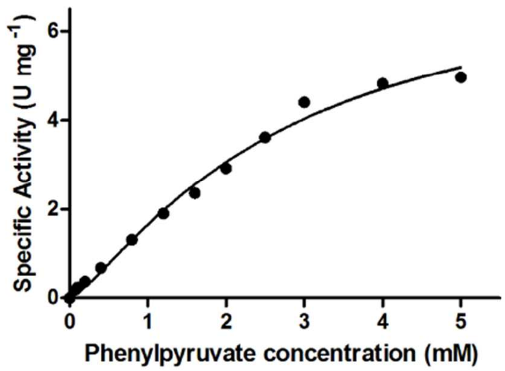
Plasmids or Primers	Description or Sequences[a] (5'→3')	source or reference
Plasmids		
pET28a	Cloning and expression vector	Novagen
pETDuet-1	Cloning and expression vector	Novagen
pET28a-NT-*Sv*PPDCα	pET28a derivate carrying *SvPPDC*α gene with an N-terminal histag	This study
pET28a-NT- *Sv*PPDCβ	pET28a derivate carrying *Sv*PPDCβ gene with an N-terminal histag	This study
pETDuet-NT-*Sv*PPDCα/β	pETDuet-1 derivate carrying *SvPPDC*α and *Sv*PPDC β gene with hexahistindine tags fused to their N termini	This study
pETDuet-SNT-*Sv*PPDCα/β	pETDuet-1 derivate carrying N-terminal his-tagged *SvPPDC*α gene and non-tagged *Sv*PPDC β gene	This study
pETDuet-NT-*Sv*PPDCα^{G167A}/β	pETDuet-NT- *SvPPDC*α/β derivate carrying a mutation G167A in *SvPPDC*α gene	This study
pETDuet-NT-*SvPPDC*$\alpha^{G167AD168AG169A}$/β	pETDuet-NT- *SvPPDC* α/β derivate carrying mutations G167A, D168A and G169A in *SvPPDC* α gene	This study
pETDuet-NT-*Sv*PPDCα/β^{E47A}	pETDuet-NT- *SvPPDC* α/β derivate carrying a mutations E47A in *Sv*PPDCβ gene	This study
pET28a-NT-*Sc*PPDC	pET28a derivate carrying *Sc*PPDC gene with an N-terminal histag	This study
Primers[b]		
28a-NT-*SvPPDC*α-F	CCTGGTGCCGCGCGGCAGCCATATGGTGACCGTACTCGAAGCGGCCACCG	
28a-NT-*SvPPDC*α-R	CAAGCTTGTCGACGGAGCTCGAATTCTCAGTCCCCCTGTCCGTCCGCGGCCAGTTC	
28a-NT- *Sv*PPDCβ-F	CTGGTGCCGCGCGGCAGCCATATGATGTCCGAGATCACCATGGCCAAGGC	
28a-NT- *Sv*PPDCβ-R	CTTGTCGACGGAGCTCGAATTCTCATGCCGGGACCGCCTCCCATTCCA	
Duet-NT-*SvPPDC*α-F	GCCATCACCATCATCACCACAGCAGCGCGATGGTGACCGTACTCGAAGCGGCCACCGCC	
Duet-NT-*SvPPDC*α-R	CGGCCGCAAGCTTGTCGACCTGCAGTCAGTCCCCCTGTCCGTCCGCGGCCAG	
Duet-NT-T7-F	CTGGCCGCGGACGGACAGGGGGACTGACTGCAGGTCGACAAGCTTGCGGCCG	
Duet-NT-T7-R	CGCGCTGCTGTGGTGATGATGGTGATGGCTGCTGCCCATATGTATATCTCCTTCTTATA	

	CTTAAC
Duet-NT- *Sv*PPDCβ-F	ATGGGCAGCAGCCATCACCATCATCACCA CAGCAGCGCGATGTCCGAGATCACCATG GCCAAGG
Duet-NT- *Sv*PPDCβ-R	GTTTCTTTACCAGACTCGAGGGTACCTCA TGCCGGGACCGCCTCCCATTCCAG
pETDuet-SNT- *SvPPDC*α/β-F	GTATAAGAAGGAGATATACATATGTCCG AGATCACCATGGCCAAGGC
pETDuet-SNT- *SvPPDC*α/β-R	GCCTTGGCCATGGTGATCTCGGACATATG TATATCTCCTTCTTATAC
SvPPDCα-G167A-F	GTGGCGCTCGCCTACATC**GCA**GACGGCG CCACCAGCGAGGGCGACTTC
SvPPDCα-G167A-R	GAAGTCGCCCTCGCTGGTGGCGCCGTC**TG** **C**GATGTAGGCGAGCGCCAC
SvPPDCα-GDG-F	GATCGTGGCGCTCGCCTACATC**GCAGCC** **GCA**GCCACCAGCGAGGGCGACTTC
SvPPDCα- GDG-R	GAAGTCGCCCTCGCTGGTGGC**TGCGGCT** **GC**GATGTAGGCGAGCGCCACGATC
*Sv*PPDCβ -E47A-F	GATCACCGACGGGCTGGCCGCC**GCA**TTC GGCGACGAACGCTGCTTC
*Sv*PPDCβ -E47A-R	GAAGCAGCGTTCGTCGCCGAA**TGC**GGCG GCCAGCCCGTCGGTGATC
pET28a-NT-*Sc*PPDC-F	CGGCCTGGTGCCGCGCGGCAGCCATATG GCACCTGTTACAATTGAAAAGTTCG
pET28a-NT-*Sc*PPDC-R	GTCGACGGAGCTCGAATTCGGATCCCTAT TTTTTATTTCTTTTAAGTGCCGCTGCTTC

[a] the introduced restriction sites are underlined and the mutation sites are shown in bold;

[b] F: forward primer; R: reverse primer.

a)



b)

Kinetic parameters of wild-type *Sc*PPDC			
Enzyme	K_m [mM]	k_{cat} [min^{-1}]	k_{cat}/K_m [$min^{-1}mM^{-1}$]
*Sc*PPDC	2.70 ± 0.69	537.60 ± 81.0	203.05 ± 23.0

Figure S1 Kinetics of *Sc*PPDC. a) Dependence of the reaction rate on phenylpyruvate concentrations for *Sc*PPDC. The solid line is a fitted curve. b) Kinetic parameters of *Sc*PPDCα/β.