Supplementary Information

Discovery and Engineering of an Aldehyde Tolerant 2-deoxy-D-ribose 5-phosphate Aldolase (DERA) from *Pectobacterium Atrosepticum*

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Supporting Information

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Table S1. Primers used in this work.

Name	Sequence
*Pa*DERA_fwd_NcoI	CATGCCATGGGCATGACCAAGCTGACCACGGCCGCGCAGC
*Pa*DERA _rev_XhoI	CCGCTCGAGTGAGTAGCTGCCCTGTGGCGCCGCTG
*Ec*DERA_ fwd _NcoI	CCATGGGCATGACTGATCTGAAAGCAAGCAGCCTGCGTGCACTG
*Ec*DERA _ rev _XhoI	CCTCGAGTGAGTAGCTGCTGGCGCTCTTAC
T7Promotor	TAATACGACTCACTATAGGG
T7Terminal	GCTAGTTATTGCTCAGCGG
*Pa*DERA_fwd_N Terminus	[Phos]ATGACCAAGCTGACCACGGCCG
*Pa*DERA _rev_N Terminus	[Phos]GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAG
*Pa*DERA_fwd_C Terminus	[Phos]CACCACCACCACCACCACTGAGATCCG
*Pa*DERA _rev _C Terminus	[Phos] TGAGTAGCTGCCCTGTGGCGCC
*Pa*DERA_fwd_remove amino acids M and G	ATGACCAAGCTGACCACGGCCG
*Pa*DERA_rev_remove amino acids M and G	GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAG
*Pa*DERA_fwd_remove amino acids S, L and E	CACCACCACCACCACCACTGAGATCCG
*Pa*DERA_rev_remove amino acids S, L and E	GTAGCTGCCCTGTGGCGCCG
*Pa*DERA_fwd _C49M	ATGATCTATCCACGTTTTATCCCCCTGGC
*Pa*DERA _rev _C49M	GATAGCAGCAGTTTTTCCTGCCGG

Table S2: Overview of enzyme properties and acetaldehyde resistance of DERA isolated from different organisms including this work.

Source	Characterisation					Acetaldehyde resistance		Reference
	Molecular Mass[a] (kDa)	Optimum pH	Optimum Temperature (℃)	K_M (mM)	V_{max}[b] or Specific Activity[c] (U mg^{-1})[d]	Activity	Reaction conditions	
Pectobacterium atrosepticum (With five extra amino acids in the sequence)	**28.8**	**8.6-9.0**	**40**	**0.22**	**17.67**	**70 % retention of activity, 1.5 h 300 mM 25 ℃**		**This work**
Pectobacterium atrosepticum (Without five extra amino acids)	**28.8**	**8.6-9.0**	**40**	**0.11**	**16.24**	**n.s**		**This work**
Streptococcus suis	27	7.0	40	0.8	18.2	23.3 % retention of activity, 2 h 200 mM 30 ℃		[1]
Rat liver	n.s.	7.5	n.s.	0.17	106.5[c]	n.s.		[2]
Environmental DNA libraries	23.9	7	35	0.038	2.9[b]	n.s.		[3]
Mesophiles								
Salmonella Typhimurium	28.7	7.3-8.4	n.s.	0.1	3500[b]	No quantitative data, but 99% activity loss reported in the presence of acetaldehyde		[4]
Steptococcus lactis subsp. diacetylactis DRC3	n.s.	6.8	45	0.6[*]	n.s.	n.s.		[5]
Klebsiella pneumoniae	27.6	7.5	37	n.s.	2.5[c]	n.s.		[6]

