Functional Characterization of UDP-D-glucur Acid 4-Epimerase Family from *Ornithogalum caudatum*

Sen Yin, Yu-Jia Sun, Ming Liu, Li-Na Li and Jian-Qiang Kong

Strain/Plasmid	Description	Source
Strain		
Trans1-T1	Escherichia coli, F-φ80(lacZ)∆M15∆lacX74hsdR(rk⁻,mk+)∆recA13 98endA1tonA	TransGen, Beijing, China
<i>Trans</i> etta (DE3)	E. coli, F [.] ompThsdSb(rb [.] mb [.])gal dcm lacY1(DE3)pRARE(argU, argW,ilex,glyT,leuW,proL))Cam ^r)	TransGen, Beijing, China
GS115	Pichia pastoris, His ⁻ , Mut ⁺	Invitrogen, Carlsbad, CA, USA
Plasmid		
pEASY TM -Blunt	General cloning vector, T7 promoter, f1 ori, Amp ^r and Kan ^r	TransGen, Beijing, China
pET-28a(+)	General expression vector, T7 promoter, f1 ori, Kan ^r	Novagen, Madison, USA
pPIC3.5K	Pichia pastoris expression vector, AOX1 promoter	Invitrogen, Carlsbad, CA, USA
pGro7	Chaperone plasmid, araB promoter, Cm ^r	
pEASY-OcUGlcAE1	<i>pEASY</i> ™-Blunt derived plasmid containing <i>OcUGlcAE1</i> gene	This study
pEASY-OcUGlcAE2	<i>pEASY</i> ™-Blunt derived plasmid containing <i>OcUGlcAE2</i> gene	This study
pEASY-OcUGlcAE3	<i>pEASY</i> ™-Blunt derived plasmid containing <i>OcUGlcAE</i> 3gene	This study
pET28aOcUGlcAE1(△1-116)	pET-28a(+) derived plasmid containing trun- <i>OcUGlcAE1</i> gene	This study
pET28aOcUGlcAE2(△1-136)	pET-28a(+) derived plasmid containing trun- <i>OcUGlcAE</i> 2 gene	This study
pET28aOcUGlcAE3(△1-128)	pET-28a(+) derived plasmid containing trun- <i>OcUGlcAE3</i> gene	This study
pPIC3.5KOcUGlcAE1	pPIC3.5K derived plasmid containing OcUGlcAE1 gene	This study
pPIC3.5KOcUGlcAE2	pPIC3.5K derived plasmid containing OcUGlcAE2 gene	This study
pPIC3.5KOcUGlcAE3	pPIC3.5K derived plasmid containing OcUGlcAE3 gene	This study

Table S1. Strains and	plasmids	used in	this study.
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e Gierre i ossible	TIVI HEIIX (da~ad)
AtUGlcAE1	31~50
AtUGlcAE2	37~59
AtUGlcAE3 34-	~56, 85~107
ZmUGlcAE3	31~53
OsUGlcAE1 49~	-66,116~138
OsUGlcAE2	NO
OsUGlcAE3	31~53
OcUGlcAE3 36~	58, 110~132

Table S2. The putative TM helix of OcUGlcAE3 and other UGlcAEs from varied organisms.

