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**Original Paper**

# Glutathione-dependent formaldehyde dehydrogenase from golden pothos (*Epipremnum aureum*) and the production of formaldehyde detoxifying plants

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**Abstract** Glutathione-dependent formaldehyde dehydrogenase (FALDH) is an enzyme involved in formaldehyde metabolism in eukaryotes. FALDH cDNA was cloned from golden pothos, which is reported to effectively purify gaseous formaldehyde from enclosed room atmosphere. FALDH cDNAs from Arabidopsis, rice and golden pothos were overexpressed in transgenic Arabidopsis, and the enzyme activity was compared to determine the one most suitable for the molecular breeding of formaldehyde-detoxifying plants. The transgenic lines exhibited modified levels of the *FALDH* transcript, i.e. 10–800% compared to the endogenous transcript, due to either an overexpression or a cosuppression phenotype. The enzyme activity in the crude leaf extract was not proportionate, but did correlate with the transcription levels with certain exceptions. The FALDHs from the three plant species indicated similar enzymatic activity on average. The capacity to detoxify exogenous formaldehyde in the transformants with the FALDHs was determined at the whole plant level. Plants overexpressing FALDH from the three plant species displayed up to a 40% increase in their efficiency to take up exogenous formaldehyde as compared with the wild-type plants. On the other hand, no difference in the survival rate was observed among the transformants and wild type plants on formaldehyde-containing agar medium. These results show the FALDH from golden pothos to be similarly or more effective for detoxifying formaldehyde in transgenic plants compared with Arabidopsis and rice, and that this cDNA is applicable to the molecular breeding of formaldehyde detoxifying plants.

**Key words:** Formaldehyde dehydrogenase (FALDH), golden pothos (*Epipremnum aureum*), sick house.

Formaldehyde is one of the main indoor air pollutants, present in tobacco smoke, furniture, industrial adhesives and varnishes, and is implicated in cases of "sick house" or "sick building" syndrome. It has been classified as a mutagen and suspected carcinogen (Wippermann et al. 1999). Formaldehyde can also arise from several biological processes and its detoxification is a general requirement in all living cells. Certain foliage plants, including golden pothos (*Epipremnum aureum*) and the spider plant (*Chlorophytum comosumn*), can reportedly purify the air of various atmospheric chemicals, including formaldehyde (Giese et al. 1994; Tada et al. 2010; Wolverton et al. 1984). Formaldehyde responsive genes have been also identified from golden pothos (Tada et al. 2010). Successful genetic engineering to improve the detoxification of such chemicals by indoor foliage plants would provide a convenient way to purify indoor air. Exogenous formaldehyde can be incorporated into the metabolism of photosynthetic cells and used as a carbon source (Achkor et al. 2003). Biochemical and genetic studies in a variety of eukaryotes indicate that the main enzyme responsible for the metabolism of formaldehyde is glutathione-dependent formaldehyde dehydrogenase (FALDH). This enzyme is ubiquitously present and highly conserved in animal and plant tissues, and catalyzes the NAD-dependent formation of *S*-formylglutathione from *S*-hydroxymethylglutathione, which forms spontaneously from formaldehyde and glutathione (Uotila and Koivusalo 1979). *S*-formylglutathione is then hydrolyzed to formate and glutathione by *S*-formylglutathione hydrolase. Formate is oxidized to $CO_2$ by formate dehydrogenase (Cossins 1964). FALDH is expressed at low levels in Arabidopsis ($5\times10^{-3}$ units $mg^{-1}$; Martínez et al. 1996), which is approximately the same amount that has been observed in the dry pea (*Pisum sativum*) seeds ($4\times10^{-3}$ units $mg^{-1}$; (Shafqat et al. 1996). The genes for FALDH in *Arabidopsis*, rice, and maize have been isolated (Dolferus et al. 1997; Fliegmann and Sandermann 1997; Martínez et al. 1996). The *Arabidopsis* gene is expressed

Abbreviations: FALDH, formaldehyde dehydrogenase; RACE, rapid amplification of cDNA ends; RT, Real-time quantitative reverse transcriptase
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at similar levels in all plant organs (Martínez et al. 1996). The over-expression of FALDH in *Arabidopsis* seedlings conferred enhanced detoxification of formaldehyde in liquid culture (Achkor et al. 2003); however, an adverse effect on root growth was observed in those transgenic plants (Espunya et al. 2006). However, the genetic properties and enzymatic activities of the FALDH in foliage plants are still unknown.