Escherichia coli	28	7.5	n.s.	0.23	58[c]	0% retention of activity	2 h 300 mM 25 ℃	[7]
Escherichia coli	n.s.	n.s.	n.s.	0.29	16[c]	Half-life = 25 min	300 mM 25 ℃	[8]
Yersinia sp. EA015	24.8	6	50	9.1	137[c]	Maximal activity at 200mM		[9]
Paenibacillus sp. EA001	24.5	6	50	145	62[c]	n.s.		[10]
Rhodococcus erythropolis	22.9	7	25[e]	4.84	17[c]	Half-life = 64.4 min	300 mM 25 ℃	[8]
Haemophilus influenza	23.6	7.5	40	0.14	70.42[b]	Maximal activity at 300 mM		[11]
Staphylococcus epidermidis	29.2	n.s.			67.1[c]	11.3% retention of activity	2h 300 mM 25 ℃	[12]
Lactobacillus brevis ECU8302	n.s.	6	40	3.34	102[b]	Half-life = 37.3 min	300 mM 25 ℃	[13]
Extremophiles (Hyperthermophilic unless specified)								
Aeropyrum pernix	24.5	6.5	n.s.	0.057[*]	4.5[c**]	n.s.		[14]
Thermococcus kodakaraensis	24.5	4	95	0.81[**]	285[b***]	n.s.		[15]
Pyrobaculum aerophilum	24.5	6	n.s.	0.066	0.25[c]	53% retention of activity	20 h 300 mM 25 ℃	[16]
Thermotoga maritima	27.8	6.5	n.s.	0.02	1[c]	46% retention of activity	20 h 300 mM 25 ℃	[16]
Hyperthermus butylicus	26.4	5.5	80	0.15	0.5[c]	75% retention of activity	8 h 300 mM 25 ℃	[17]
Aciduliprofundum boonei	26.6	7	80	0.12	n.s.	70% retention of activity	4 h 250 mM 25 ℃	[18]
Haloarcula japonica (Halophilic)	26.6	6.4	60	1.02	8.92[b]	35% retention of activity	5h 300 mM 25 ℃	[19]

[a] – of monomer, [b]- V*max*, [c]- specific activity

[d] - One unit is defined as the amount of DERA required to cleave 1 µmol DR5P per minute

[e] - increase of activity till 65 ℃; extreme loss in activity at 67 ℃ and 70 ℃

[*] - Measured at 37 ℃

[**] - Measured at 50 ℃

[***] - Measured at 95 ℃

n.s. – Not specified

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ATGACCAAGCTGACCACGGCCGCGCAGCGCGCGCTGGCGTTGATGGATTTAACCACGCTGAATGAGGATG
ATACGGATGAAAAAGTGACGGCACTCTGCCGTCAGGCAAATAGCCCGGCAGGAAAAACTGCTGCTATCTG
TATCTATCCACGTTTTATCCCCCTGGCGAAAAAAATCCTGCGTGAGCAGGGTACGCCGGATATCCGTATT
GCGACGGTGACCAACTTCCCGCACGGCAATGATGATGTTGACATCGCTGTTGCAGAAACCAGAGCGGCGA
TAGCCTATGGCGCTGATGAAGTTGATGTCGTATTCCCTTACCGAGCACTGATTGCAGGCAATGCGCAAAT
TGGTTTTGAGCTGGTGCAGGCATGCAAAGCCGTATGTCAGGATGCCCACGTGCTGCTGAAGGTGATCATC
GAGACAGGCGAGCTGAAACAGGAAACGCTGATACGTCAGGCGAGCGAGATTGTCATTGATGCGGGTGCCG
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GACGATCCGCGATAAAGGCGTGGGTGAGTATGTCGGCTTTAAAGCCGCTGGCGGCGTGCGTAACGCGGAA
GACGCCGCCATTTATCTGCAACTGGCGGACGATATCATGGGCGCTGAATGGGCGAATGCGCAGCATTTTC
GCTTTGGCGCATCCAGTTTGCTGGCGAGCCTGCTGACAACGCTCGGACACGCGGCAGCGGCCCCACAGGG
CAGCTACTAA
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Figure S1: DNA sequence of *Pectobacterium_atrosepticum* DERA.

A)

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MTDLKASSLR ALKLMDLTTL NDDDTDEKVI ALCHQAKTPV GNTAAICIYP RFIPIARKTL
KEQGTPEIRI ATVTNFPHGN DDIDIALAET RAAIAYGADE VDVVFPYRAL MAGNEQVGFD
LVKACKEACA AANVLLKVII ETGELKDEAL IRKASEISIK AGADFIKTST GKVAVNATPE
SARIMMEVIR DMGVEKTVGF KPAGGVRTAE DAQKYLAIAD ELFGADWADA RHYRFGASSL
LASLLKALGH GDGKSASSYH HHHHHStop
```

```
MGMTKLTTAA QRALALMDLT TLNEDDTDEK VTALCRQANS PAGKTAAICI YPRFIPLAKK
ILREQGTPDI RIATVTNFPH GNDDVDIAVA ETRAAIAYGA DEVDVVFPYR ALIAGNAQIG
FELVQACKAV CQDAHVLLKV IIETGELKQE TLIRQASEIV IDAGADFIKT STGKVPVNAT
PESASIMLKT IRDKGVGEYV GFKAAGGVRN AEDAAIYLQL ADDIMGAEWA NAQHFRFGAS
SLLASLLTTL GHAAAAPQGS YSLEHHHHHHStop
```

B)

