

# Molecular evidence of sorbitol dehydrogenase in tomato, a non-Rosaceae plant

Kazuhiro Ohta <sup>a</sup>, Ryo Moriguchi <sup>a</sup>, Koki Kanahama <sup>a</sup>, Shohei Yamaki <sup>b</sup>,  
Yoshinori Kanayama <sup>a,\*</sup>

<sup>a</sup> Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

<sup>b</sup> Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

Received 9 August 2005; received in revised form 25 September 2005

Available online 9 November 2005

## Abstract

The enzyme NAD-dependent sorbitol dehydrogenase (SDH) is well characterized in the Rosaceae family of fruit trees, which synthesizes sorbitol as a translocatable photosynthate. Expressed sequence tags of *SDH*-like sequences have also been generated from various non-Rosaceae species that do not synthesize sorbitol as a primary photosynthetic product, but the physiological roles of the encoded proteins in non-Rosaceae plants are unknown. Therefore, we isolated an *SDH*-like cDNA (SDL) from tomato (*Lycopersicon esculentum* Mill.). Genomic Southern blot analysis suggested that *SDL* exists in the tomato genome as a single-copy gene. Northern blot analysis showed that *SDL* is ubiquitously expressed in tomato plants.

Recombinant *SDL* protein was produced and purified for enzymatic characterization. *SDL* catalyzed the interconversion of sorbitol and fructose with NAD (H). *SDL* showed highest activity for sorbitol among the several substrates tested. *SDL* showed no activity with NADP<sup>+</sup>. Thus, *SDL* was identified as a *SDH*, although the *K<sub>m</sub>* values and substrate specificity of *SDL* were significantly different from those of *SDH* purified from the Japanese pear (*Pyrus pyrifolia*), a Rosaceae fruit tree. In addition, tomato was transformed with antisense *SDL* to evaluate the contribution of *SDL* to *SDH* activity in tomato. The transformation decreased *SDH* activity to approximately 50% on average. Taken together, these results provide molecular evidence of *SDH* in tomato, and *SDL* was renamed *LeSDH*.

© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** *Lycopersicon esculentum* Mill.; Solanaceae; Tomato; cDNA cloning; Recombinant protein expression; Antisense transformation; Sorbitol dehydrogenase; Carbohydrate; Sorbitol; Fructose

## 1. Introduction

Sorbitol is important in translocating photosynthate in fruit trees of the Rosaceae family (Kanayama et al., 1992; Kanayama, 1998; Sakanishi et al., 1998). NAD-dependent sorbitol dehydrogenase (SDH) catalyzes the oxidation of sorbitol to fructose. SDH has been purified from Japanese pear fruit (Oura et al., 2000). The expression analysis of *SDH* cloned from apple cDNA demonstrated the importance of *SDH* in the metabolism of sorbitol that is

translocated to fruit (Yamada et al., 1998, 1999; Park et al., 2002).

SDH has also been found in plants that are not in the Rosaceae and that synthesize sucrose for translocation of photosynthate. SDH activity was detected in a crude extract from germinating soybean seeds (Kuo et al., 1990), and SDH was partially purified from developing maize endosperm (Doehlert, 1987) and from the shoot axes of *Viscum album*, a parasitic plant (Wanek and Richter, 1993).

Recently, plant genome mapping projects have revealed that *SDH*-like sequences are widespread in the plant kingdom, and are present in the expressed sequence tag (EST) databases of several plant species (Fig. 1). Nevertheless,

\* Corresponding author. Tel.: +81 22 7178642; fax: +81 22 7178878.  
E-mail address: [kanayama@bios.tohoku.ac.jp](mailto:kanayama@bios.tohoku.ac.jp) (Y. Kanayama).