In this study, we cloned *FALDH* cDNA from golden pothos and investigated the FALDH enzyme activity in comparison with this activity in Arabidopsis and rice. We have generated transgenic Arabidopsis plants overexpressing either the *FALDH* gene constructs, measured the enzyme activity as well as the rates for formaldehyde uptake and detoxification, and tested formaldehyde tolerance in transgenic plants with enhanced FALDH levels. The results indicate that the activity of golden pothos FALDH is comparable to that of the other reported FALDHs in the detoxification of exogenous formaldehyde in the environment.

## Materials and methods

### Plant materials

Golden pothos (*Epipremnum aureum*), rice (*Oryza sativa* ver. Nipponbare) and *Arabidopsis thaliana* (ecotype Columbia) were grown at 25°C, 25°C and 22°C, respectively, under a 16 h/8 h light/dark cycle with approximately 80 $\mu$mol $m^{-2}$ $s^{-1}$ light intensity.

### Cloning and over-expression of FALDH cDNAs

Total RNA was prepared from golden pothos, rice and Arabidopsis leaves using an RNeasy plant mini kit (Qiagen, Tokyo, Japan) and cDNAs were synthesized using a Marathon cDNA Amplification kit (Clontech, Palo Alto, CA) according to the protocol supplied. A partial *FALDH* sequence from golden pothos was amplified using the PCR primers 5′-acctggagcggcaaggatcctga-3′ and 5′-gttccccagcccttgtggcagc-3′, which were designed from the conserved *FALDH* sequences from rice (XM_468385) and *A. thaliana* (NM_123761) using the cDNA as a template. PrimeSTAR HS DNA polymerase (Takarabio, Ohtsu, Japan) was used as the polymerase for PCR. Amplified fragments were cloned into pCR2.1 (Invitrogen, Carlsbad, CA) and sequenced. Then the full length cDNA was obtained by 5′- and 3′-RACE (*Rapid amplification of cDNA ends*) using a Marathon cDNA Amplification kit. As gene specific primers, 5′-aaccaggacttgctgtatcggtttctc-3′ and 5′-aaggagtgactgaggttcaacctgggg-3′, respectively, were designed based on the cloned partial sequences. Amplified fragments were cloned into pCR2.1 and sequenced. Then the full length cDNA of golden pothos *FALDH* was amplified by PCR using the specific primers 5′-caccatggctacagaggcccag-3′ and 5′-ggaaagcatttacacctgcgtgtc-3′, which were designed based on the combined full length cDNA sequences, cloned into pENTR/D-TOPO (Invitrogen, Carlsbad, CA) and sequenced. *FALDH* cDNAs of Arabidopssis and rice were also amplified by PCR using the specific primers 5′-caccatggcgactcaaggtcaggttatc-3′ and 5′-tcatttgctggtatcgaggacac-3′, and 5′-CACCATGGCTTCCTCGACCCAGGGCCA-3′ and 5′-tcatttatcagttgccagaacacac-3′, respectively, cloned into pENTR/D-TOPO and sequenced. Plasmid DNA was extracted from *E. coli* and from *Agrobacterium* suspension culture using a QIAprep Spin Miniprep kit (Qiagen). DNA sequencing was conducted using a Big Dye terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Tokyo, Japan) according to the manufacturer's instructions. Sequence analysis was performed using an ABI3100 capillary DNA sequencer (Applied Biosystems, Foster City, CA). Raw sequence data was analyzed using GENETYX software (Genetyx, Tokyo, Japan), and the sequences were searched against the NCBI non-redundant EST database using Blastn. The nucleotide sequence data of the golden pothos *FALDH* gene is available in the DDBJ database under accession no. AB618795. The expression vectors were generated using LR Recombinase™ and *in vitro* recombination between the entry clones and the destination vector. The destination vector pGWB2 (the gift of Dr. Tsuyoshi Nakagawa, Shimane University) contains the CaMV35S promoter for cDNA expression.