```
MGMTKLTTAA QRALALMDLT TLNEDDTDEK VTALCRQANS PAGKTAAICI YPRFIPLAKK
ILREQGTPDI RIATVTNFPH GNDDVDIAVA ETRAAIAYGA DEVDVVFPYR ALIAGNAQIG
FELVQACKAV CQDAHVLLKV IIETGELKQE TLIRQASEIV IDAGADFIKT STGKVPVNAT
PESASIMLKT IRDKGVGEYV GFKAAGGVRN AEDAAIYLQL ADDIMGAEWA NAQHFRFGAS
SLLASLLTTL GHAAAAPQGS YSLEHHHHHHStop
```

```
MGMTKLTTAA QRALALMDLT TLNEDDTDEK VTALCRQANS PAGKTAAIMI YPRFIPLAKK
ILREQGTPDI RIATVTNFPH GNDDVDIAVA ETRAAIAYGA DEVDVVFPYR ALIAGNAQIG
FELVQACKAV CQDAHVLLKV IIETGELKQE TLIRQASEIV IDAGADFIKT STGKVPVNAT
PESASIMLKT IRDKGVGEYV GFKAAGGVRN AEDAAIYLQL ADDIMGAEWA NAQHFRFGAS
SLLASLLTTL GHAAAAPQGS YSLEHHHHHHStop
```

Figure S2: A) Amino acid sequences of *Pa*DERA (WT) and *Ec*DERA. The grey shaded region highlights sequence similarity around position C47(*Ec*DERA) and C49 (*Pa*DERA). The cloning strategy introduced