RESEARCH PAPER

Molecular cloning and characterization of UDP-glucose: furaneol glucosyltransferase gene from grapevine cultivar Muscat Bailey A (Vitis labrusca × V. vinifera)

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Abstract

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furaneol) is an important aroma compound in fruits, such as pineapple and strawberry, and is reported to contribute to the strawberry-like note in some wines. Several grapevine species are used in winemaking, and furaneol is one of the characteristic aroma compounds in wines made from American grape (Vitis labrusca) and its hybrid grape. Furaneol glucoside was recently isolated as an important furaneol derivative from the hybrid grapevine cultivar, Muscat Bailey A (V. labrusca × V. vinifera), and this was followed by its isolation from some fruits such as strawberry and tomato. Furaneol glucoside is a significant ‘aroma precursor of wine’ because furaneol is liberated from it during alcoholic fermentation. In this study, a glucosyltransferase gene from Muscat Bailey A (UGT85K14), which is responsible for the glucosylation of furaneol was identified. UGT85K14 was expressed in the representative grape cultivars regardless of species, indicating that furaneol glucoside content is regulated by the biosynthesis of furaneol. On the other hand, furaneol glucoside content in Muscat Bailey A berry during maturation might be controlled by the expression of UGT85K14 along with the biosynthesis of furaneol. Recombinant UGT85K14 expressed in Escherichia coli is able to transfer a glucose moiety from UDP-glucose to the hydroxy group of furaneol, indicating that this gene might be UDP-glucose: furaneol glucosyltransferase in Muscat Bailey A.

Key words: Furaneol, glucosyltransferase, grape, Muscat Bailey A, Vitis, wine

Introduction

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furaneol) is an important aroma compound in many fruits, such as pineapple (Rodin et al., 1965), strawberry (Ohloff, 1969), and raspberry (Honkanen et al., 1980), and is the most essential contributor to strawberry aroma (Pyysalo et al., 1979; Pérez et al., 1996). In strawberry, furaneol is metabolized into a methyl derivative (2,5-dimethyl-4-methoxy-3(2H)-furanone) (Pyysalo et al., 1979; Pérez et al., 1996), a glucosylated derivative (furaneol β-d-glucopyranoside) (Mayerl et al., 1989), and a malonylated derivative (furaneol β-d-glucopyranoside malonate) (Raab et al., 2006). Among the enzymes involved in the biosynthesis of furaneol and its derivatives, several cDNAs, such as quinone oxidoreductase and O-methyltransferase, were also isolated from strawberry (Wein et al., 2002; Lunkenbein et al., 2006; Raab et al., 2006; Zorrilla-Fontanesi et al., 2012). Regarding the enzymes catalysing furaneol glucosylation,
the only glucosyltransferase gene (FaGT2) was isolated from strawberry (Landmann et al., 2007). The protein encoded by this gene participates in the detoxification of xenobiotics, and possesses broad substrate specificity and high apparent Kin values for natural products. The analogous gene product of FaGT2 in grape was reported to be a bi-functional resveratrol/hydroxycinnamic acid glucosyltransferase (80% identity) (Hall and De Luca, 2007).

Furaneol is also present in concentrations well exceeding the odour threshold in American grape species, such as Vitis labrusca and its hybrids, and contributes to the strawberry-like aroma in wines made from those grape species (Rapp et al., 1980; Schreier and Paroschy, 1981; Shure and Acree, 1994; Kobayashi et al., 2013). Although furaneol is not present in large amounts in European wine grapes, such as V. vinifera, it is also important because it may enhance the fruity note of some wines (Ferreira et al., 2002). In a recent study, furaneol glucoside was identified in the hybrid grapevine cultivar, Muscat Bailey A (V. labrusca × V. vinifera), and it was demonstrated that furaneol glucoside was accumulated in larger amounts in American grape species than European grape as well as furaneol aglycon (Sasaki et al., 2015). Several odourless glycoside conjugates of aroma compounds as well as glutathione derivatives have been conceived as aroma precursors in wine because aroma compounds are liberated from them by alcoholic fermentation (Strauss et al., 1987; Noble et al., 1988; Wein et al., 2002; Kobayashi et al., 2011). Furaneol glucoside is also a significant aroma precursor of the strawberry-like note in several wines (Pinho and Bertrand, 1995; Sasaki et al., 2015).