### Plant transformation

*A. thaliana* wild-type (WT) plants (ecotype Columbia) were transformed by floral dipping (Clough SJ and Bent AF 1998). Hygromycin-resistant $T_1$ and $T_2$ seedlings were selected on 1/2 Murashige-Skoog (MS) medium supplemented with 20 mg $l^{-1}$ hygromycin, 1.0% sucrose and 0.8% agar, and then transferred to 1/2 MS medium supplemented with 1.0% sucrose and 0.8% agar.

### Real-time quantitative reverse transcriptase (RT)-PCR

Real-time quantitative PCR was performed to quantify the transcriptional levels of the introduced and endogenous *FALDH* genes in plants. Briefly, single strand cDNA was synthesized from total RNA using a Quanti Tect Reverse Transcription kit (Qiagen) according to the manufacturer's instructions. Real-time quantitative PCR was performed using a Quanti Tect SYBR Green PCR kit (Qiagen) with a Light Cycler (Roche Diagnostics, Basel, Switzerland). The Light Cycler was programmed as follows: 2 min at 50°C and 15 min at 95°C, followed by 55 cycles of 10 s at 98°C, 5 s at 60°C, and 1 min at 72°C. Amplification of *TUB5* was used as an internal control to normalize all data. The primers used are as follows. Golden pothos *FALDH*: 5′-gaagggaacagcctttggtggc-3′ and 5′-tcccaacgtcagattatgggtg-3′, rice *FALDH*: 5′-ccgaagccaagttccatggctc-3′ and 5′-ccttcatgcagcaggtcaaacg-3′, Arabidopsis *FALDH*: 5′-gaatacataacacacaacttgacc-3′ and 5′-acgaggacacaacgaaggcaag-3′, *TUB5*: 5′-TGACTTTCTCAGTGTTTCCTTCTCC-3′ and 5′-GAGGCGGAGGACGAGATGAAG-3′

### Enzymatic activity

Crude proteins were extracted from 100 $\mu$g of Arabidopsis leaves in 1 ml of 0.1 M sodium phosphate buffer (pH 8.0) using a mortar and pestle on ice. FALDH activity was determined by the decrease in the formaldehyde concentration in the presence of 10 $\mu$l of leaf extracts, 10 mM NAD and 10 mM glutathione in 0.1 M sodium phosphate (pH 8.0). After incubation at 25°C for 240 min, the formaldehyde concentration in the reaction mixture was determined spectrophotometrically using a

NANOCOLOR Tube test kit, Formaldehyde 8 (Macherey-Nagel, Germany) and the compact Photometer PF-12 (Macherey–Nagel). The formaldehyde concentration in the presence of 10 $\mu$l of 0.1 M sodium phosphate, instead of leaf extract, was also determined as the blank. The concentration of dissolved formaldehyde was calculated by subtraction of the formaldehyde concentration in the blank from that in the samples. The protein concentration was determined using a Bio-Rad Protein Assay (Bio-Rad, CA).

### *Evaluation of formaldehyde detoxification ability*

To determine whether the transgenic plants would exhibit a higher capacity for formaldehyde uptake, transgenic lines and WT plants were cultured in liquid medium or in an airtight chamber supplemented with formaldehyde. For liquid culture, three each of the $T_2$ and WT seedlings at 17 days old were weighed and then transferred into 2 ml of 1/2 MS liquid medium supplemented with 2 mM formaldehyde in test tubes. After 24 and 48 h of rotation culture at 23°C under 16 h/8 h light/dark cycles with approximately 110 $\mu$mol m$^{-2}$ s$^{-1}$ light intensity, the formaldehyde concentration in the medium was determined. For cultivation in a chamber, 4 plates each of the 3 week-old $T_2$ lines and WT plants (9 plants/plate) on 1/2 MS agar medium were put into airtight chambers (27×26×37 cm) supplemented with 8 $\mu$l of a 18.5% formaldehyde solution in a sample tube, into which a strip of filter paper was placed to facilitate evaporation. The air in the chamber was occasionally circulated by a propeller driven by a battery-powered motor. The formaldehyde concentration in the chambers was determined 48 h after the treatment with a gas detector tube for formaldehyde (Komyo, Kanagawa, Japan).

### *Formaldehyde tolerance assay*

To determine whether the transgenic plants would display a higher tolerance for formaldehyde, the growth of the transgenic lines and WT plants on formaldehyde-containing agar medium was observed. Five-day-old $T_2$ and WT seedlings were transplanted to 1/2 MS agar plates supplemented with 3, 4, 5 or 6 mM formaldehyde. At 7 days after transplantation, the survival rate of individual lines was determined.