Table S3. Primers used in this research.	
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Primers	Sequences(5'-3')	Description
ECIALE1 1		Forward primer used for OcUGlcAE1
FGICAE1-1	AGAGGGAAAAAGAAAGAIGAAG	amplification in the first round
PCIANE1 1		Reverse primer used for OcUGlcAE1
KGICAEI-I	CICCIACCAAIAACAAAAAICG	amplification in the first round
ECIANE1 2		Forward primer used for OcUGlcAE1
FGICAEI-2	AIGAGGAIACIGGAGGAGGAGG	amplification in the second round
RClcAF1-2	ΤΤΑΓΑΔΑΤΤΓΔΑΓΟΟΟΤΟΤΟ	Reverse primer used for OcUGlcAE1
KORALI-2		amplification in the second round
FClcAF2-1	<u>ΟΤΤΟΓΑ ΑΤΟ Α ΑΤΟ ΑΤΟ Α Α Ο ΑΤ</u>	Forward primer used for OcUGlcAE2
I GICAL2-1	endermentermentermenter	amplification in the first round
RGlcAF2-1	TTCCCCCCCCACCACACC	Reverse primer used for OcUGlcAE2
	Treddeederkerkinde	amplification in the first round
FGlcAF2-2	ATCCCCCCTCCATCCTCCTC	Forward primer used for OcUGlcAE2
	mocedereemedreere	amplification in the second round
RGlcAE2-2	CTACTCTTTGTGCCCTCCTC	Reverse primer used for OcUGlcAE2
		amplification in the second round
FGlcAF3-1	CTCTATCTCTCTCTCTCTCT	Forward primer used for OcUGlcAE3
TGICAE5-1	crementererererer	amplification in the first round
RCIcAE3.1	GCAGCTCCCCGTTCTTCC	Reverse primer used for OcUGlcAE3
		amplification in the first round
FClcAF3-2	ATGGACGCGATGATCTCGCC	Forward primer used for OcUGlcAE3
I GIGIELO-2		amplification in the second round
RClcAF3-2	TCAGCTCGAGTTACTCCTCG	Reverse primer used for OcUGlcAE3
	Tenderedharmereered	amplification in the second round
F28aClcAF1	GGTCGCGGATCCGAATTCATGGCCCTC	Forward primer used for pET28aOcUGlcAE1
12000107121	AAGAAGCGCGGCGA	$(\Delta 1-116)$ construction
R28aClcAF1	GAGTGCGGCCGCAAGCTTCAAATTCTT	Reverse primer used for pET28aOcUGlcAE1
	GCCCCCTCTCG	$(\Delta 1-116)$ construction
F28aClcAF2	GGTCGCGGATCCGAATTCATGAAGAAG	Forward primer used for pET28aOcUGlcAE2
1 200 GIGI YEZ	CGAGGGGACGGTGT	$(\Delta 1-136)$ construction
R28aClcAF2	GAGTGCGGCCGCAAGCTTCTCTTTGTGC	Reverse primer used for pET28aOcUGlcAE2
	CCTCCTCCTC	$(\Delta 1-136)$ construction
F28aGlcAF3	GGTCGCGGATCCGAATTCATGTCGGCC	Forward primer used for pET28aOcUGlcAE3
12000101110	GCCCTCAAGCGACG	$(\Delta 1-128)$ construction
R28aClcAF3	GAGTGCGGCCGCAAGCTTGCTCGAGTT	Reverse primer used for pET28aOcUGlcAE3
	ACTCCTCGCAC	$(\Delta 1-128)$ construction
F3 5kGlcAF1	ACTAATTATTCGAAGGATCCGCCACCA	Forward primer used for pPIC3.5KOcUGlcAE1
F3.5KGICAE1	TGAGGATACTGGAGGAGGA	construction
R3.5kGlcAF1	GCGCGGCCGCCCTAGGGAATTCTTACA	Reverse primer used for
NJ.JKGICAEI	AATTCTTGCCCCCTC	pPIC3.5KOcUGlcAE1construction
F3 5kGlcAF2	ACTAATTATTCGAAGGATCCGCCACCA	Forward primer used for pPIC3.5KOcUGlcAE2
10.0KGICAEZ	TGCCGGCTCCATCGTCGTC	construction
R3 5kClc AF2	GCGCGGCCGCCCTAGGGAATTCCTACT	Reverse primer used for pPIC3.5KOcUGlcAE2
NJ.JKGICAEZ	CTTTGTGCCCTCCTC	construction

d for	pPIC3	.5KO	cUGI	lcA

F2 5kCleAF2	ACTAATTATTCGAAGGATCCGCCACCA	Forward primer used for pPIC3.5KOcUGlcAE3
F5.5KGICAE5	TGGACGCGATGATCTCGCC	construction
R3.5kGlcAE3	GCGCGGCCGCCCTAGGGAATTCTCAGC	Reverse primer used for pPIC3.5KOcUGlcAE3
	TCGAGTTACTCCTCG	construction
5'AOX1	GACTGGTTCCAATTGACAAGC	Forward primer used for PCR identification of
		pPIC3.5k derived plasmids
214 02/1		Reverse primer used for PCR identification of
3'AOXI	GCAAAIGGCAIICIGACAICC	pPIC3.5k derived plasmids
FO LICE AF1		Forward primer used for RT-qPCR analysis of
FOcUGLcAE1	CITCAACCIIGGCAACACCI	OcUGlcAE1
ROcUGLcAE1	GGTCCTGGCGTAACTGATGT	Reverse primer used for RT-qPCR analysis of
		OcUGlcAE1
FOcUGLcAE2	TCCTCTCCTCCTCCTTCCTC	Forward primer used for RT-qPCR analysis of
		OcUGlcAE2
		Reverse primer used for RT-qPCR analysis of
KUCUGLCAEZ	GCCIGICCCICICAGIGAG	OcUGlcAE2
FO-LICL - A F2		Forward primer used for RT-qPCR analysis of
FOCUGLCAE3	ACATIGCIGGCCIIGITACC	OcUGlcAE3
DO-LICL - AE2	CCGTACACCGTGAAGAACCT Reverse primer used for RT-qPCR analy OcUGlcAE3	Reverse primer used for RT-qPCR analysis of
ROCUGLCAE3		OcUGlcAE3
FGAPDH2	ACTTGGTGTCCACCGACTTC	Forward primer used for RT-qPCR analysis of
		GAPDH1
		Reverse primer used for RT-qPCR analysis of
KGAPDH2		GAPDH1



Figure S1. The predicted transmembrane helices of OcUGlcAE1 (A); OcUGlcAE2 (B) and OcUGlcAE3 (C).