In this article, the isolation of UDP-glucose: furaneol glucosyltransferase (GlyT_07) from the grapevine cultivar, Muscat Bailey A, as well as the phylogenetic analysis—revealing that GlyT_07 belongs to the UGT85K subfamily of plant secondary glycosyltransferase—is reported. Recombinant GlyT_07 expressed in Escherichia coli was shown to catalyse the glucosyltransferase reaction from UDP-glucose to the hydroxyl group of furaneol (Fig. 1), and to possess a remarkable specific activity for furaneol.

Materials and methods

Plant materials and chemicals

Several cultivars of grapevines, including Muscat Bailey A (V. labrusca × V. vinifera), Concord (V. labrusca), Pinot Noir, Cabernet Sauvignon, Chardonnay, and Riesling (V. vinifera), were grown in Yamanashi, Akita, and Fukushima Prefectures, Japan, and harvested at maturity. Furaneol and 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone (homofuraneol) were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Cytidin chloride, kaempferol, 4-nitrophenol and trans-zeatin were from Wako Pure Chemical Industries (Osaka, Japan); trans-cinnamic acid, 4-coumaric acid, naringenin, quercetin, trans-resveratrol, geraniol, and linalool were from Sigma-Aldrich Japan (Osaka, Japan); acetone cyanohydrin was from Junsei Chemical Co. (Tokyo, Japan). Furaneol glucoside was isolated and purified from strawberry juice according to a previous report with slight modification (Mayerl et al., 1989; Sanz et al., 1994), and its structure was confirmed by NMR measurement (Sasaki et al., 2015).

Phylogenetic analyses

Forty-one putative glucosyltransferase genes were selected, which were strongly expressed at the late ripening stage of berries from the grapevine species expression data in a previous report (Sweetman et al., 2012) (Supplementary Table S1, available at JXB online). Phylogenetic analysis was performed using the 26 candidates, and this was followed by the exclusion of putative genes involved in the biosynthesis of proteins and lipids. Sequence alignments were generated based on a comparison of the amino acid sequences using the ClustalW program (Thompson et al., 1994) with the following values: 10 for gap opening penalty and 0.1 for gap extension penalty in pairwise alignment; 10 for gap opening penalty and 0.2 for gap extension penalty in multiple alignment; Gonnet for protein weight matrix; available residue-specific penalties; available hydrophilic penalties; 4 for gap separation distance; and 30% delay divergent cutoff. Those alignments were adopted to construct neighbour-joining phylogenetic trees using MEGA 6.06 with the scope of all selected taxa, amino acid substitution type, Poisson model, uniform rates, homogeneous pattern among lineages, and complete deletion for gaps or missing data treatment (Tamura et al., 2011). The scale bar of 0.1 indicates a 10% change, and each number shown next to the branches is the percentage of replicate trees in which the related taxa clustered in the bootstrap test with 1,000 replicates.

Isolation of putative furaneol glucosyltransferase cDNAs from Muscat Bailey A

Total RNA was extracted from Muscat Bailey A grape skins by the cetyltrimethylammonium bromide (CTAB) method, and purified with an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). DNase treatment was conducted prior to column purification of RNA according to the manufacturer’s instructions (Qiagen). cDNAs were synthesized using SuperScript III reverse transcriptase (Life Technologies Inc., Rockville, MD, USA). PCR was carried out with Phusion High-Fidelity DNA Polymerase (New England Biolabs Inc., Ipswich, MA, USA) with cDNAs as templates. PCR primers were designed from the sequence data in the previous gene expression analysis data (Sweetman et al., 2012). PCR products of approximately 1.5 kb were subcloned into pT7 Blue vector via TA cloning using a DNA Ligation Kit (Takara-Bio Inc., Japan), and were confirmed by sequencing.