## Results

### *Cloning of golden pothos* FALDH *cDNA*

A portion of golden pothos FALDH cDNA was amplified by PCR using primers designed from *FALDH* sequences which were conserved in rice and *A. thaliana.* Subsequently, the primers specific for the cloned partial *FALDH* cDNAs were used to clone the 5′ and 3′ ends of the cDNA, and fragments of approximately 0.45 and 1.2 kb, respectively, were obtained by 5′- and 3′-RACE. The combined sequence consisted of 1411 nucleotide and encoded a polypeptide of 379 amino acids, with an estimated molecular mass of 40.7 kDa and a theoretical isoelectric point (PI) of 6.47. The deduced amino acid sequence of *FALDH* cDNA for golden pothos is shown in Figure 1 in alignment with the amino acid sequences from Arabidopsis, maize and rice. The amino

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AtFALDH    1:MA--TQGQVITCKAAVAYEPNKPLVIEDVQVAPPQAGEVRIKILYTALCHTDAYTWSGKD
EaFALDH    1:MA--TEAQVITCKAAVAWEANKPLAIEDVLVAPPQAGEVRIKILFTALCHTDAYTWSGKD
OsFALDH    1:MASSTQGQVITCKAAVAWEANRPMTIEDVQVAPPQAGEVRVKILFTALCHTDHYTWSGKD
ZmFALDH    1:MASPTQGQVITCKAAVAYEPNKPLVIEDVQVAPPQAGEVRVKILFTALCHTDHYTWSGKD
             **  *..********** * *.*.  ****.********** ***.******* *******

AtFALDH   59:PEGLFPCILGHEAAGIVESVGEGVTEVQAGDHVIPCYQAECRECKFCKSGKTNLCGKVRS
EaFALDH   59:PEGLFPCILGHEAAGIVESVGEGVTEVQPGDHVIPCYQAECRECKFCKSGKTNLCGKVRA
OsFALDH   61:PEGLFPCILGHEAAGIVESVGEGVTEVQPGDHVIPCYQAECRECKFCKSGKTNLCGKVRA
ZmFALDH   61:PEGLFPCILGHEAAGIVESVGEGVTDVQPGDHVIPCYQAECKECKFCKSGKTNLCGKVRS
             ************************.**.***********.****************

AtFALDH  119:ATGVGIMMNDRKSRFSVNGKPIYHFMGTSTFSQYTVVHDVSVAKIDPTAPLDKVCLLGCG
EaFALDH  119:ATGVGIMMNDRKSRFSINGKPIYHFMGTSTFSQYTVVHDVSVAKIDPKAPLEKVCLLGCG
OsFALDH  121:ATGVGVMMNDRKSRFSINGKPIYHFMGTSTFSQYTVVHDVSVAKINPQAPLDKVCLLGCG
ZmFALDH  121:ATGVGVMMNDMKSRFSVNGKPIYHFMGTSTFSQYTVVHDVSVAKINPQAPLDKVCLLGCG
             ***** ****.***** ***************************** * ***.********

AtFALDH  179:VPTGLGAVWNTAKVEPGSNVAIFGLGTVGLAVAEGAKTAGASRIIGIDIDSKKYETAKKF
EaFALDH  179:VATGLGAVWNTAKVEAGSIVAIFGLGTVGLAVAEGAKTAGASRIIGIDIDSKKFDVAKNF
OsFALDH  181:VSTGLGAVWNTAKVEAGSIVAIFGLGTVGLAVAEGAKSAGASRIIGIDIDSKKFDVAKNF
ZmFALDH  181:VPTGLGAVWNTAKVESGSVVAVFGLGTVGLAVAEGAKAAGASRVIGIDIDNKKFDVAKNF
             * ************* ** **.*************** *****.******.**...**.*

AtFALDH  239:GVNEFVNPKDHDKPIQEVIVDLTDGGVDYSFECIGNVSVMRAALECCHKGWGTSVIVGVA
EaFALDH  239:GVTEFVNPKDYEKPIQQVLVDLTDGGVDYSFECIGNVSVMRAALECCHKGWGTSVIVGVA
OsFALDH  241:GVTEFVNPKDHDKPIQQVIVDLTDGGVDYSFECIGNVSVMRSALECCHKGWGTSVIVGVA
ZmFALDH  241:GVTEFVNPKEHDKPIQQVLVDLTDGGVDYSFECIGNVSIMRAALECSDKGWGTSVIVGVA
             **.******...****.* ******************.**.****..************

