

1 **Title**

2 Characterization of prostaglandin F_{2α} 9-dehydrogenase from *Rhodotorula kratochvilovae*

3 **Authors**

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5 Hideaki Nagano^{1,2}, Si-Bum Park^{1,2}, Hiroko Watanabe¹, Akinori Ando¹, Makoto Ueda², Jun
6 Ogawa^{1,*}

8 Supplement data

10 Table S1 Result of screening for microorganisms reducing PGE₂ to PGF_{2α}

	Number of screened strains	PGF _{2α} producing activity ^a
12 Yeasts	109	22
13 Molds	261	23
14 Basidiomycetes	83	8
15 Bacteria	78	0
16 Actinomycetes	84	0
17 Lactic acid bacteria	70	0
18 Total	685	53

19 ^a The number of strains producing PGF_{2α} more than 5.6×10⁻² mM (2% of substrate)

Effects of coenzymes on PGF_{2α} production by *R. kratochvilovae* NBRC 0389 CFE

Rhodotorula kratochvilovae NBRC 0389 (AKU 4826) was inoculated into 3 mL medium [5 % (w/v) malt extract and 0.3 % yeast extract, pH 5.5] and cultivated at 28 °C with shaking at 300 rpm overnight. The culture broth was all added into 100 mL of the same medium, and it was cultured at 28 °C with shaking at 120 rpm overnight. After cultivation, the cells were harvested by centrifugation at 3,000 rpm for 10 min and washed once with 100 mM Tris-HCl buffer (pH 8.0) or appropriate buffer for following reaction.

Cells were suspended in 100 mM Tris-HCl (pH8.0) at 33% (w/v) and disrupted by sonication with Ultrasonic Disruptor UD-211 (TOMY SEIKO, Tokyo, Japan) at Output 3 and Duty 50 for 20 min in iced water. After disruption, the lysate was centrifuged at 15,000 *g* for 10 min at 4 °C to remove cell debris. The supernatant was used as cell-free extracts (CFE).

Effects of NADH and NADPH on the PGF_{2α} production catalyzed by *R. kratochvilovae* NBRC 0389 were evaluated with using its CFE. Reaction mixtures (500 μL) contained 100 mM Tris-HCl (pH 8.0), 2.8 mM PGE₂, 20 % (v/v) *R. kratochvilovae* NBRC 0389 CFE, and appropriate concentrations of NADH, NADPH, glucose, and GDH. The reaction and lipids extraction were performed in the same way as the screening. Yields of PGs were quantified by HPLC.

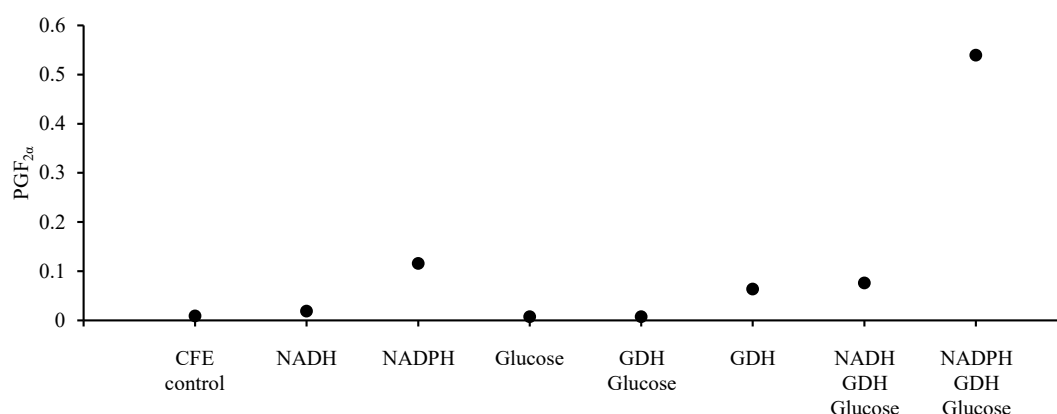


Fig. S1 *R. kratochvilovae* NBRC 0389 CFE produced PGF_{2α} only when there was NADPH in reaction mixture. NADH and NADPH were added at 3 mM, glucose was at 160 mM, and GDH was at 14 U/ml. n=1

Analysis of recombinant PDH activities by resting cell and cell-free extracts reactions

E. coli harboring pET-21b/His-tag-PDH was cultured in 5 mL LB medium at 28 °C with shaking at 300 rpm overnight, and the culture broth was all added into 100 mL of the same medium. The cells were cultured until OD₆₀₀ was 0.4, and 1 mM IPTG was added to the culture. The culture medium was incubated at 28 °C with 120 rpm shaking overnight. The cells were harvested by centrifugation and washed twice with 0.75 % NaCl solution. The enzyme expression was checked by SDS-PAGE. The evaluation of the resting cell and cell-free extracts activity were performed in the same way as evaluation of PGE₂-reducing activity. The reaction time was changed to 24 hours, and the PGE₂ concentration was changed to 2.8 mM. The expression of the PDH gene was confirmed by SDS-PAGE (Fig. S2). The PDH-expressing strain was reacted with PGE₂. As a result, PGF_{2α} was produced about 1.5 mM in the resting cells and about 2.1 mM in cell-free extracts (Fig. S2).

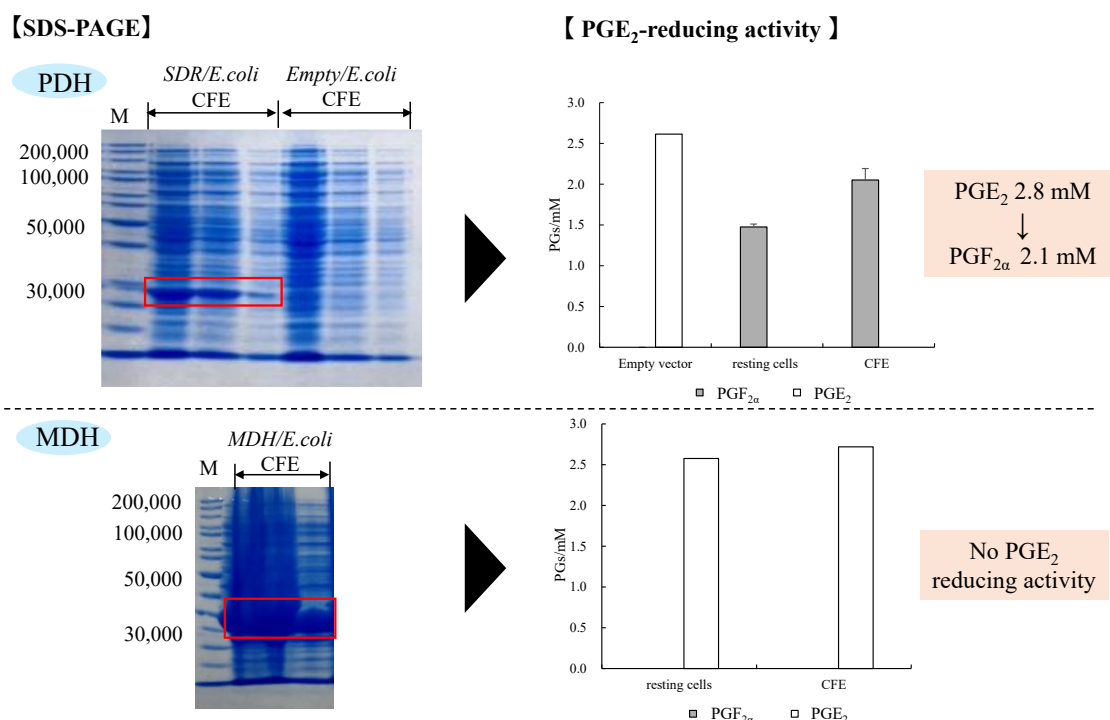
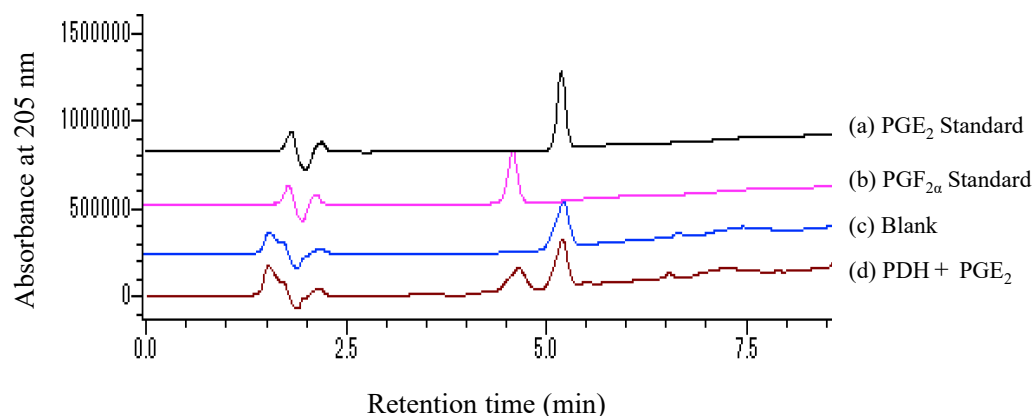


