Supplementary Materials

Systematic unravelling of the biosynthesis of poly(L-diaminopropionic acid) in *Streptomyces albulus* PD-1

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Supplementary Table S1. Strains and plasmids used in this study

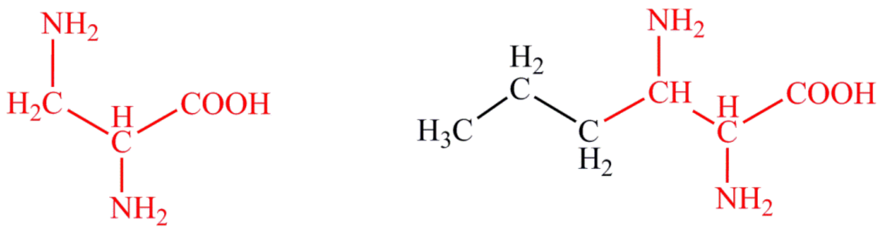
Strains or plasmids	Descriptions	Source
S. albulus PD-1	Producer of ε-PL and PDAP	CGMCC No. M2011043
S. albulus PD-3	△*pdaps* :: Am^{R}	This study
S. lividans TK 24	A model streptomycete used for PDAPs expression	Lab stock
E. coli DH 5α	*E. coli* strain used for cloning	Lab stock
E. coli BL 21 (DE3)	*E. coli* strain used for the expression A domain of PDAPs	Lab stock
E. coli Origami (DE3)	*E. coli* strain used for expression of enzymes involved in L-DAP synthesis	Lab stock
pET-28a	Plasmid used for the expression A domain of PDAPs	Lab stock
pET-22b	Plasmid used for the expression of NjxA and NjxB	Lab stock
pKC1139	Used for the gene disruption of putative *pdaps*	Lab stock
pKC1139-*pdap'*	pKC1139 carrying homologous sequence of *pdaps*	This study
pIJ86	Used for the heterologous expression of PDAPs in *S. lividans* TK24	Lab stock

Supplementary Table S2. List of similarity between NjxA/NjxB and the reported proteins related to L-DAP biosynthesis

Organism	Homologous gene (NjxA/NjxB)	Similarity (%NjxA /%NjxB)	Query cover (NjxA%/NjxB%)	Related products	References
Streptomyces sp. strain ATCC 11861	VioB/VioK	33/31	84/87	viomycin	Antimicrobial agents and chemotherapy, 2003, 47(9): 2823-2830
S. mutabilis subsp. *capreolus* ATCC 23892	CmnB/CmnK	34/32	82/98	capreomycin	Applied and environmental microbiology, 2007, 73: 4162-4170
Bacillus thuringiensis sub. *kurstaki* YBT-1520	Zwa5A/Zwa5B	30/30	82/79	zwittermicin A	FEBS letters, 2008, 582: 3125-3131
Staphylococcus aureus Newman	SbnA/SbnB	31/27	95/90	staphyloferrin B	Molecular microbiology, 2009 74: 594-608