AtFALDH  299:ASGQEISTRPFQLVTGRVWKGTAFGGFKSRTQVPWLVEKYMNKEIKVDEYITHNLTLGEI
EaFALDH  299:ASGQEISTRPFQLVTGRVWKGTAFGGFKSRSQVPWLVDKYMKKEIKVDEYITHNLTLGEI
OsFALDH  301:ASGQEISTRPFQLVTGRVWKGTAFGGFKSRSQVPWLVEKYLNKEIKVDEYVTHSMNLTDI
ZmFALDH  301:ASGQEISTRPFQLVTGRVWKGTAFGGFKSRTQVPWLVDKYMKKEIKVDEYITHNMNLADI
             ****************************** ****** **.  ********.**.   *  *

AtFALDH  359:NKAFDLLHEGTCLRCVLDTSK   379
EaFALDH  359:NKGFDFMHEGSCLRCVLDTQV   379
OsFALDH  361:NKAFDLLHEGGCLRCVLATDK   381
ZmFALDH  361:NDAFHLLHEGGCLRCVLAMQI   381
             *..*...*** ****** .
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Figure 1. Alignment of the deduced amino acid sequences of the FALDHs from Arabidopsis (AtFALDH, NM_123761), golden pothos (EaFALDH), rice (Os11FALDH, XM_468385), and maize (ZmFALDH, BT016362).

acid sequence from golden pothos FALDH displayed 91.1, 87.9 and 90.3% identity with FALDH from Arabidopsis, maize and rice, respectively.

### *Comparison of* FALDH *transcript levels and enzyme activities in transgenic plants*

The *FALDH* cDNAs of golden pothos, Arabidopsis and rice were amplified by PCR using specific primers. Three *FALDH* cDNAs were respectively cloned into the expression vector pGWB2, in which the cDNA is under the control of the CaMV35S promoter, and introduced into Arabidopsis by a floral dipping method. Transgenic $T_1$ plants were selected on hygromycin-containing medium, and the transcriptional level of *FALDH* genes in $T_1$ and WT plants was determined by real time quantitative RT-PCR analysis. The relative transcriptional levels in terms of the total sum of the transgene and endogenous gene transcripts in individuals from the $T_1$ plants are shown in Figure 2A. Some of them exhibited a moderate to high increase in *FALDH* transcription levels, whereas exhibited cosuppressed expression. The transcriptional levels of the *FALDH* transgenes from golden pothos and rice in the overexpression lines (Ea and Os transformant lines, respectively) were 1.3 to 84 (14.8 on average) and 1.6 to 14 (10.0 in average) fold those of the WT plants, respectively. The transcriptional levels of the *FALDH* trans- and endogenous-genes in Arabidopsis (At transformant lines) were 1.4 to 14 (6.3 on average) fold those of the WT plants in the overexpressed lines. The lowest transcriptional levels of *FALDH* genes in the