Heterologous expression of UDP-glucose: furaneol glucosyltransferase in E. coli

Sequences for the ORF of candidate glucosyltransferase genes were amplified by PCR using specific primers (GlyT07_Fw and Rv for GlyT_07, GlyT17_Fw and Rv for GlyT_17, GlyT21_Fw and Rv for GlyT_21) (Supplementary Table S2, available at JXB online). The PCR products were subcloned into the expression vector pET45b (Merck, Darmstadt, Germany) to create N-terminal fusion proteins with His₆-tag, and transformed into E. coli NiCo21 (DE3) (New England BioLabs Inc.). Transformed bacteria were grown at 37 °C in 200 ml of LB medium containing 50 μg/ml ampicillin, and protein expression was induced with 1 mM isopropyl β-D-thiogalactoside at OD₆₀₀ of 0.6. After incubation at 16 °C overnight, the induced cells were harvested by centrifugation. The bacterial cells were sonicated in the extraction buffer (50 mM Tris-HCl, pH 8.0, containing 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, and
20 mM imidazole), and the supernatant from the centrifugation at 9200 g was affinity-purified on an Ni-NTA agarose matrix (Qiagen) according to the manufacturer’s instructions. Enzyme purity was confirmed by SDS-PAGE on 10% (w/v) slabs (e-PAGEL, ATTO Co., Tokyo, Japan), and protein concentration was determined by the Bradford method (Coomassie Plus Protein Assay, Thermo Scientific, Rockford, IL, USA).

**Enzyme assay**

The standard reaction mixture (100 μl) contained 50 mM Tris-HCl, pH 7.5, 1 mM UDP-sugar (UDP-glucose or UDP-galactose), 1 mM acceptor substrate (furaneol, homofuraneol, 4-nitrophenol, acetone cyanohydrin, 4-coumaric acid, trans-cinnamic acid, naringenin, quercetin, kaempferol, cyanidin, trans-resveratrol, linalool, geraniol, and trans-zeatin), and the purified enzyme (2 μg of protein). The reaction was incubated at 30 °C for 30 min, and terminated by adding 100 μl of 1 M HCl. After centrifugation at 15 000 g for 10 min, the supernatant was diluted with an equal volume of 0.1% (v/v) formic acid. Then, the mixture was filtered through a 0.45 μm cellulose acetate filter (DISMIC-25CS, Advantec, Tokyo, Japan), and the filtrate was subjected to HPLC-MS/MS or HPLC-MS analyses.

**HPLC-MS/MS analyses**

The enzymatic characterization of furaneol glucosyltransferase was mostly carried out by HPLC-MS/MS. HPLC (CBM-20A, Shimadzu, Kyoto, Japan) was performed with an Atlantis T3 column (3.0 mm × 150 mm; Waters, Milford, MA, USA) at 40 °C. The solvents were 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B), and the flow rate was 0.2 ml min⁻¹. The linear gradient program for solvent B was as follows: 0 min, 5%; 3 min, 20%; 10 min, 40%; 15 min, 50%; and 30 min, 80%. All mass spectrometric data were acquired in the electrospray ionization positive detection mode using an MS/MS system (3200 QTRAP, AB SCIEX, Massachusetts, USA). Nitrogen was used as curtain gas at 15 psi, nebulizing gas (GS1) at 70 psi, dry gas (GS2) at 15 psi, and source temperature was set at 5500 V and 700 °C, respectively. Mass transitions m/z 129.1 to 57.1 for furaneol, m/z 291.1 to 129.1 for its glucoside, m/z 143.1 to 101.1 for homofuraneol, and m/z 305.0 to 143.0 for its glucoside were set for the multiple reaction monitoring (MRM) experiment. Collision energies for furaneol, its glucoside, homofuraneol, and its glucoside were 13 eV, 19 eV, 15 eV, and 19 eV, respectively.

**Enzyme kinetics**

To determine the kinetic parameters, enzyme assay was performed in triplicate at each substrate concentration with 2 μg of the purified enzyme at 30 °C for 30 min. The substrate concentrations used were 3.2 μM to 10 mM furaneol or homofuraneol with UDP-glucose at 10 mM for acceptor kinetics, and 3.2 μM to 10 mM UDP-glucose with furaneol at 10 mM for donor kinetics. The kinetic parameters were calculated from the Hanes–Woolf plot.

**HPLC-MS analyses**

The acceptor substrate specificity of the enzyme was analysed by HPLC-MS. HPLC (CBM-20A) was performed with the same column as that for HPLC-MS/MS analysis at 40 °C. The solvents were 0.1% aqueous formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B), and the flow rate was 0.2 ml min⁻¹. The linear gradient program for solvent B was as follows: 0–3 min, 10%; and 25 min, 60%. All mass spectrometric data were obtained in the electrospray ionization positive and negative detection modes using an LCMS-2020 system (Shimadzu). The HPLC-MS conditions were as follows: probe voltage, 3,500 V; nebulizing and drying gas flow rate, 1.5 l min⁻¹; CDL temperature, 250 °C; and block heater temperature, 200 °C.