extra amino acids (MG in the beginning, SLE at the end) to the recombinant *Pa*DERA. B) Amino acid ssequences of *Pa*DERA and *Pa*DERA C49M.

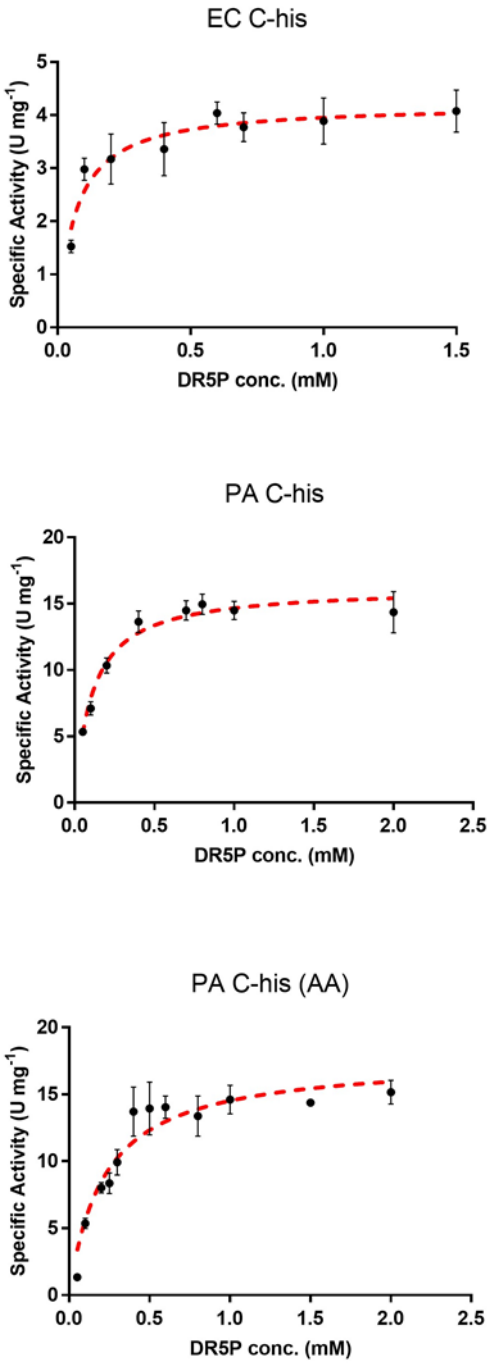

Figure S3: Michaelis-Menten curves of different DERA variants including *Ec*DERA, *Pa*DERA and *Pa*DERA with extra amino acids.

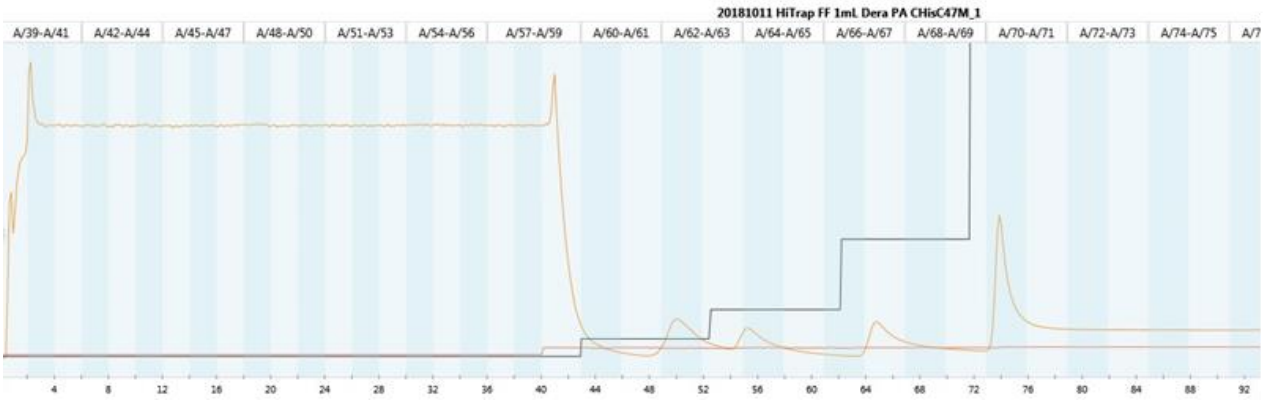

Figure S4: The purification of *Pa*DERA C49M, chromatogram obtained from NGC shows the using of isocratic elution approach.

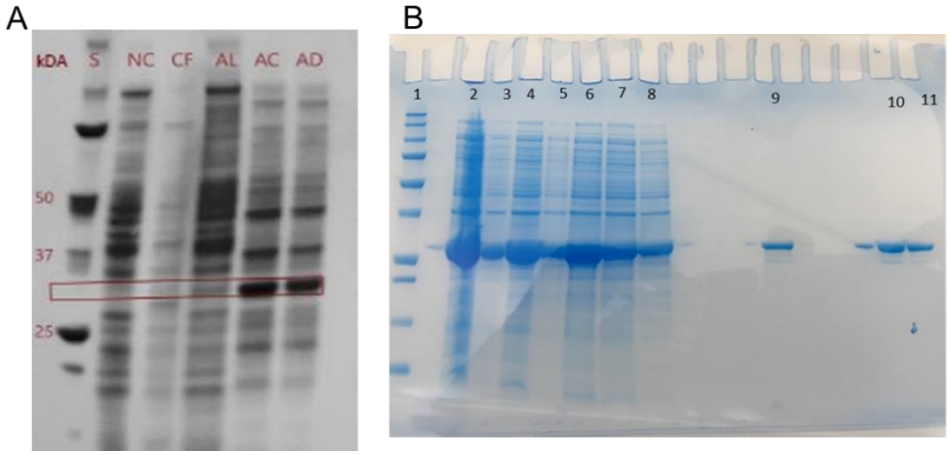

Figure S5: **A.** SDS-PAGE analysis of the purified *Pa*DERA using gradient elution (elution buffer: 1 M Imidazole, pH 7 and running buffer: 100 mM KPi, pH 7). **B.** SDS-PAGE analysis of the purified PaDERA C49M. Lane 1: protein markers; lane 2: Whole cell with 20% w/v KPi buffer; lane 3: Disrupted