Supplementary Table S3. Sequences of primer pairs used in this study

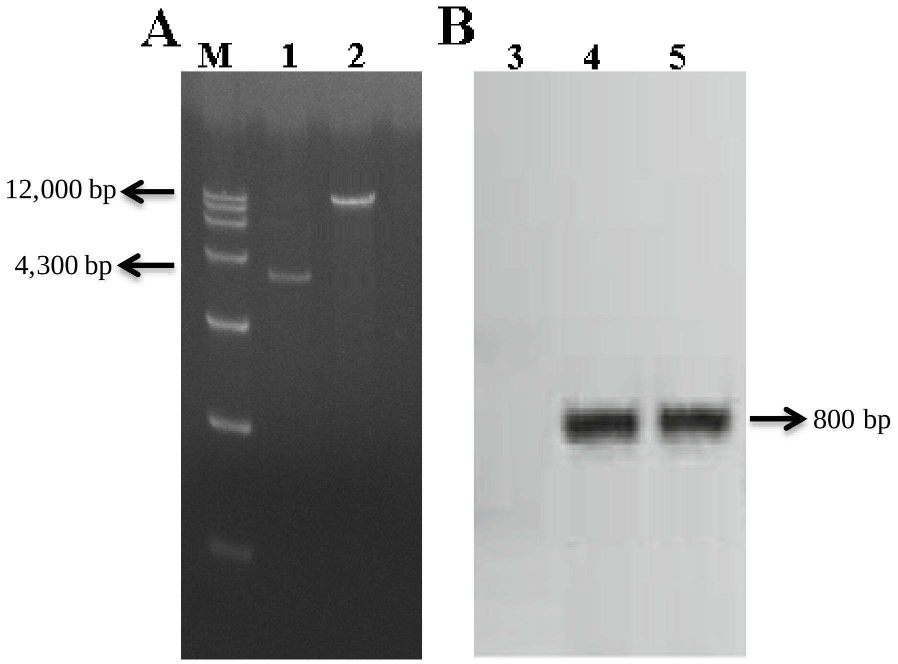
Target products	Primer sequences (5′-3′)
Homologous fragment of *pdaps*	CGGAATTCCCAACCGGCTCGCCCACCACCTCAT
	GATAAGCTTCCTCGAACAGGTGCGTCTGCAAGCT
Complete fragment of *pdaps*	GTGACGGGCGGGACCGTCGCTGACCG
	TCACCGTGCAGGACTGGTGGGGTGGG
pdaps with its own promotor	ATGGATCCAGCGCCAGTTGTCCCAGTGGACCTC
	GATAAGCTTTCACCGTGCAGGACTGGTGGGGTG
A domain of *pdaps*	CGGAATTCGTGACGGGCGGGACCGTCGCTGA
	GATAAGCTTGGACGTCGAGGAACGGCGGCACCATG
njxA	CGGAATTCGATGACCATCATCTCCGTTCCCA
	GATAAGCTTTCAGGCCGTCAGGTCCGTGGC
njxB	CGGAATTCGGTGACCACCACACGTCCCGCC
	GATAAGCTTTCAGCCGTAGCGGCGTAGCTC