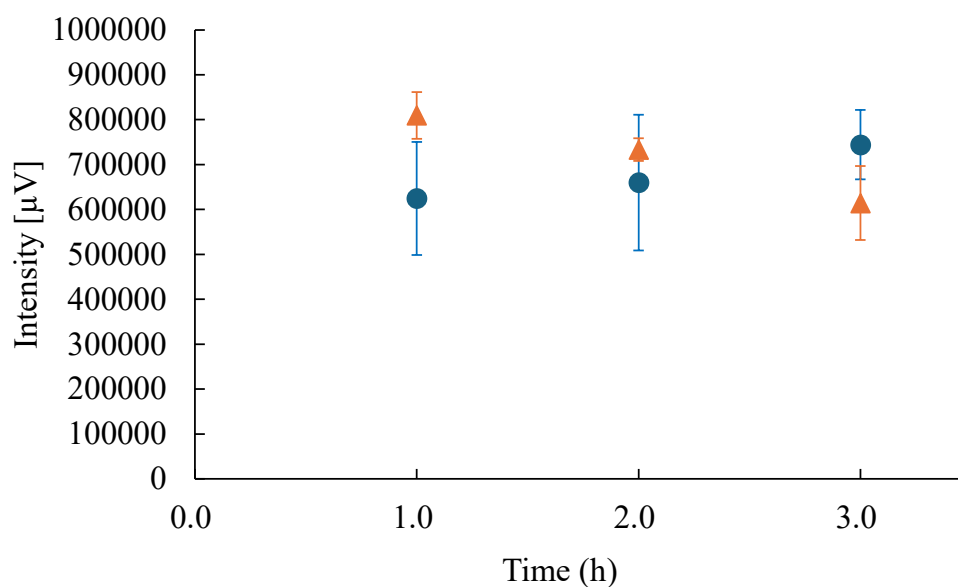
Fig. S2 SDS-PAGE analysis of cell-free extracts of the recombinant *E. coli* BL21 (DE3) expressing PDH gene and MDH gene. M: PageRuler Unstained Protein Ladder (thermo scientific, MA, USA). The uncropped SDS-PAGE gels are shown in Fig. S6.

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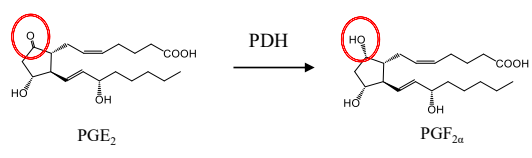
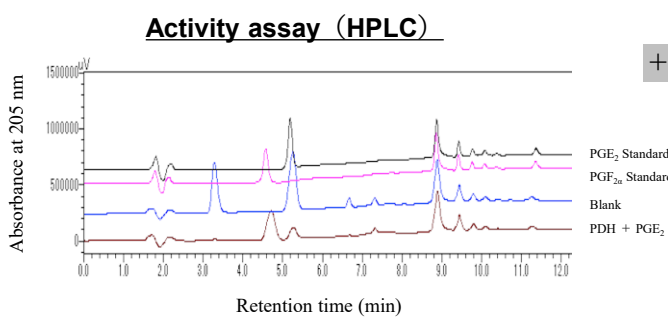
Fig. S3 HPLC chromatograms of enzymatic reaction of PDH. The chromatograms showed PGE₂ standard (a), PGF_{2α} standard (b), reaction solution without PDH (c), and reaction solution containing PDH (d). The reaction solution consisted of 20 mM KPB (pH 8), 5 mM PGE₂, 2 mM NADPH, 100 mM glucose, and 15 U/mL GDH, and the reaction was performed at 28 °C for 2 h with shaking at 200 rpm.



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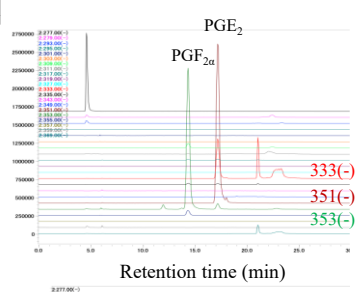
Fig. S4 The degradability of PGE₂. Blue circles and orange triangles represent KPB (pH 8) and Tris-HCl buffer (pH 9.5), respectively. Each value represents the mean $\pm$ standard deviation of three independent experiments.

69 a) PGE₂

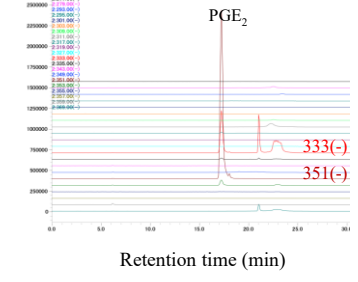


+ PDH

Result of LC/MS



- PDH



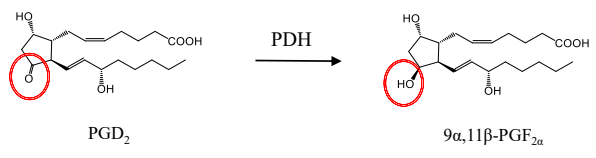
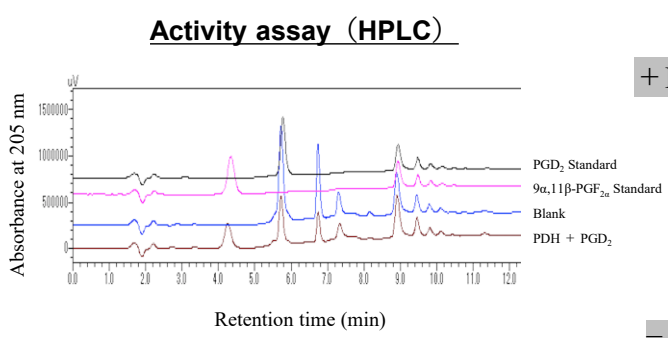
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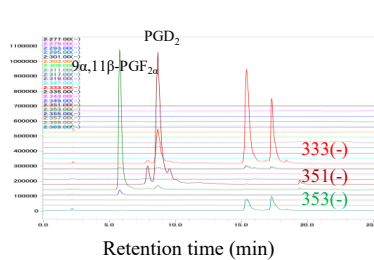
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74 b) PGD₂