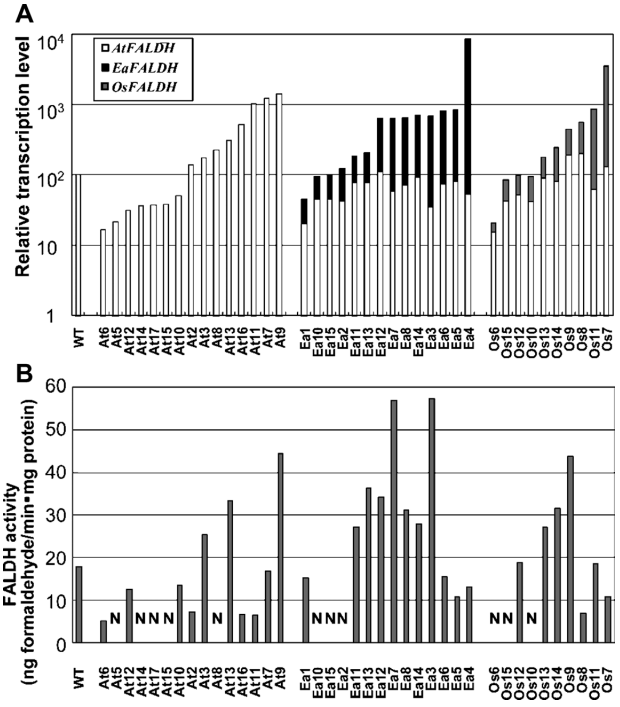


Figure 2. Transcriptional levels of *FALDH* genes and FALDH activity in transgenic and WT plants. Transformants with *FALDH* from Arabidopsis, golden pothos and rice were designated as At, Ea and Os lines, respectively. (A) The transcriptional level of *FALDH* genes in $T_1$ and WT plants was determined by real time quantitative RT-PCR analysis using the *TUB5* gene as an internal standard. The relative transcriptional levels of the transgene and endogenous gene transcripts are represented as relative values of 100. (B) FALDH enzyme activity in crude leaf extracts from transformants and WT plants was determined. N: not determined.

cosuppression lines (At6) were 17% of the WT plants. FALDH enzyme activity in leaf extracts from these transgenic and WT plants was also determined (Figure 2B). Although there was an overall correlation observed between the increase in the enzyme activity and the transcription levels, certain lines (At7, At11, At16, Ea4, Ea5, Ea6, Os8, Os7 and Os11) with high expression levels did not exhibit enhanced enzyme activity in comparison to the WT plants. The lines (At6, At12 and At10) with reduced levels of *FALDH* transcription, indicating the cosuppression phenomenon, displayed reduced enzyme activity in 5–13% of the WT plants. The growth of some of the transgenic lines with both elevated and suppressed *FALDH* expression was severely reduced (data not shown). Transformants which displayed growth comparable to the WT plants and higher FALDH enzyme activity (At9, Ea3 and Os9) were selected as representative transgenic lines for each construct. They were used for further experiments to examine the capacity to detoxify exogenous formaldehyde and to survive on formaldehyde-containing medium.

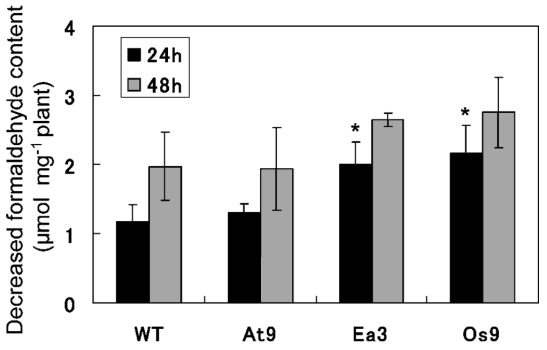


Figure 3. Detoxification of liquid formaldehyde by the *FALDH* transformants. The WT plants and the transgenic lines At9, Ea3 and Os9 germinated and grown on agar medium for 17 days were transferred into liquid medium (2 ml) supplemented with 2 mM of formaldehyde. Formaldehyde concentrations of the culture medium were measured at 24 and 48 h after treatment. The values are represented as the decreased formaldehyde content in the plant-containing medium compared to plant free medium. Values are the average of three independent experiments ± standard deviation. * is significantly different from WT with $P<0.05$ as calculated by the Student's *t*-test. $n=3$.

### *Detoxification of exogenous formaldehyde by the* FALDH *transformants*

To compare the ability of the transformants to metabolize exogenous formaldehyde in liquid medium, transformants and WT plants at 17 days old were transferred into liquid medium supplemented with 2 mM of formaldehyde. Formaldehyde concentrations of the culture medium were measured at 24 and 48 h after the treatment and the decreased formaldehyde contents in the medium were determined (Figure 3). At 24 h, Ea3 and Os9 exhibited the significantly increased rate for formaldehyde uptake (about 40% increase of WT plants). At9 exhibited slightly better rate for formaldehyde uptake. At 48 h, similar tendency was observed in their formaldehyde uptake. Thus, among the three transgenic lines tested, Ea3 and Os9 effectively detoxified formaldehyde compared with the WT plants.

Next, to compare the ability of the transformants to metabolize gaseous formaldehyde, WT plants and the transformants At9, Ea3 and Os9 were put into airtight chambers supplemented with gaseous formaldehyde. As a control, a chamber with an agar plate with no plants was also prepared. Forty-eight hours after treatment, the formaldehyde concentration in the chambers was measured (Figure 4). The formaldehyde concentration in the chambers with Ea3 and Os9 exhibited the lowest value, followed by the At9 and WT plants, showing that gaseous formaldehyde was effectively metabolized by the *FALDH* transformants, especially the Ea and Os transformants.