**Quantitative RT-PCR analyses**

Total RNA was extracted from grape skins by the CTAB method, and purified with an RNeasy Plant Mini Kit. DNase treatment was conducted before column purification of RNA, and cDNAs were synthesized from 500 ng of total RNA using PrimeScript RT Master Mix (Takara-Bio Inc., Shiga, Japan) according to the manufacturer’s instructions. Real-time quantitative RT-PCR was performed with SYBR Premix Ex Taq II (Takara-Bio Inc.) using an ABI Prism 7300 real-time PCR system (Life Technologies Inc., Rockville, MD, USA). Two sets of PCR primers were designed from the common sequence between the furaneol glucosyltransferases from *V. labrusc*ua and *V. vinifera*, using Primer 3 software (http://bioinfo.ut.ee/primer3/) (Fw1 and Rv1, Fw2 and Rv2) (Supplementary Table S2 available at *JXB* online). As the results of gene expression analyses using either primer sets are approximately the same, the data using the first primer set (Fw1 and Rv1) are described in Figs 5 and 6. For normalization, 18S rRNA was used as the external standard (GenBank accession number: AF207053) (Supplementary Table S2).

**Preparation of grape juice sample**

Juice was prepared by crushing grape berries with a juicer (IFM-620DG, Iwatani Co., Osaka, Japan) following by pressing to 60% of the total berry weight. Total soluble solids in juice were measured with a refractometer (Pocket Pa-1, Atago Co., Tokyo, Japan), and expressed as °Brix. The grape juice sample was diluted with an equal volume of 0.1% (v/v) formic acid. Then, the mixture was filtered through a 0.45 μm cellulose acetate filter, and the filtrate was subjected to HPLC-MS/MS analyses. Validation of this analysis was performed previously (Sasaki et al., 2015).

**Extraction of furaneol and its glucoside from grape tissues**

The extraction of furaneol and its glucoside was performed as described (Sasaki et al., 2015). Grape berry tissue was frozen and then pulverized in liquid nitrogen using a mixer mill (MM 400, Retsch, Haan, Germany). Sample extraction was carried out according to a previous report with slight modification (Pérez et al., 1999). The powder (0.20 g fresh weight) was extracted with water (1.0 ml) for 16 h at 4 °C, followed by adding 4-methoxy-2,5-dimethyl-3(2H)-furanone as an internal standard, and then the mixture was centrifuged. The supernatant was diluted with one-tenth its volume of 0.1% (v/v) formic acid, and subjected to HPLC-MS/MS analyses as described above.

**Results**

**Identification of UDP-glucose: furaneol glucosyltransferase from the hybrid grape cultivar Muscat Bailey A (V. labrusca × V. vinifera)**

The candidate genes for furaneol glucosyltransferase were selected from the gene expression analysis data in a previous study (Sweetman et al., 2012). In brief, putative glycosyltransferases genes possessing the ripening-related expression pattern in berries were picked out (41 clones) (Supplementary Table S1). Then, the genes involved in protein or lipid glycosylation were excluded from the first screening for the 41 clones. Phylogenetic analysis was performed on the other 26 genes (Fig. 2). The phylogenetic tree is divided into several clusters, such as putative flavonoid 3-O-glycosyltransferases and putative anthocyanidin glucosyltransferases. The candidate genes for furaneol glucosyltransferase were randomly picked out from the uncharacterized genes, and *GlyT_07*, *GlyT_17*, and *GlyT_21*, were selected initially. Those genes
were isolated from Muscat Bailey A berry skin, and were confirmed by sequencing. The genes of Groups B and N could not be isolated as a full-length gene from Muscat Bailey A. The isolated cDNAs, GlyT_07, GlyT_17, and GlyT_21, had 1449, 1374, and 1389 base pairs, and encoded polypeptides composed of 482, 457, and 462 amino acids, respectively. Furthermore, they contained a common motif, the plant secondary product glycosyltransferase (PSPG) box, for plant secondary product glucosyltransferases (Ross et al., 2001; Caputi et al., 2012). These clones, GlyT_07, 17, and 21, were subsequently assigned to UGT85K14, UGT74B6, and UGT712E1 by the UGT Nomenclature Committee (Mackenzie et al., 1997), and their DDBJ accession numbers are LC021362, LC021363, and LC021364, respectively.