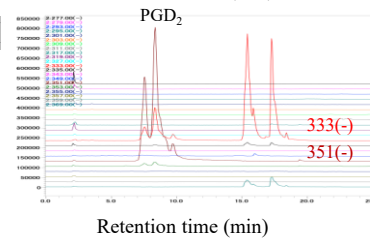


+ PDH

Result of LC/MS



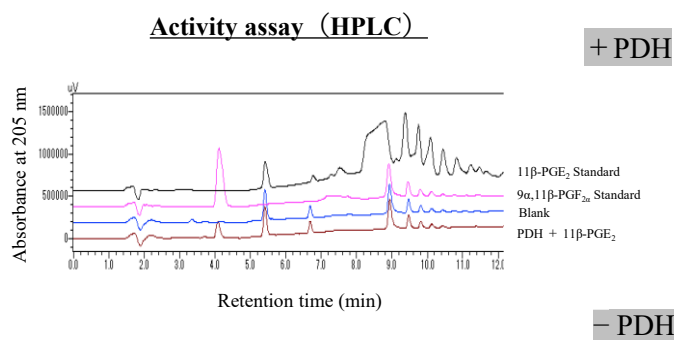
- PDH



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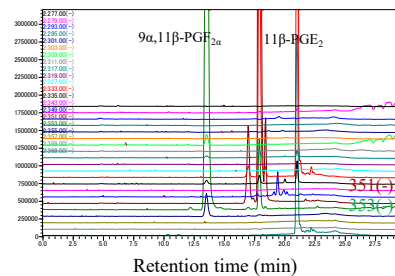
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77 c) 11 β -PGE₂

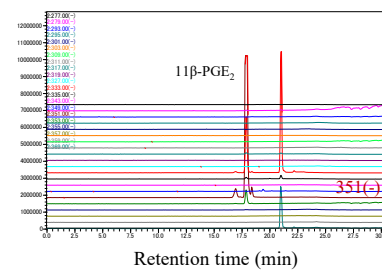
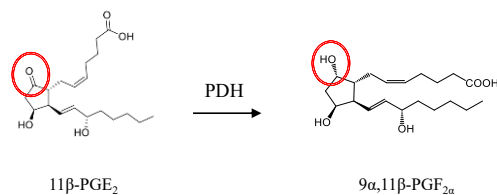


+ PDH

Result of LC/MS



- PDH

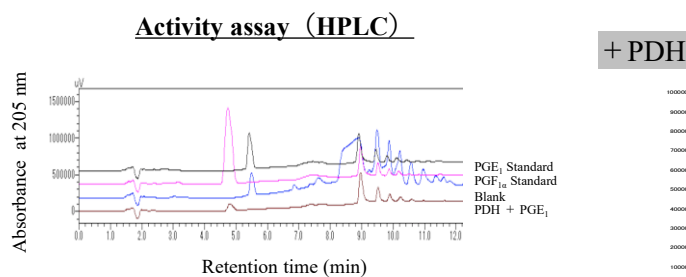


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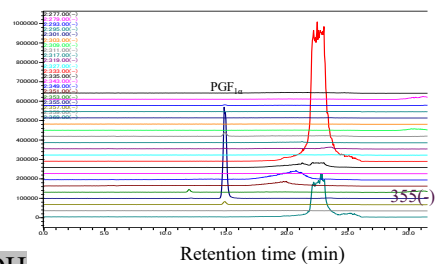
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81 d) PGE₁

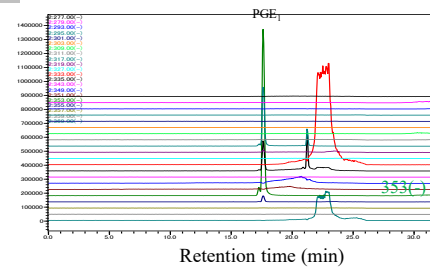
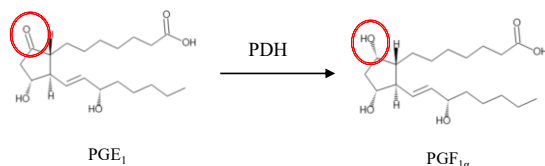


+ PDH

Result of LC/MS



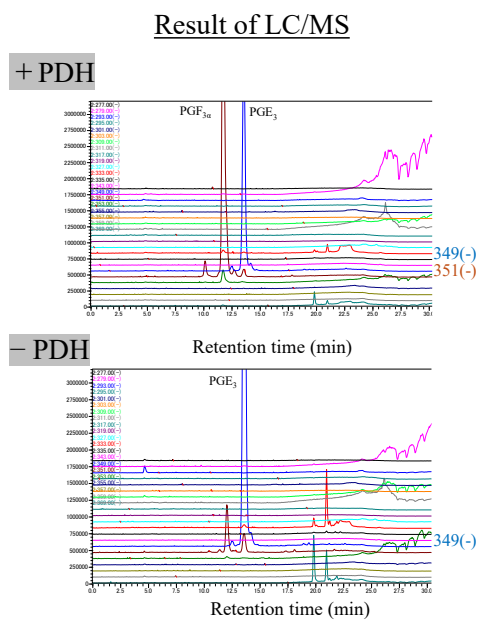
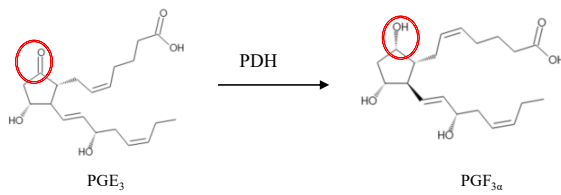
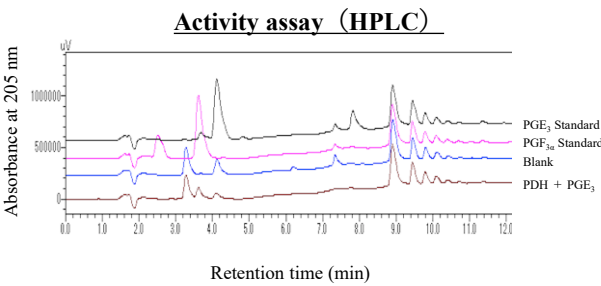
- PDH



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84 e) PGE₃

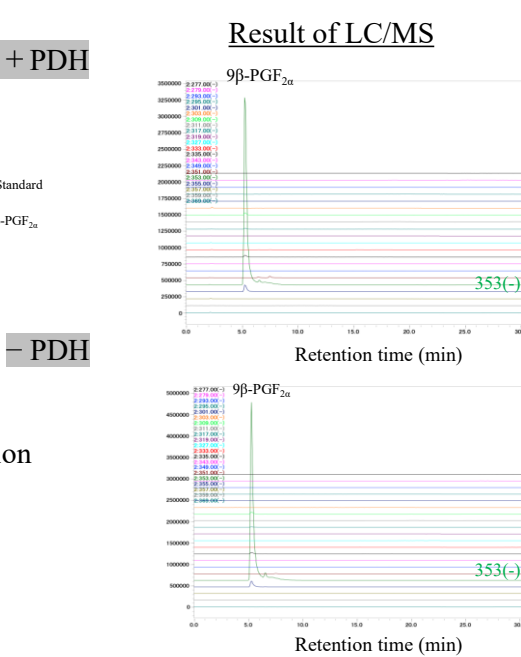
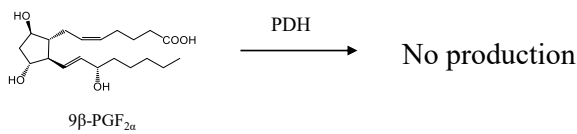
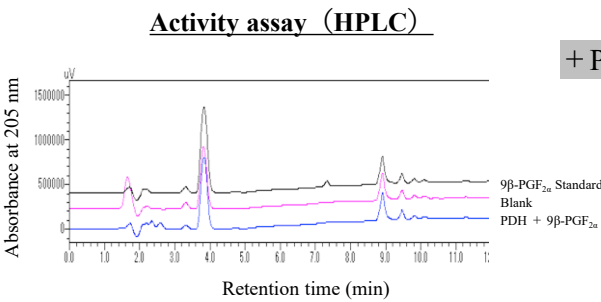