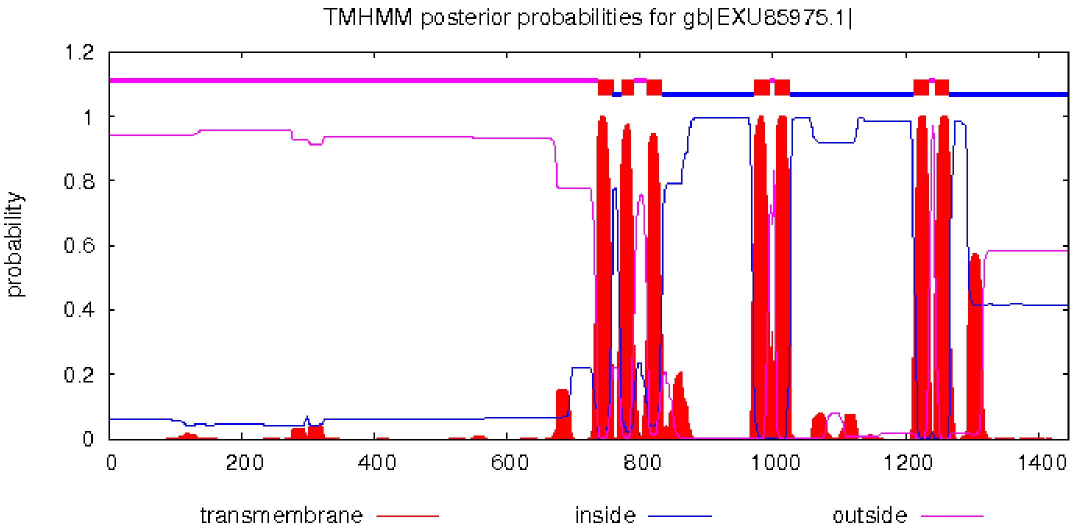
L-DAP

β-Lysine

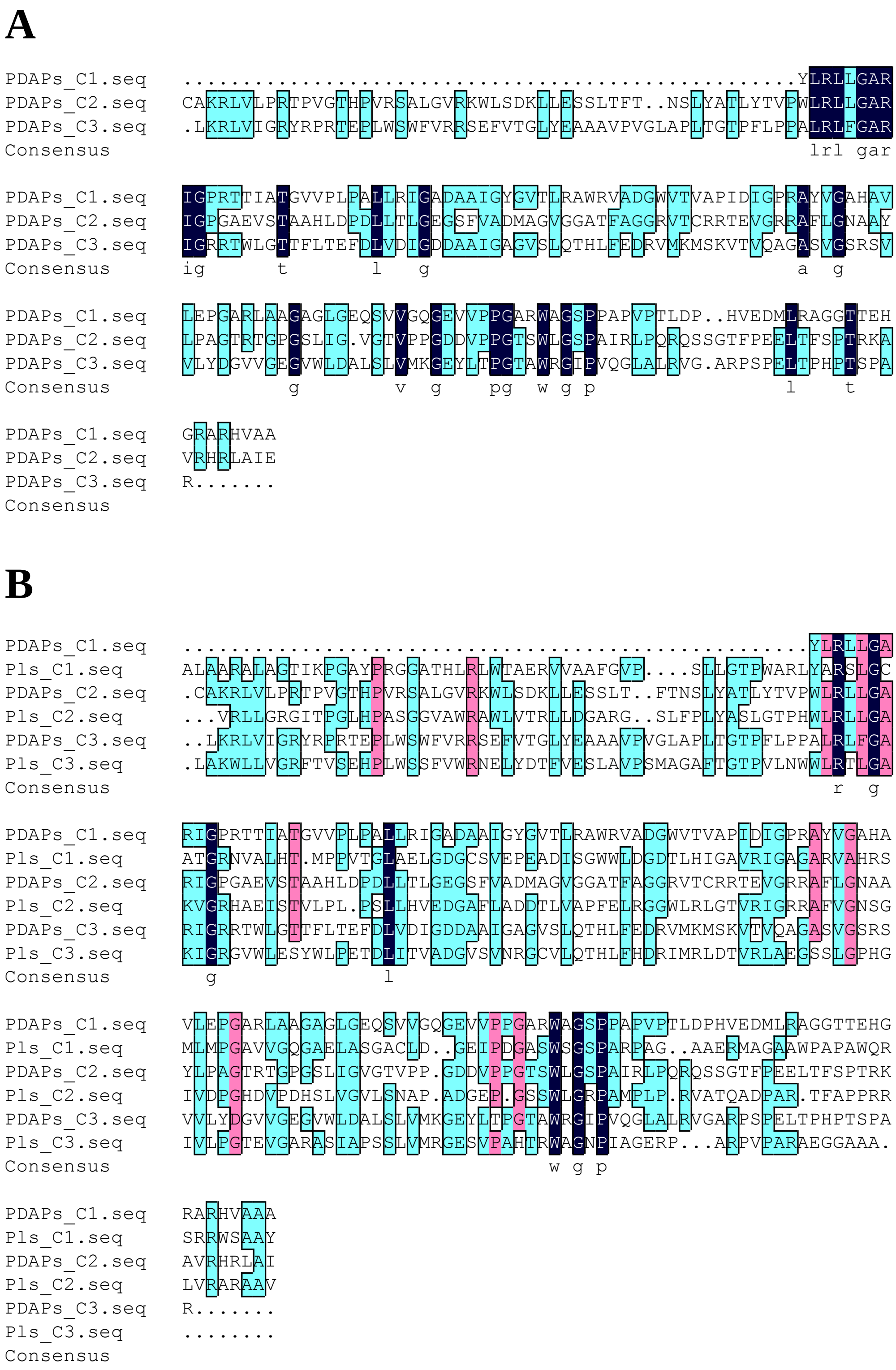
Supplementary Fig. S1. Structures of L-DAP and β-lysine