Characterization of furaneol glucosyltransferase using recombinant protein

To demonstrate the enzymatic function of the isolated genes, ORFs were expressed in E. coli as N-terminal fusion proteins with His_6-tag. The recombinant protein was detected at the predicted molecular mass, 55kDa in SDS-PAGE (Supplementary Fig. S1 available at JXB online). Purified recombinant protein was incubated with furaneol in the presence of UDP-glucose at 30 °C, and the reaction mixture was subjected to HPLC-MS/MS. UDP-galactose could be also utilized as a sugar donor substrate. However, the relative enzymatic activity for UDP-galactose was much lower than that for UDP-glucose (1.6%).

The substrate specificity of UGT85K14 was analysed by using various natural compounds (Supplementary Fig. S2). Furaneol and its analogue compound, 2,5-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone (homofuraneol), could be utilized as a substrate, whereas the other compounds (4-nitrophenol, acetone cyanohydrin, 4-coumaric acid, trans-cinnamic acid, naringenin, quercetin, kaempferol, cyanidin, trans-resveratrol, linalool, geraniol, and trans-resveratrol) were not glucosylated, indicating that the isolated UGT85K14 protein was UDP-glucose: furaneol glucosyltransferase (1.32 nmol s^{-1} mg^{-1} protein). Then, the UDP-sugar substrate specificity of UGT85K14 was analysed by HPLC-MS/MS. UDP-galactose could be also utilized as a substrate. However, the relative enzymatic activity for UDP-galactose was much lower than that for UDP-glucose (1.6%).

The kinetic parameters of glucosyltransferase using furaneol and homofuraneol as substrates were determined based on pseudo single substrate kinetics using UDP-glucose as the sugar donor substrate. On the other hand, those using UDP-glucose as the substrate were determined using furaneol as the acceptor substrate. The apparent Km values for furaneol and UDP-glucose were calculated to be 156 μM and 321 μM, respectively. The apparent Km value for homofuraneol, an analogue, was calculated to be 132 μM, which is similar to that for furaneol. The kcat and the kcat/Km ratio for furaneol were 0.072 s^{-1} and 461 M^{-1} s^{-1}, respectively. Those enzymatic features—particularly the remarkable substrate specificity—indicated that this enzyme might be one of the plant secondary product glucosyltransferases. The optimum temperature was approximately 25–30 °C, and the optimum pH was approximately 6.0–7.0 (Fig. 4).
Identification of furaneol glucosyltransferase gene from grape

Furaneol glucosyltransferase gene from other grapevine cultivars Pinot Noir (V. vinifera) and Concord (V. labrusca)

Several grapevine species for wines, such as V. vinifera, V. labrusca, and their hybrid, are cultivated worldwide. In addition to Muscat Bailey A (V. labrusca × V. vinifera), the corresponding genes from V. vinifera (cultivar, Pinot Noir) and V. labrusca (cultivar, Concord) harvested in Japan have been identified and characterized. Sequence analyses showed that the gene isolated from V. labrusca was identical with that from Muscat Bailey A, whereas that from V. vinifera had 98% identity. It was also demonstrated that the recombinant protein encoded by the gene from V. vinifera possessed similar characteristics to that from Muscat Bailey A, such as substrate specificity and the apparent Km values for furaneol (261 μM) and UDP-glucose (67 μM).

Relationship between UGT85K14 expression and contents of furaneol and its glucoside in grape berry

The concentrations of furaneol and its glucoside were analysed by HPLC-MS/MS, and UGT85K14 expression was quantified by real-time PCR using two specific primer sets. In previous work, it was demonstrated that the contents of furaneol and its glucoside were higher in Concord (V. labrusca) than Muscat Bailey A (V. labrusca × V. vinifera), and were almost undetectable in several cultivars of V. vinifera (Sasaki et al., 2015). In contrast, UGT85K14 expression was detected in all the cultivars regardless of species (Fig. 5), suggesting that the content of furaneol glucoside was regulated by the biosynthesis of furaneol.