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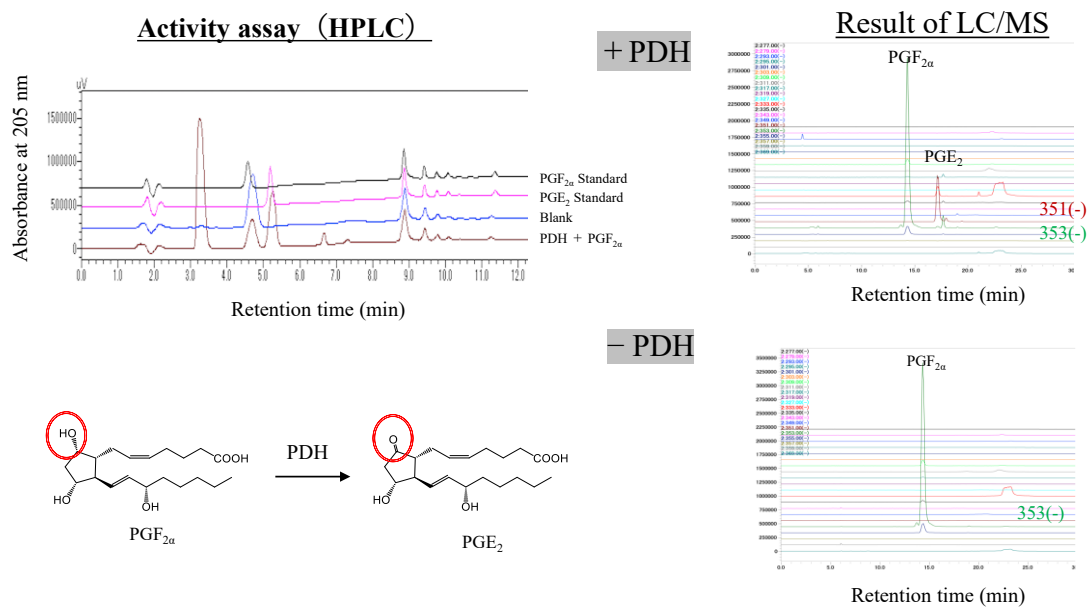
88 f) PGF_{2β}



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91 g) PGF_{2α}

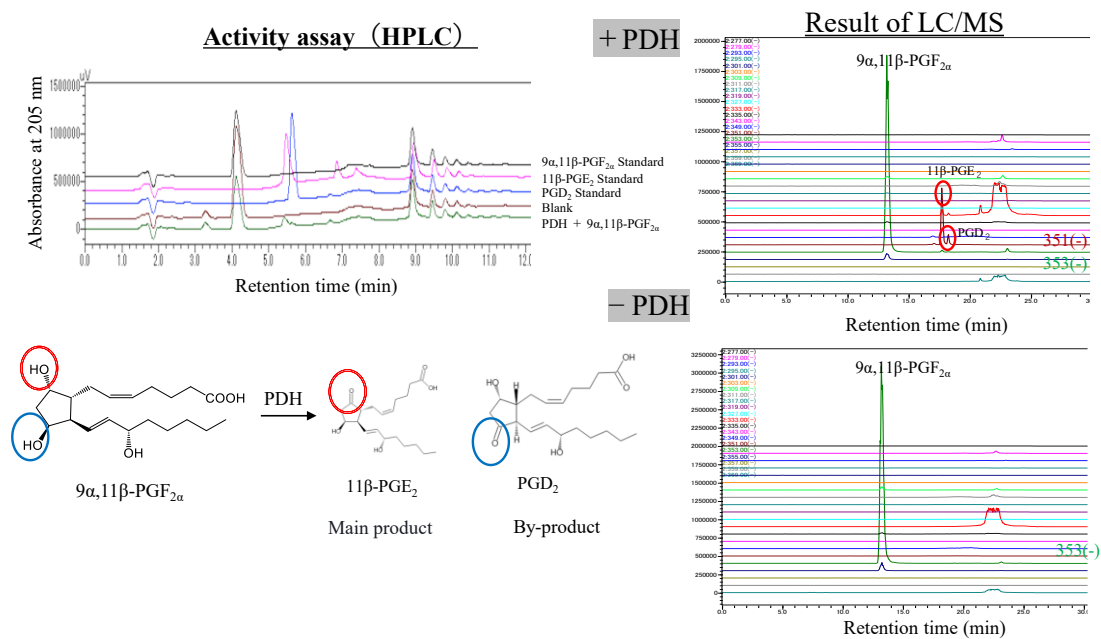


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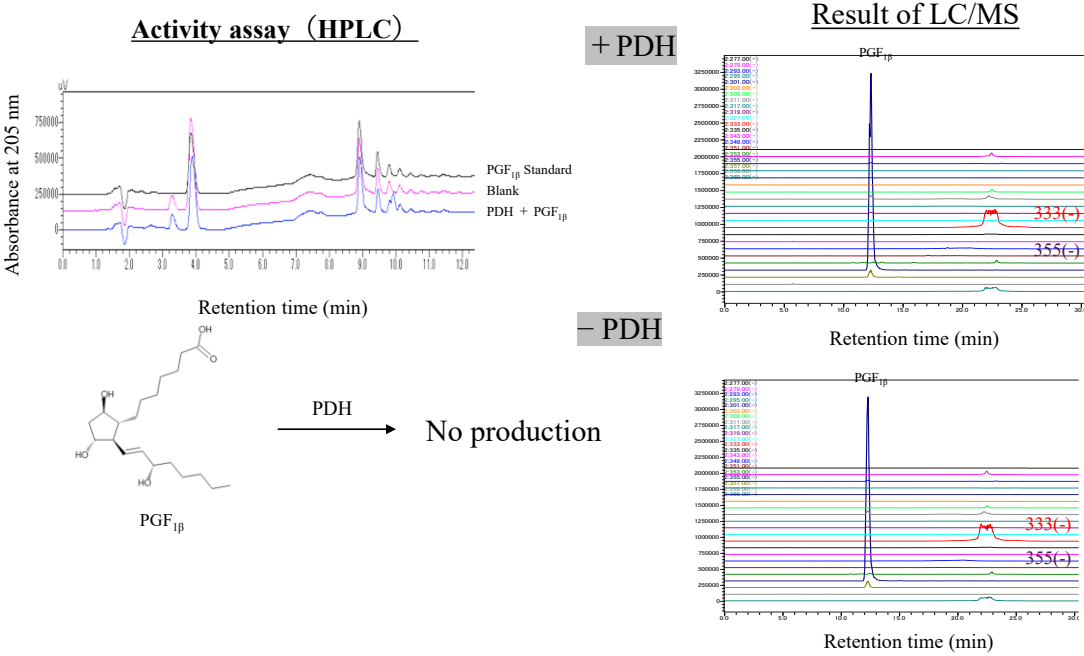
95 h) 9α,11β-PGF_{2α}