Supplementary Fig. S2. Analysis for putative *pdaps* disruption by PCR (A) and Southern-blot (B). For PCR analysis, the start and the end sequences of putative *pdaps* were used as primers for confirmation of the *pdaps* disruption. 1. using the chromosome of wild-type strain *S. albulus* PD-1 as the PCR template; 2. using the chromosome of putative *pdaps* disruption strain *S. albulus* PD-3 as a template. For Southern-blot analysis, the genomes were digested by *Sac*I and apramycin resistance gene was used as a probe. 4. wild-type strain *S. albulus* PD-1; 5 and 6. putative *pdaps* disruption strain *S. albulus* PD-3.



Supplementary Fig. S3. Analysis of transmembrane helices in PDAPs



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A

PDAPs_C1.seq    ....................................................YLRLLGAR
PDAPs_C2.seq    CAKRLVLPRTPVGTHPVRSALGVRKWLSDKLLESSLTFT..NSLYATLYTVPWLRLLGAR
PDAPs_C3.seq    .LKRLVIGRYRPRTEPLWSWFVRRSEFVTGLYEAAAVPVGLAPLTGTPFLPPALRLFGAR
Consensus                                                            lrl gar

PDAPs_C1.seq    IGPRTTIATGVVPLPALLRIGADAAIGYGVTLRAWRVADGWVTVAPIDIGPRAYVGAHAV
PDAPs_C2.seq    IGPGAEVSTAAHLDPDLLTLGEGSFVADMAGVGGATFAGGRVTCRRTEVGRRAFLGNAAY
PDAPs_C3.seq    IGRRTWLGTTFLTEFDLVDIGDDAAIGAGVSLQTHLFEDRVMKMSKVTVQAGASVGSRSV
Consensus       ig      t       l  g                                a  g

PDAPs_C1.seq    LEPGARLAAGAGLGEQSVVGQGEVVPPGARWAGSPPAPVPTLDP..HVEDMLRAGGTTEH
PDAPs_C2.seq    LPAGTRTGPGSLIG.VGTVPPGDDVPPGTSWLGSPAIRLPQRQSSGTFPEELTFSPTRKA
PDAPs_C3.seq    VLYDGVVGEGVWLDALSLVMKGEYLTPGTAWRGIPVQGLALRVG.ARPSPELTPHPTSPA
Consensus                g        v  g    pg  w g p                 l    t

PDAPs_C1.seq    GRARHVAA
PDAPs_C2.seq    VRHRLAIE
PDAPs_C3.seq    R.......
Consensus
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B

PDAPs_C1.seq    .....................................................YLRLLGA
Pls_C1.seq      ALAARALAGTIKPGAYPRGGATHLRLWTAERVVAAFGVP....SLLGTPWARLYARSLGC
PDAPs_C2.seq    .CAKRLVLPRTPVGTHPVRSALGVRKWLSDKLLESSLT..FTNSLYATLYTVPWLRLLGA
Pls_C2.seq      ...VRLLGRGITPGLHPASGGVAWRAWLVTRLLDGARG..SLFPLYASLGTPHWLRLLGA
PDAPs_C3.seq    ..LKRLVIGRYRPRTEPLWSWFVRRSEFVTGLYEAAAVPVGLAPLTGTPFLPPALRLFGA
Pls_C3.seq      .LAKWLLVGRFTVSEHPLWSSFVWRNELYDTFVESLAVPSMAGAFTGTPVLNWWLRTLGA
Consensus                                                               r  g