### *Formaldehyde tolerance of* FALDH *transformants*

To determine whether the transformants had a higher tolerance to formaldehyde, 5 day-old seedlings of WT

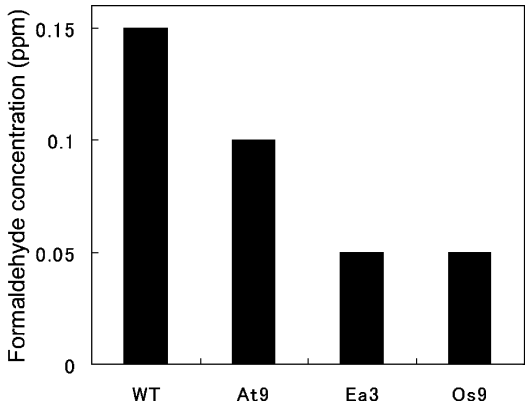


Figure 4. Detoxification of gaseous formaldehyde by the *FALDH* transformants. The WT plants and the transgenic lines At9, Ea3 and Os9 (36 plants each) germinated and grown on agar medium for 21 days were put into airtight chambers supplemented with gaseous formaldehyde. Average shoot fresh weights of WT, At9, Ea3 and Os9 plants at this stage were 52.6, 55.1, 53.8 and 51.3 mg, respectively. The formaldehyde concentration in the chambers was measured at 48 h after treatment. The experiment was repeated twice with similar results. The formaldehyde concentration in the control (i.e. a chamber with an agar plate with no plants) was 1.2 ppm.

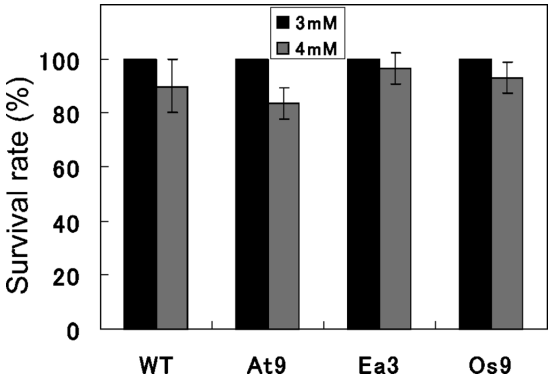


Figure 5. Formaldehyde tolerance of the *FALDH* transformants. Five-day-old seedlings were transplanted to agar plates supplemented with 3, 4, 5 or 6 mM formaldehyde. At 7 days after transplanting, the survival rate of individual lines was determined. At 5 and 6 mM formaldehyde, all plants died, then data at 3 and 4 mM formaldehyde were represented. Values are the average of three independent experiments ± standard deviation. $n=3$

plants and the transformants At9, Ea3 and Os9 were transferred to 1/2 MS medium supplemented with 3, 4, 5 and 6 mM formaldehyde. After incubation for a further 7 days, their survival rate was determined (Figure 5). At 3 mM formaldehyde, all the transgenic lines and WT plants survived. At 5 or 6 mM formaldehyde, all of the transformants and WT plants died as the result of its phytotoxic effects, displaying chlorotic patches throughout their tissues. At 4 mM formaldehyde, each of the transformant and WT plants displayed chlorotic regions in their leaves, but there was no significant difference in their survival rate, indicating no effective difference in formaldehyde tolerance.

## Discussion

If it were possible to utilize indoor foliage plants to remove formaldehyde, it would be a convenient way to purify indoor air at low energy and cost. Although certain foliage plants were reported to have a relatively high capacity for the removal of formaldehyde, an enhancement of formaldehyde detoxification by genetic engineering is needed for practical applications. FALDH is known to play a central role in formaldehyde detoxification in plants (Achkor et al. 2003; Fliegmann and Sandermann 1997). The enzymatic properties of the FALDHs from a variety of plants are reportedly similar to those found in the homolog enzymes from mammals, invertebrates, and microorganisms (Martínez et al. 1996; Shafqat et al. 1996; Uotila and Koivusalo 1979; Wippermann et al. 1999). However, FALDH activity in foliage plants has not been reported. In this study, we cloned *FALDH* cDNA from golden pothos and investigated the FALDH enzyme activity in comparison with this activity in rice in transgenic Arabidopsis plants.