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98 i) PGF_{1β}

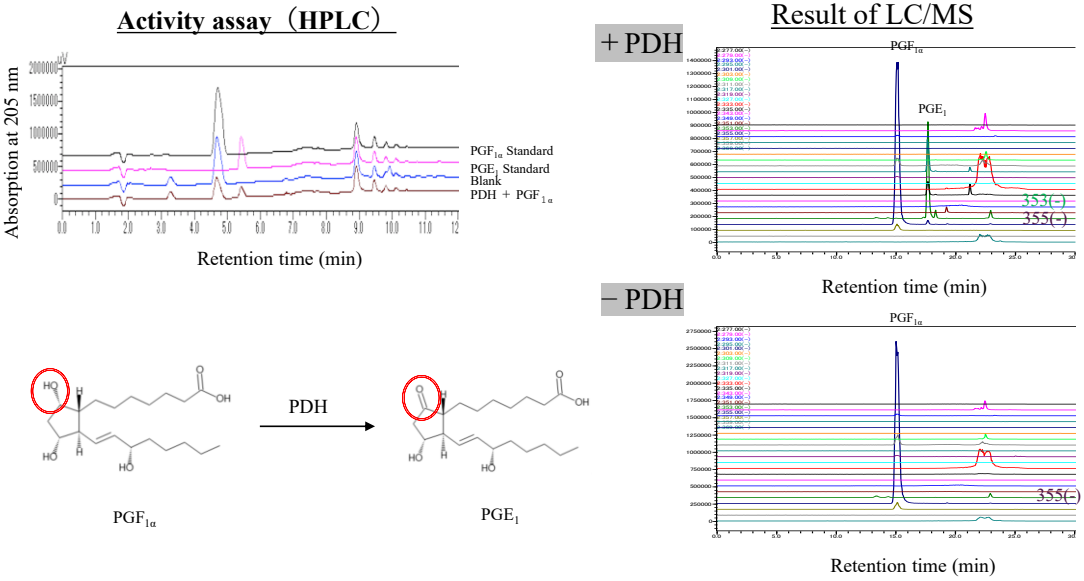


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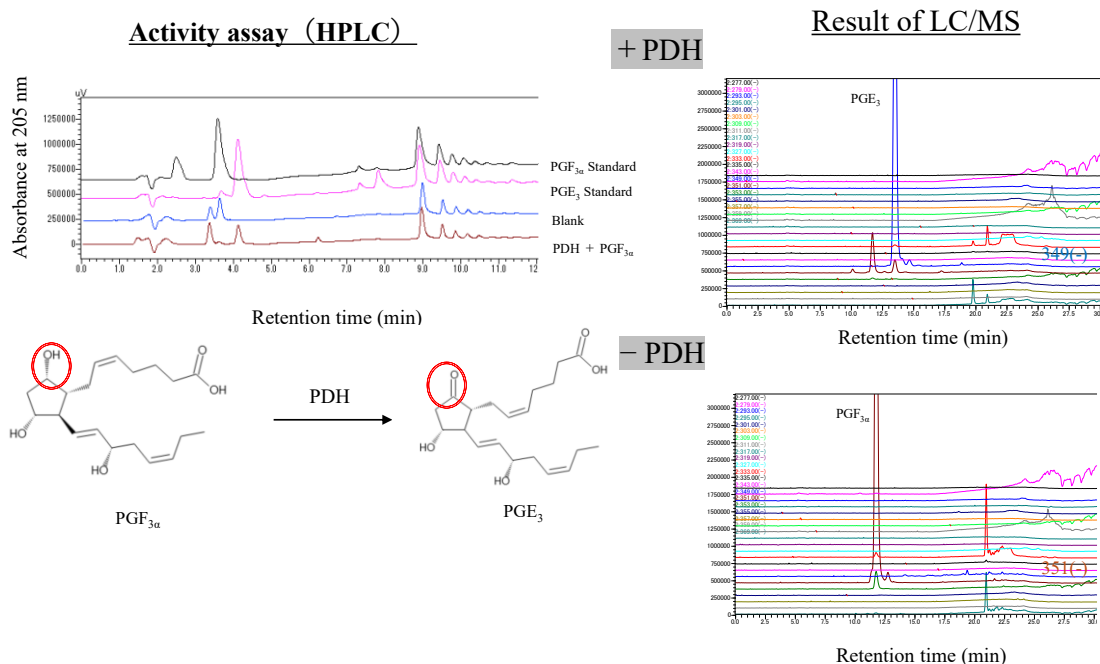
102 j) PGF_{1α}



103

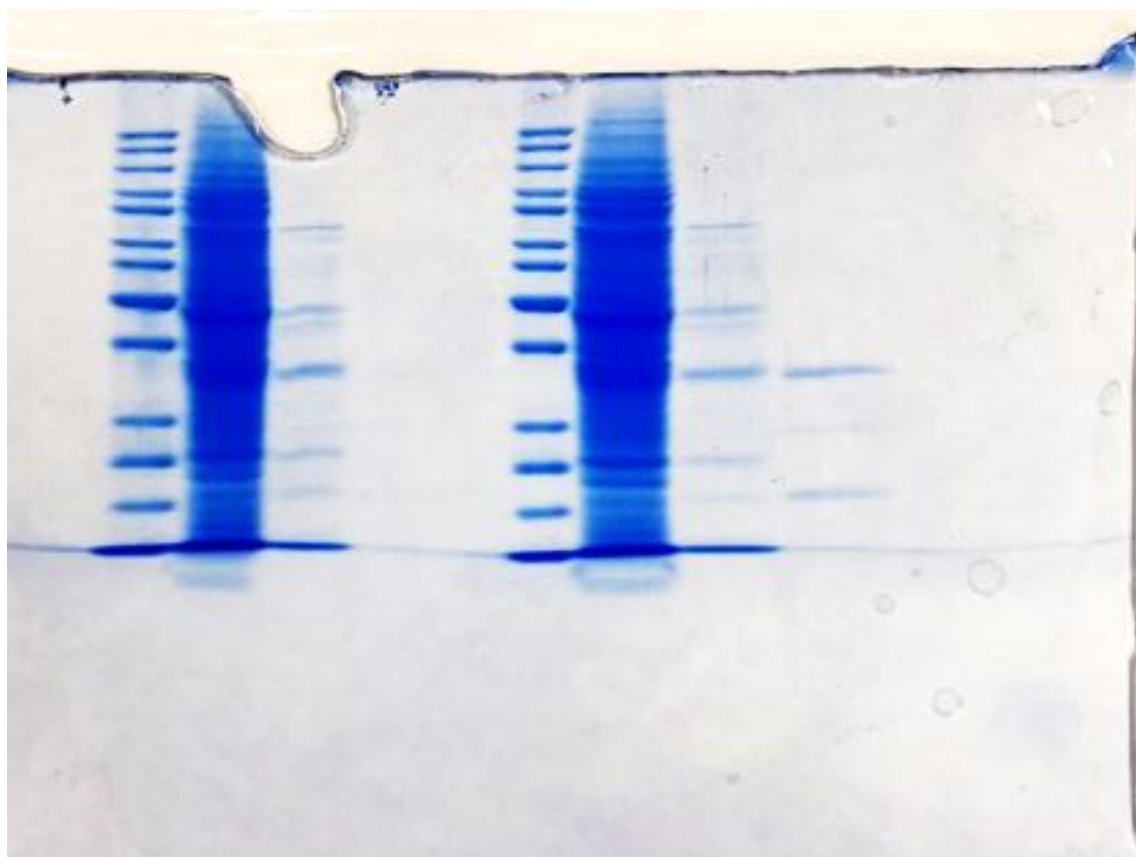
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The substrate specificity of PDH was evaluated using enzyme reaction solutions. The reaction solution consisted of 7.2 μM (186 mg/L; 26 U/L) PDH, 20 mM KPB (pH 8.0), 5 mM substrate, 100 mM glucose, 15 U/mL GDH, and 2 mM NADPH in the reduction reaction. The oxidation reaction consisted of 4.8 μM (= 124 mg/L (= 17 U/L) PDH, 20 mM KPB (pH 8.0), 5 mM substrate, and 5 mM NADP^+ .