A previous study has revealed that furaneol and its glucoside were mainly accumulated in Muscat Bailey A berry flesh (Sasaki et al., 2015). As the ratio of flesh weight to berry weight was approximately 95%, the concentrations of furaneol and its glucoside in the skin were higher than those in the flesh (Fig. 6A). In accordance with this result, UGT85K14 expression in the skin was higher than that in the flesh; however, UGT85K14 expression in the seed was higher than that in the flesh (Fig. 6B). HPLC-MS/MS analyses also showed that furaneol glucoside content, as well as furaneol content, increased at the late ripening stage (Fig. 6C) (Kobayashi et al., 2013), whereas UGT85K14 expression was high in mid-September and reduced thereafter (Fig. 6D).

Discussion

As aroma is one of the most important factors affecting wine quality, many aroma compounds have been isolated from a large variety of wines and grapevines. Some aroma
compounds are reported to exist in the odourless form, such as glutathione and glycoside conjugate, in grapevine (Rapp et al., 1980; Schreier and Paroschy, 1981; Shure and Acree, 1994; Kobayashi et al., 2013) as well as many other plants (Krammer et al., 1991; Ohta et al., 1991; Fujita and Nakayama, 1992; Chassagne et al., 1996; Nishikitani et al., 1996). Glycosylation is one of the major modifications for natural products, and contributes to the transport, accumulation, and detoxification of some unstable and toxic acceptor molecules, followed by changes of physiological properties, such as increased solubility in water and stability. Furaneol is an important aroma compound in many fruits, and is also responsible for the characteristic aroma of several wines as a strawberry-like note. Recently, it was shown that furaneol glucoside was a precursor of wine aroma in the hybrid grapevine cultivar Muscat Bailey A (V. labrusca × V. vinifera) (Sasaki et al., 2015). In this study, UDP-glucose: furaneol glucosyltransferase gene (GlyT_07, UGT name: UGT85K14) was isolated from Muscat Bailey A, and its heterologous expression in E. coli was confirmed.

The candidate genes (GlyT_07, 17, 21) were randomly selected from the uncharacterized genes that possessed the ripening-related expression pattern, using the gene expression analysis data (Sweetman et al., 2012). All the three clones possessed the 44 amino acid signature motif in the C-terminal region designated as the PSPG-box (Ross et al.,...
that furaneol glucoside is controlled by the *UGT85K14* expression along with the biosynthesis of furaneol.

Several genes involved in the biosynthesis of furaneol and its derivative have been isolated from strawberry and grape. *O*-Methyltransferase gene (*FaOMT*) catalysing the methylation of furaneol was isolated from strawberry (*Fragaria × ananassa*) (*Zorrilla-Fontanesi et al., 2012*). The enone oxidoreductase gene (*FaQR*) catalysing the formation of furaneol was also isolated from strawberry (*Raab et al., 2006*). The expression patterns of those genes were found to coincide with the accumulation patterns of their metabolic products. As the metabolic product catalysed by OMT, 2,5-dimethyl-4-methoxy-3(2H)-furaneone, has not been identified yet in grape, it is not clear whether the *FaOMT* orthologous genes are present or not in grape. On the other hand, *FaQR* orthologous genes may exist and regulate the contents of furaneol and its derivatives in grape. Actually, a homologous gene of *FaQR* was found in the genome data of grape (http://www.plantgdb.org/VvGDB/) (accession no. GSIVL011013734001), and the partial amino acid sequence showed 73% identity with that of *FaQR*.

**UDP-glucose: furaneol glucosyltransferase (*UGT85K14*)** was isolated from only grape in this study. As furaneol glucoside was isolated from other fruits such as strawberry, *UGT85K14* could serve as a powerful tool for isolating glucosyltransferases of furanones from them. Actually, a homologous gene of *UGT85K14* was found in the genome data of strawberry (*Fragaria vesca* ssp. *vesca*) (accession No. XP_004306404), and the amino acid sequence of that gene showed 66% identity with that of *UGT85K14*. Therefore, that gene may be involved in the biosynthesis of furaneol glucoside in strawberry. The metabolic pathway of furaneol has not been exhaustively clarified in plant yet. The identification of genes involved in the pathway might help further understanding of furaneol metabolites.

### Supplementary data

Supplementary data are available at *JXB* online.

- **Table S1.** Putative glycosyltransferase genes strongly expressed at the late ripening stage.
- **Table S2.** PCR primers.
- **Figure S1.** SDS-PAGE analysis of the soluble recombinant His-tagged *UGT85K14*.
- **Figure S2.** Chemical structures of acceptors for substrate specificity analysis.

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