Figure S2. SDS-PAGE analyses of total soluble proteins isolated from *E. coli* cells expressing OcUGlcAE1 (Δ 1–116) (**A**); OcUGlcAE2 (Δ 1–136) (**B**) or OcUGlcAE3 (Δ 1–128) (**C**). Lanes 1 and 2 referred to the total extract of cells harboring the empty vector and the recombinant vector, respectively. The arrows indicated the overproduced proteins



Figure S3. HPLC profiles of reaction mixtures generated by *E. coli* expressing the empty vector alone (1), pET28aOcGlcAE1 (Δ 1–116) (2), pET28aOcGlcAE2 (Δ 1–136) (3) or pET28aOcGlcAE3 (Δ 1–128) (4) in the presence of UDP-GlcA and NAD⁺.



Figure S4. HPLC profiles of OcUGlcAE3-catalyzed reaction mixtures co-injected with (**2**) or without UDP-GalA (**1**). The red arrows stand for UDP-GalA.



Figure S5. HPLC profiles of OcUGlcAE3-catalyzed reaction mixtures co-injected with (1) or without UDP-GlcA (2).

AtUGICAE1	1	MSRLDDIPSSPGKFKMEKSSYLHRLRFQSSLTKF 34	4
AtUGICAE2	1	MPLSATADTSKTVK LERYNSYLRKTHSTKVLNASSK 3	6
AtUGIcAE3	1	MPSIEDELFPSTPGKFK	7
ZmUGIcAE3	1		0
OsUGIcAE1	1	- MAPOLTGA PGTAGAAGGAASVKPQFHHFHHHRLATRHHHPSPTSLLSKL 4	9
OsUGIcAE2	1	- MMPHSGWVDAAAKGWKLGGGGGG ALMVRRVASGKLUSASSHLLFRAT 4	7
OsUGIcAE3	1		Ō
OcUGICAE3	1	MDAMIS, PSTPGKEK, PEKPHHHLHHHHHBPLEBLOHSPLEKC 4	ĩ
ocourierieu			1
AtUGICAE1	35	ALFSFFLUCTISTLFLRSP PSINPSSPSDP	2
AtUGICAE2	37	VEFRATELVALVEVETFATNYPPESDSRAAAAHHEHRRSFESTGEFS 8	13
AtUGICAE3	28	CEASTSTMFLWALFLIALTASYLSFOSFVOSGSRYLTAS 6	i6
ZmUGIcAE3	31	CFASTSTMFLWALFLVAMTASYLSFQSFVDTSSKYFAAS6	<u>;9</u>
OsUGICAE1	50	A EWSVCSLSLLLAELLLSPSAAPAPRAAPDSPRRSLHTSSPSA 9	12
OsUGIcAE2	48	ILATL <u>CLVCLFTVHYPSLLSHSFHLSSAAAAAANGKHRAASRS</u> SH <mark>R</mark> SLLGS 9	37
OsUGICAE3	31	CFASTSTMFLWALFLVAMTASYLSFQSFVDTSSKYFAAS 6	<u>;9</u>
OcUGICAE3	42	GLIVVCASVVVLLLCLAPSHPSSSSSSSSSSPFAASRIERRSLSASS 8	38
		Gx×G××G	
AtUGICAE1	72	11 TYGGPAWEKRLRSSARIRTSTNNGITVLVTGAAGEVGTHV	12
AtUGICAE2	84	SSSSSS <mark>SSIGGAAWEKRVR</mark> QSSTAKRPHGLSVLVTGAAGEVGSHC 12	27
AtUGICAE3	66)3
ZmUGIcAE3	69	WGGLHWERQIRASAVPRRPPGSAAGAGMSVLVTGAAGFVGTHC 11	12
OsUGIcAE1	92	AATWG GAAWEKKV RASARVRRANG RGL TVLVT GAAG FVGCHA 13	34
OsUGIcAE2	98	S - A AVAYGGA AWEKEVRRSA APRRDG GLSVLVTGA A GEVGAHC 13	39
OsUGIcAE3	69	WGGLHWERQIRASAAPRRPPGSAAGAGMSVLVTGAAGFVGTHC 11	12
OcUGIcAE3	88	SSSWGGAEWEKRVRYSARIRRVGGFSVLVTGAGGFVGTHV 12	28

Figure S6. N-terminal alignment of OcUGlcAE3 and other UGlcAEs from varied organisms. The red box represents the G××G××G motif.