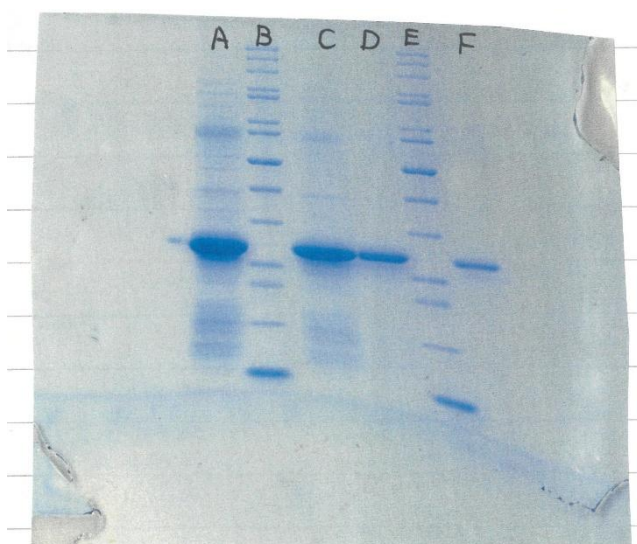
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118 a)



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120 b)



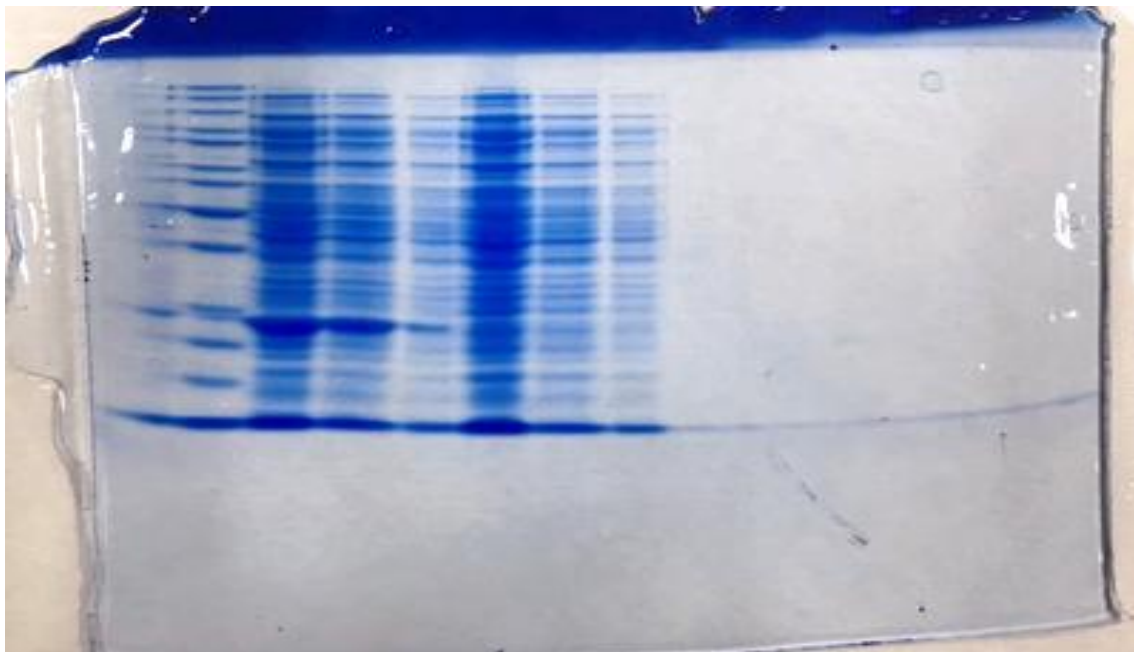
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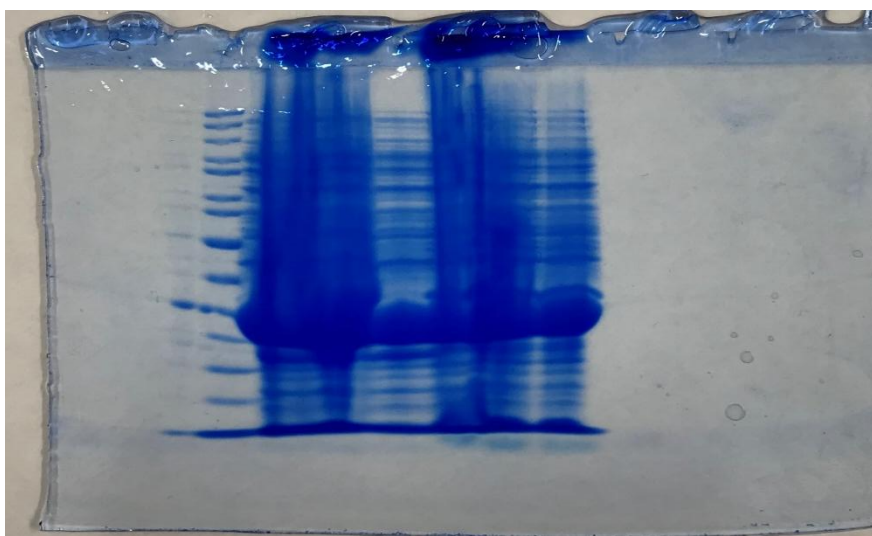
125 c)



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127

128 d)



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130 **Fig. S6** The uncropped SDS-PAGE gels of the figures. a) Fig.2a), b) Fig. 2b), c) Fig. S2 PDH, d) Fig.
131 S2 MDH