PDAPs_C1.seq    RIGPRTTIATGVVPLPALLRIGADAAIGYGVTLRAWRVADGWVTVAPIDIGPRAYVGAHA
Pls_C1.seq      ATGRNVALHT.MPPVTGLAELGDGCSVEPEADISGWWLDGDTLHIGAVRIGAGARVAHRS
PDAPs_C2.seq    RIGPGAEVSTAAHLDPDLLTLGEGSFVADMAGVGGATFAGGRVTCRRTEVGRRAFLGNAA
Pls_C2.seq      KVGRHAEISTVLPL.PSLLHVEDGAFLADDTLVAPFELRGGWLRLGTVRIGRRAFVGNSG
PDAPs_C3.seq    RIGRRTWLGTTFLTEFDLVDIGDDAAIGAGVSLQTHLFEDRVMKMSKVTVQAGASVGSRS
Pls_C3.seq      KIGRGVWLESYWLPETDLITVADGVSVNRGCVLQTHLFHDRIMRLDTVRLAEGSSLGPHG
Consensus         g               l

PDAPs_C1.seq    VLEPGARLAAGAGLGEQSVVGQGEVVPPGARWAGSPPAPVPTLDPHVEDMLRAGGTTEHG
Pls_C1.seq      MLMPGAVVGQGAELASGACLD..GEIPDGASWSGSPARPAG..AAERMAGAAWPAPAWQR
PDAPs_C2.seq    YLPAGTRTGPGSLIGVGTVPP.GDDVPPGTSWLGSPAIRLPQRQSSGTFPEELTFSPTRK
Pls_C2.seq      IVDPGHDVPDHSLVGVLSNAP.ADGEP.GSSWLGRPAMPLP.RVATQADPAR.TFAPPRR
PDAPs_C3.seq    VVLYDGVVGEGVWLDALSLVMKGEYLTPGTAWRGIPVQGLALRVGARPSPELTPHPTSPA
Pls_C3.seq      IVLPGTEVGARASIAPSSLVMRGESVPAHTRWAGNPIAGERP...ARPVPARAEGGAAA.
Consensus                                           w g p

PDAPs_C1.seq    RARHVAAA
Pls_C1.seq      SRRWSAAY
PDAPs_C2.seq    AVRHRLAI
Pls_C2.seq      LVRARAAV
PDAPs_C3.seq    R.......
Pls_C3.seq      ........
Consensus
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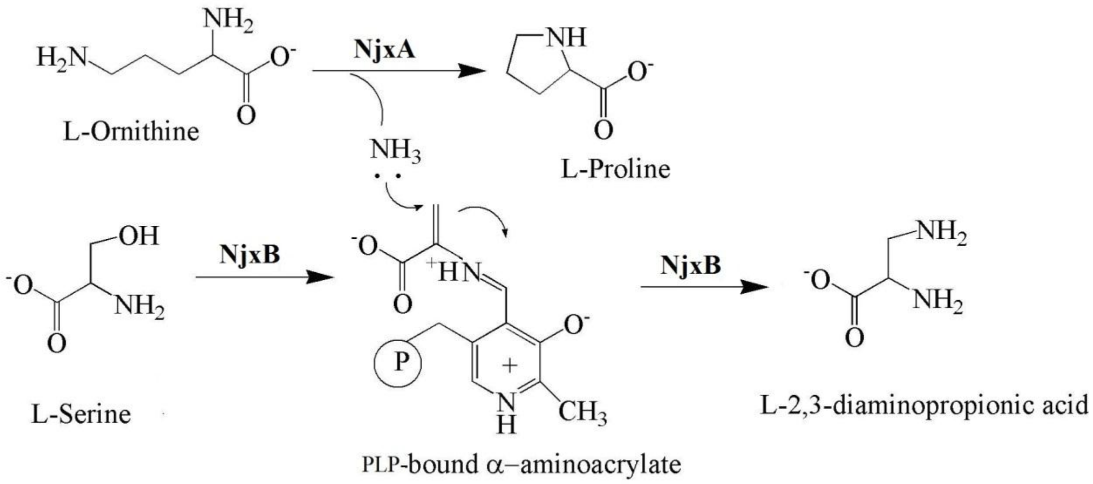
Supplementary Fig. S4. (A) Alignment of the three tandem domains of PDAPs. (B) Alignment of the amino acid sequences between the tandem domains of PDAPs and Pls.



Supplementary Fig. S5. The putative synthesis mechanism of L-DAP in *S. albulus* PD-1.