		10	20	30	40	50	
SDL	1	MGKGG	---	SD--	ENMA	AWLLGVNTLK	IQPNLPALG
Malus	1	MGKGGSD	HADRCCEAT	NDVQQ	ENMA	AWLLGVNTLK	IQPKLPNLG
Arabidopsis	1	MGKGGMS	GS	KVEE	ENMA	AWLVGINTLK	IQPFLPSVG
Zea	1	MGKAQGSDA	AA	AGSEVE	ENMA	AWLVAKNTLK	IMPFKLPVVG
Citrus	1	---	---	---	---	---	---
Medicago	1	---	---	---	---	---	---
Gossypium	1	---	---	---	---	---	---
Pinus	1	---	---	S--	DNMA	AWLTGINTLR	IQPFKLPPLG
		60	* 70	80	* 100		
SDL	51	PHDVRVRMKA	VGICGSDVHY	LKTMRCADFV	VKEPMVIGHE	CAGIIEEVGS	100
Malus	51	PHDVRVRLRA	VGICGSDVHI	FKNMRCVDFI	VKEPMVIGHE	CAGIIEEVGS	100
Arabidopsis	51	PHDVRVRMKA	VGICGSDVHY	LKTMRCADFV	VKEPMVIGHE	CAGIIEEVGS	100
Zea	51	PHDVRVRMKA	VGICGSDVHY	LEMTLAFV	VKEPMVIGHE	CAGIIEEVGS	100
Citrus	51	---	VGICGSDVHY	LKTMRCADFV	VKEPMVIGHE	CAGIIEEVGS	100
Medicago	51	---	KA	VGICGSDVHY	LKTMRCADFV	VKEPMVIGHE	CAGIIEEVGS
Gossypium	51	---	---	---	---	---	---
Pinus	51	PHDAKVRMKA	VGICGSDVHY	LKTMRCADFV	VKEPMVIGHE	SAGIIEEVGS	100
		110	+ + + 130	+ 140	150		
SDL	101	EVKTLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
Malus	101	EVEHLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
Arabidopsis	101	EVEHLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
Zea	101	GVTHLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
Citrus	101	EVKTLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
Medicago	101	QVTHLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
Gossypium	101	EVKTLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
Pinus	101	EVEHLVPGDR	VALEPGISCW	RCNLCKEGRY	NLCPEMKFFA	TPPVHGLSLAN	150
		160	170	* 180	190	200	
SDL	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
Malus	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
Arabidopsis	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
Zea	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
Citrus	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
Medicago	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
Gossypium	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
Pinus	151	QVHPADLCF	KLDPNVSLEE	GAMCEPLSVG	VHACRRANVG	PETNVLVLMGA	200
		210	220	230	240	250	
SDL	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
Malus	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
Arabidopsis	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
Zea	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
Citrus	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
Medicago	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
Gossypium	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
Pinus	201	GPIGLVTLA	ARAFGAPRIV	IVDVDDYRLS	VAKSLGADII	VKVSTNIQDV	250
		260	270	280	290	300	
SDL	251	AEEVAKIQK	LEN-GVDVTF	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
Malus	251	AEEVAKIQK	LEN-GVDVTF	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
Arabidopsis	251	GSVEEIQKA	MGS-NIDVTF	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
Zea	251	AEVEERIRAA	MGS-DIDVSL	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
Citrus	251	AEEVEKIDKA	MGT-GIDVSL	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
Medicago	251	AEEVKQIHNV	LCA-GVDVTF	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
Gossypium	251	PEEVTRICEV	MGA-GVDVTF	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
Pinus	251	DEEVTRICQA	MGS-NIDVTF	DCAGFNKTMS	TALSAATRPGG	KVCLVGMGHI	300
		310	320	330	340	350	
SDL	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
Malus	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
Arabidopsis	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
Zea	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
Citrus	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
Medicago	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
Gossypium	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
Pinus	301	EMTVPLTPAA	AREVDVVGIF	RYKNTWPLCL	EFLRSGKIDV	KPLITHRFGF	350
		360	370	380	390	400	
SDL	351	SOKEVEEAFE	TSARGGDAIK	VMFNL	.....	.....	400
Malus	351	SOKEVEEAFE	TSARGGDAIK	VMFNL	.....	.....	400
Arabidopsis	351	SOKEVEEAFE	TSARGGDAIK	VMFNL	.....	.....	400
Zea	351	SOKEVEEAFE	TSARGGDAIK	VMFNL	.....	.....	400
Citrus	351	---	---	---	.....	.....	400
Medicago	351	---	---	---	.....	.....	400
Gossypium	351	SOKEVEEAFE	TSARGGDAIK	VMFNL	.....	.....	400
Pinus	351	---	---	---	.....	.....	400

Fig. 1. Multiple alignment of the deduced tomato SDL amino acid sequence, the apple SDH sequence, and the SDH-like sequences in EST from species of different plant families. Black shading indicates identical amino acids. Asterisks and plus signs indicate conserved amino acid residues in the catalytic zinc binding site and in the structural zinc binding site, respectively. The zinc-containing alcohol dehydrogenase signature is underlined. SDL (accession number AB183015); *Malus domestica*, apple SDH (AB016256); *Arabidopsis thaliana* (AF370161); *Zea mays* (BT016754); *Citrus paradisi* × *Poncirus trifoliata* (CX668813); *Medicago sativa* (CB894631); *Gossypium raimondii* (CO082515); *Pinus taeda* (CO361351).

little information exists about the physiological roles of the proteins encoded by these genes. The ultimate goal of this research was to understand the significance of the wide-

spread *SDH*-like genes. As a first step, we now provide molecular evidence of *SDH* genes in non-Rosaceae plants. This study is a molecular and biochemical characterization

Download English Version:

<https://daneshyari.com/en/article/5167933>

Download Persian Version:

<https://daneshyari.com/article/5167933>

[Daneshyari.com](https://daneshyari.com)