132

PDH (LC877254)	1	----	MAPFTYLLITGATRSLLG	YTRALLATKPDVWVACARNPSSASDLQALAEESN-K	55
Uncharacterized protein RHOBADRAFT_43041 (<i>Rhodotorula graminis</i> WP1) (XP_018271667)	1	----	MSTFTYLLITGASRSLLG	YARMLLAARPEARVVAARNPSAATDLQALANEDAN-K	55
Hypothetical protein JCM3775_005796 (<i>Rhodotorula graminis</i>) (GAA5922623)	1	----	MSTFTYLLITGASRSLLG	YTRALLAARPEARVVAARNPSAATDLQALANEDAN-K	55
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21589)	1	----	MSTFTYLLITGASRSLLG	YTRALLAARPEARVVAARNPSSAPDLQALAKEDAN-K	55
Uncharacterized protein RHOBADRAFT_43042 (<i>Rhodotorula graminis</i> WP1) (XP_018271668)	1	----	MPFTYLLITGASRSLLG	YAKPDLASSPDVWVVAARNPSSAEGDLQALANDAN-K	54
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21588)	1	----	MSTFTYLLITGASRSLLG	YSKALLASDPRIWVAARNPSSASDLQALANEAN-K	55
Dehydrogenase/reductase SDR family member 1 (<i>Homo sapiens</i>) (Q96LJ7)	1	MAAPMNGQVCVVTCASRGIRGIA--	DLCKAGATVVTGRH---	LDTLRVVQAQSLG	55
Carbonyl reductase [NADPH] 1 (<i>Homo sapiens</i>) (P16152)	1	--MSSGIHVALITGNGKIG	HAIVRDE	CLFSGDVLTARDVTRGQAAVQLQAEGLS--	56
			** *		
PDH (LC877254)	56	GRVHVLLQLVEDAEQVRAAGELEKSGFLGEAGAL	DAIVN---	AGVA--LAHEVQPSQIT	111
Uncharacterized protein RHOBADRAFT_43041 (<i>Rhodotorula graminis</i> WP1) (XP_018271667)	56	GRVHVLLQLVEDKAQIAKAAKDLEACGFLGEAGGID	ALIH---	AGVA--LNHQTKPSOLE	111
Hypothetical protein JCM3775_005796 (<i>Rhodotorula graminis</i>) (GAA5922623)	56	GRVHVLLQLVEDKAQIAKAAKDLEACGFLGEAGGID	ALIH---	AGVA--LNHQTKPSOLE	111
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21589)	56	DRVYLLQLVEDKEQVAKAAKELEASGFLQ--	NGIDALIH---	AGVA--LNHQVKPSOLE	109
Uncharacterized protein RHOBADRAFT_43042 (<i>Rhodotorula graminis</i> WP1) (XP_018271668)	55	GRVYLLQLVENKEQVANAAKELEDGSGFLGE--	SGIDALVN---	AGVA--ADAFKPSSELE	109
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21588)	56	GRVHLLQLVEDKDKVAQVKELEASGFE--	NGIDALVL---	AGVA--AESKYKPSSELE	109
Dehydrogenase/reductase SDR family member 1 (<i>Homo sapiens</i>) (Q96LJ7)	56	QCQVFVVCSSQSEVRSLEFQVDRE---	QCGRIDLVNNAYAVQTLINTRNKAFWET	111	
Carbonyl reductase [NADPH] 1 (<i>Homo sapiens</i>) (P16152)	57	PRFH--QIDIDLQSL-RALRDRFLRKEY---	GGIDLVNN--AGIA-----	FKVADPT	101
PDH (LC877254)	112	ARDV--LNLRLINV--	GVLVNVTKAFLEPR--	QKQKQIFVSSM-----	151
Uncharacterized protein RHOBADRAFT_43041 (<i>Rhodotorula graminis</i> WP1) (XP_018271667)	112	PEDV--LDNLKINV--	GVLVNVTKAFLEPIE--	QKQKQIFALSSM-----	151
Hypothetical protein JCM3775_005796 (<i>Rhodotorula graminis</i>) (GAA5922623)	112	PEDV--LDNLKINV--	GVLVNVTKAFLEPIE--	QKQKQIFALSSM-----	151
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21589)	110	PEHV--LDNLNVNV--	GVLVNVTKAFLELIG--	QKQKQIFVSSLSV-----	149
Uncharacterized protein RHOBADRAFT_43042 (<i>Rhodotorula graminis</i> WP1) (XP_018271668)	110	PEHV--LSNIGVNV--	GVLVNVTKQFVLEIK--	QKQKQIFVLSV-----	149
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21588)	110	PEHV--LNLGVNL--	GVLVNVTKAFLELVR--	QKQKQIFVTSV-----	149
Dehydrogenase/reductase SDR family member 1 (<i>Homo sapiens</i>) (Q96LJ7)	112	PSMWDDINNVGLRGHYCSVYGARLMPE--	ASQGLIVVLSIP-----		152
Carbonyl reductase [NADPH] 1 (<i>Homo sapiens</i>) (P16152)	102	RFHI--QAEVTMTNFGSTRVCTELE	ELIKPGRVNVSSISM	VRALKSCSPQLQKQF	158
PDH (LC877254)	152	-----	CGSIEVWGNTS-----	TAPSLRLKVALNMYSKKLAPLG--	189
Uncharacterized protein RHOBADRAFT_43041 (<i>Rhodotorula graminis</i> WP1) (XP_018271667)	152	-----	CASIEQWGNVNM-----	TPAPSVSRATLNMMWSKLAPLG--	189
Hypothetical protein JCM3775_005796 (<i>Rhodotorula graminis</i>) (GAA5922623)	152	-----	CASIEQWGNVNM-----	TPAPSVSRATLNMMWSKLAPLG--	189
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21589)	150	-----	CASIEQWGNVNM-----	TPAPSVSRATLNMMWSKLAPLG--	187
Uncharacterized protein RHOBADRAFT_43042 (<i>Rhodotorula graminis</i> WP1) (XP_018271668)	150	-----	CGSIEEFQKNTM-----	VTQVCISRAAVNMYTAKLASPLG--	187
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21588)	150	-----	CGSVEEFQKNTV-----	ATQVCISRAVNTMTAKLASPLG--	187
Dehydrogenase/reductase SDR family member 1 (<i>Homo sapiens</i>) (Q96LJ7)	153	-----	GSLQYMFN-----	VP--GVCKKACDCLAACVHBLR--	185
Carbonyl reductase [NADPH] 1 (<i>Homo sapiens</i>) (P16152)	159	RSETITEELVGLMNKFVEDTKKGHVQKEGWPS	SPVYTRKIGVTVLSRIHARRLSEQRRL	218	
			** *		
PDH (LC877254)	190	FTVVVFH--	PGVUKTDM--	NKNGEITVQEAADAALKNVFLKVT--	234
Uncharacterized protein RHOBADRAFT_43041 (<i>Rhodotorula graminis</i> WP1) (XP_018271667)	190	FTAIMFH--	PGVUKSDM--	NSGAGEITIEEAADFVKNVFLAAL--	234
Hypothetical protein JCM3775_005796 (<i>Rhodotorula graminis</i>) (GAA5922623)	190	FTAIMFH--	PGVUKSDM--	NSGAGEITIEEAADFVKNVFLAAL--	234
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21589)	188	YTVIMFH--	PGVUKTDM--	NQSKGITTEEAADLAVKNVFLAAL--	232
Uncharacterized protein RHOBADRAFT_43042 (<i>Rhodotorula graminis</i> WP1) (XP_018271668)	188	FTVIMFH--	PGVUSTDI--	NNKSGEITEVEENTALAKNVFQAAK--	232
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21588)	188	FTVIMFH--	PGVUSTGI--	NGHSGTLTVEEAALAKNVFQAAK--	232
Dehydrogenase/reductase SDR family member 1 (<i>Homo sapiens</i>) (Q96LJ7)	186	VSCVSLW--	PGVOTELLKEHMAKEEVLQDPVLKQFKSAFSS	SETTELSELGCKCVVALATDP	243
Carbonyl reductase [NADPH] 1 (<i>Homo sapiens</i>) (P16152)	219	DKILLNACCGVWRTDM--	APPKATKSPEEGETPPVYLALLPEDAEG		263
PDH (LC877254)	235	AFLRYSGETMP-----			245
Uncharacterized protein RHOBADRAFT_43041 (<i>Rhodotorula graminis</i> WP1) (XP_018271667)	235	AFLRYSGEKMP-----			245
Hypothetical protein JCM3775_005796 (<i>Rhodotorula graminis</i>) (GAA5922623)	235	AFLRYSGEKMP-----			245
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21589)	233	AFLRYSGEKMP-----			243
Uncharacterized protein RHOBADRAFT_43042 (<i>Rhodotorula graminis</i> WP1) (XP_018271668)	233	SFLRYTGEKMP-----			243
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21588)	233	SFLRYTGEKMP-----			243
Dehydrogenase/reductase SDR family member 1 (<i>Homo sapiens</i>) (Q96LJ7)	244	NILSLSGKVLPSCOLARRYGLRDVDGRPVQDYL	SLSSVLSHVSGLWLASVLPFLRVPK	303	
Carbonyl reductase [NADPH] 1 (<i>Homo sapiens</i>) (P16152)	264	PHGQFVSEKRV-----			276
PDH (LC877254)	246	-----			246
Uncharacterized protein RHOBADRAFT_43041 (<i>Rhodotorula graminis</i> WP1) (XP_018271667)	246	-----			246
Hypothetical protein JCM3775_005796 (<i>Rhodotorula graminis</i>) (GAA5922623)	246	-----			246
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21589)	244	-----			244
Uncharacterized protein RHOBADRAFT_43042 (<i>Rhodotorula graminis</i> WP1) (XP_018271668)	244	-----			244
Dehydrogenase (<i>Rhodotorula diobovata</i>) (TNY21588)	244	-----			244
Dehydrogenase/reductase SDR family member 1 (<i>Homo sapiens</i>) (Q96LJ7)	304	FIALYTSKF-----			313
Carbonyl reductase [NADPH] 1 (<i>Homo sapiens</i>) (P16152)	277	-----			277