Prior to over-expressing the *FALDH* cDNAs from Arabidopsis, rice and golden pothos in Arabidopsis, each *FALDH* cDNA was cloned into the expression vector pDEST17 (Invitrogen) to generate a His-tag fusion protein in *E. coli*; however, no fusion proteins were detected by SDS-PAGE in *E. coli* cultured at 37, 30 and 20°C (data not shown). Therefore, comparison of the three FALDH enzyme activities was performed using the transgenic plants as the host. The data reflect the performance of each enzyme introduced into the indoor plants for detoxifying formaldehyde.

Although there was an overall correlation observed between the increase in the enzyme activity in the leaf extract and the transcription levels in all of the FALDH cDNA from golden pothos, Arabidopsis and rice, certain of the transformants with high transcription levels exhibited reduced enzyme activity compared to the WT plants (Figure 2). Post transcriptional gene silencing may have occurred in these transformants, although we do not have any proof of this. Achkor et al. (2003) reported that *FALDH* overexpressing plants exhibited from 11- to 18-fold the WT FALDH activity, and the capacity of plants overexpressing *FALDH* displayed a 25% increase in the detoxification rate compared with the WT plants (Achkor et al. 2003). In this study, the Ea transformants exhibited 6.3- to 14-fold the WT *FALDH* transcriptional level, and 1.4- to 3.2-fold the WT FALDH activity. These differences in FALDH enzyme activity may be due to minor differences in the expression vectors employed, although the 35S promoter was used for both constructs.

We attempted to detect the FALDH protein by Western blot analysis using an anti-rat human ADH5 antiserum (#H00000128-A01 (Funakoshi, Tokyo, Japan)), which is the most closely related to plant FALDH among the

commercially available antisera, but failed to detect a signal (data not shown).

In transgenic Arabidopsis overexpressing *FALDH* and plants with reduced levels of *FALDH*, a reduction in root length been reported (Achkor et al. 2003). In this study, most of the transformants expressing *FALDH* from golden pothos, Arabidopsis and rice exhibited reduced shoot and root growth. It is difficult to compare formaldehyde detoxification at the whole plant level in such plants because of their disparate plant growth. In this study, we selected transformants (At9, Ea3, Os9) displaying normal growth and elevated FALDH activity and compared formaldehyde detoxification in the transformants with WT plants (in) at the whole plant level. The relatively lower FALDH enzyme activity in our transformants might be the reason we obtained transformants with normal growth. The transformants with *FALDH* from the golden pothos (Ea3) and rice (Os3) were the most effective in detoxifying aqueous and gaseous formaldehyde (Figures 3, 4) at a similar developmental stage and size. To the best of our knowledge, this is the first report on an enhancement of the ability of detoxifying gaseous formaldehyde by FALDH-overexpressing plants. Furthermore, no difference was observed in formaldehyde tolerance of the transformants and WT. In summary, the FALDH from golden pothos appears to be similar or more effective for detoxifying formaldehyde in transgenic plants. Thus, the *FALDH* cDNA is one of the promising gene sources for producing formaldehyde detoxifying plants.

Recently, the bacterial ribulose monophosphate pathway was introduced into Arabidopsis and tobacco to produce formaldehyde detoxifying plants (Chen et al. 2010). The transgenic plants transformed with 3-hexulose-6-phosphate synthase and 6-phospho-3-hexuloisomerase displayed an enhanced capacity for formaldehyde assimilation and tolerance to exogenous formaldehyde. Their formaldehyde detoxifying ability was higher than that of Arabidopsis FALDH-overexpressing transformants. However, in FALDH mediated formaldehyde metabolism, an enhancement of S-formylglutathione hydrase and formate dehydrogenase might also be needed for the removal of the harmful metabolites which are formed. Furthermore, the combined introduction of FALDH mediated formaldehyde metabolic pathway in addition to the bacterial ribulose monophosphate pathway may result in which are more practically applicable to formaldehyde detoxification. The genes for *S*-formylglutathione hydrolase and formate dehydrogenase have been cloned from Arabidopsis (Haslam et al. 2002; Olson et al. 2000). The production of transformants overexpressing these genes is in progress.

## References


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