cells before centrifuging, lane 4: Supernatant after centrifuging, lane 5: Pellet after centrifuging, lane 9: purified enzyme before desalting step, lane 10 and 11: purified enzyme after desalting step. Protein bands were visualized by Coomassie brilliant blue.

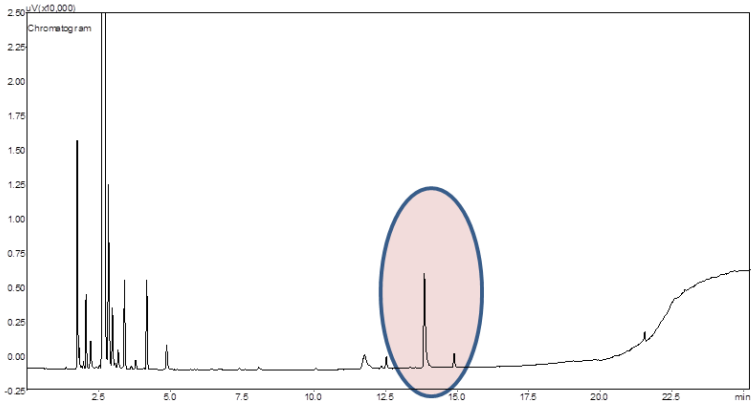

Figure S6: GC chromatogram of the products of the*Pa*DERA C49M catalyzed aldol condensation of acetaldehyde. Reaction conditions: 3mg/mL of whole cells containing *Pa*DERA were resuspended in 10 mL TEA (100 mM, pH 7.0) with 100 mM acetaldehyde. The reaction mixture was stirred at 30 ℃ for 48 h. The product 2,4,6- trideoxy-D-erythrohexapyranoside is indicated.

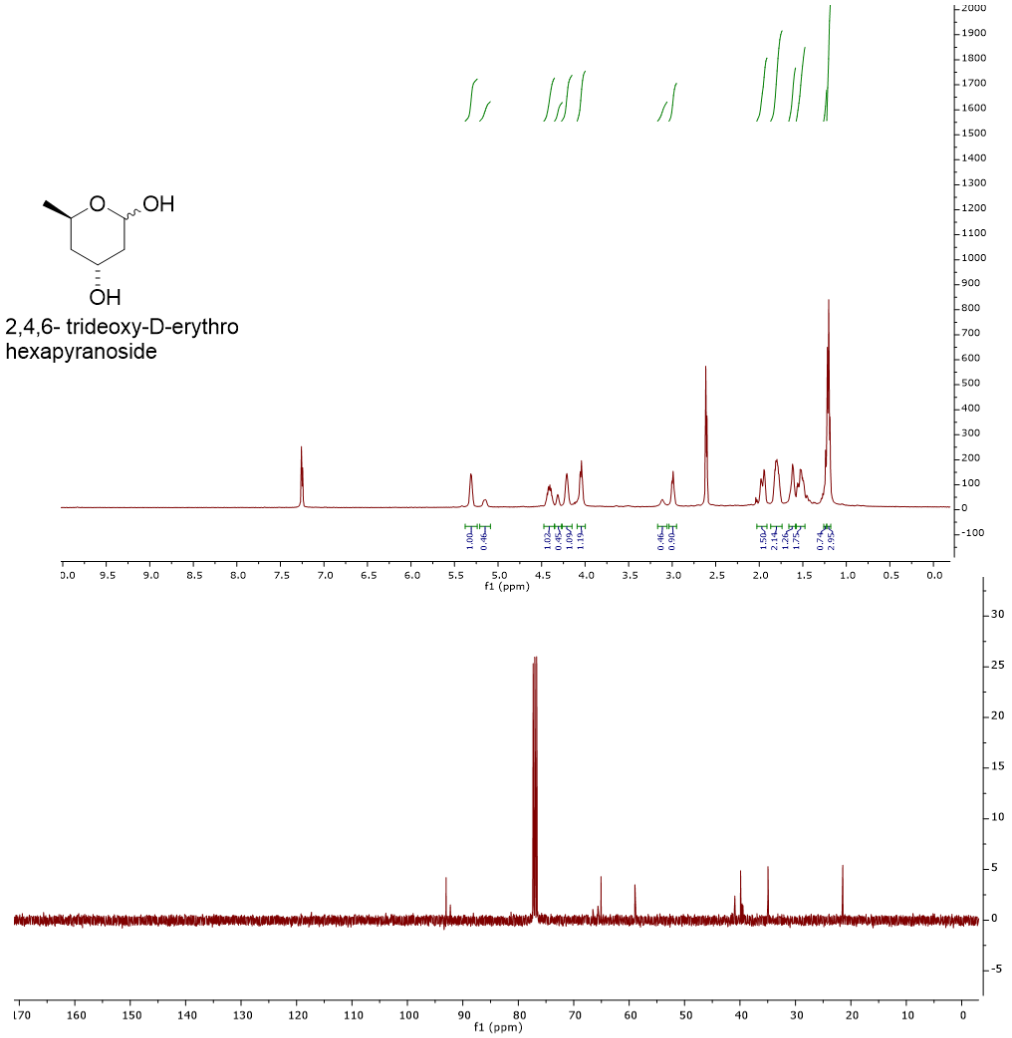

Figure S7: ^{1}H NMR and^{13}C NMR analysis for aldol product.

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