b)

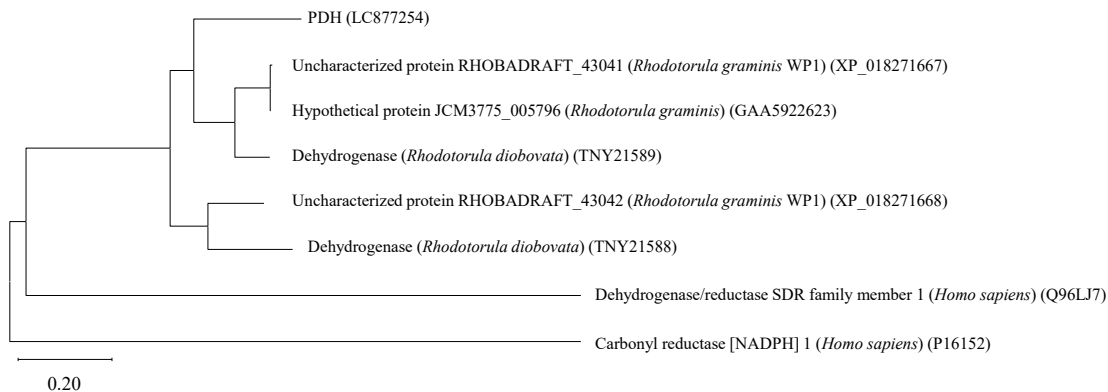


Fig. S7 Sequence analysis of PDH. a) Sequence alignment of PDH, selected sequences of similar proteins, human carbonyl reductase 1, and human dehydrogenase/reductase SDR family member 1. typical cofactor-binding motif TGXXX[AG]XG and an active-site motif YXXXXK are shown by asterisks. Multiple sequence alignment was performed using the MUSCLE algorithm implemented in MEGA. The phosphate group of NADPH was predicted to bind to T12, R13, and R37 of PDH by AlphaFold 3 (Abramson 2024). b) Phylogenetic tree analysis by the maximum likelihood method.

Reference

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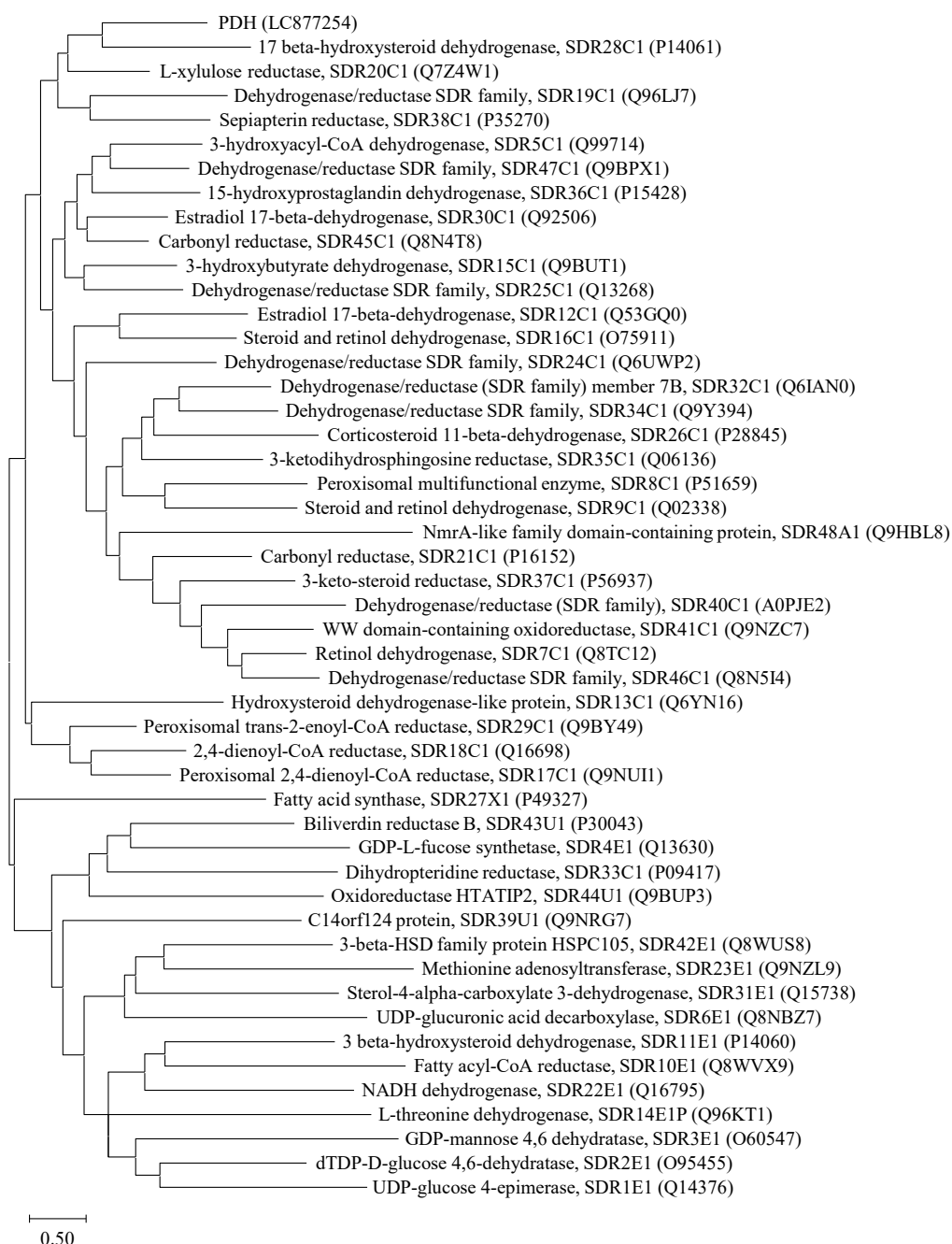


Fig. S8 Phylogenetic tree analysis of PDH and human 48 SDRs. PDH was compared with 48 kinds of SDR (Persson 2009). Multiple sequence alignment was performed using the MUSCLE algorithm implemented in MEGA. Phylogenetic analysis was performed using the maximum likelihood method.