JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

# Biotransformation of Mogrosides from *Siraitia grosvenorii* Swingle by *Saccharomyces cerevisiae*

Chun-Hui Chiu, Reuben Wang, Cho-Ching Lee, Yi-Chen Lo,* and Ting-Jang Lu*

Institute of Food Science and Technology, National Taiwan University, 59, Lane 144, KeeLung Road, Sec. 4 Taipei 106, Taiwan, ROC

**ABSTRACT:** Mogrosides are a group of triterpenoidal saponins from the fruit of *Siraitia grosvenorii* Swingle; they are intensely sweet and have consequently been used as a substitute for sugar by the food industry. The lack of efficient methods to produce specific mogrosides has hindered investigation of the relationship between their structure and bioactivity, e.g., down-regulation of blood glucose levels, anti-inflammation, and antiviral infection. Here, we attempt to selectively convert the major saponin mogroside V, a mogrol pentaglucoside, into mogroside III E, a triglucoside, via the β-glucosidases of the budding yeast *Saccharomyces cerevisiae*. We report that the β-glucopyranosyl and β-glucopyranosyl-(1→2)-β-D-glucopyranosyl attached on C-3 and -24 of mogrol, respectively, were resistant to hydrolysis by yeast β-D-glucosidases. We further screened 16 mutants bearing single defective glucanase or glucosidase genes, thereby demonstrating that Exg1 is a major enzyme of the initiation of mogroside V conversion. Deletion of the *KRE6* gene unexpectedly facilitated the production of mogroside III E in yeast culture. This paper demonstrates that yeast knockout mutants are a valuable tool for saponin modification and for studying the specificity of glucosidase function.

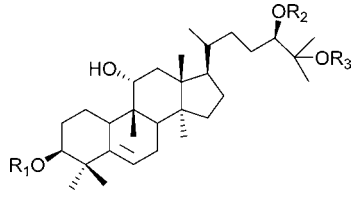
**KEYWORDS:** *biotransformation, mogroside, yeast, beta-glucosidase, EXG1, KRE6*

## INTRODUCTION

The fruit of *Siraitia grosvenorii* Swingle, Lo Han Kuo (LHK), contains a mixture of curcurbitane-type triterpene saponins, known as mogrosides, which consist of several glycosylated sugar moieties which form β-linkages with mogrol (MG; aglycone) (Table 1).[1] In traditional Chinese medicine, *S. grosvenorii* fruit is considered to be a nontoxic sweetener, capable of moisturizing lungs and soothing coughs, reducing blood pressure, and preventing constipation.[2] Mogrosides have recently been classified as "generally recognized as safe" (GRAS) substances by the U.S. Food and Drug Administration (FDA).[3] Several studies have reported that mogroside extracts have various biological activities, including antioxidative,[4] anti-inflammatory,[5] anticarcinogenic,[6] and anti-infective (against human herpes virus IV[7]) properties. In addition, the bioactivities of certain specific mogrosides have been described. For example, mogroside V (MG-V) (which contains five glucose molecules attached to mogrol) was shown to regulate insulin secretion in an in vitro cell model system,[8] and mogroside III (MG-III) inhibited intestinal maltase and suppressed the rise in blood glucose after a single oral administration of maltose in rats.[9] However, studies into the bioactivities of specific mogrosides have been hindered by a lack of techniques for converting these complex chemicals.

Several attempts have been made to perform mogroside transformation. Crude LHK extracts have been hydrolyzed by chloric acid into various forms of mogroside, including MG-IV A, MG-III $A_1$, MG-III $A_2$, MG-II $A_1$, MG-II $A_2$, and MG-I A.[10] In addition, crude enzyme extracts derived from human intestinal bacteria were used to transform MG-III into MG-II $A_1$ and MG,[11] and maltase or cellulase were used to convert MG into MG-IV E, MG-III E, MG-II E, MG-I $E_1$, and MG-I $E_2$ under stringent conditions.[12] However, chemical reactions, such as acid hydrolysis, result in low yields and poor mogroside specificity.[13] An alternative approach is bioconversion, which

**Table 1. Chemical Structure of Mogrosides**




| compd name | $R_1$ (C-3) | $R_2$ (C-24) | $R_3$ | MW |
|---|---|---|---|---|
| mogroside V (MG-V) | Glc6-Glc- | Glc6-Glc2-Glc | H | 1286 |
| siamenoside I (S-I) | Glc- | Glc6-Glc2-Glc- | H | 1124 |
| mogroside IV (MG-IV) | Glc6-Glc- | Glc2-Glc- | H | 1124 |
| mogroside IV A (MG-IV A) | Glc6-Glc- | Glc6-Glc- | H | 1124 |
| mogroside III (MG-III) | Glc- | Glc6-Glc- | H | 962 |
| mogroside III $A_1$ (MG-III $A_1$) | H | Glc6-Glc2-Glc- | H | 962 |
| mogroside III $A_2$ (MG-III $A_2$) | Glc6-Glc- | Glc | H | 962 |
| mogroside III E (MG-III E) | Glc- | Glc2-Glc- | H | 962 |
| mogroside II A (MG-II A) | H | Glc2-Glc- | H | 800 |
| mogroside II $A_1$ (MG-II $A_1$) | H | Glc6-Glc- | H | 800 |
| mogroside II $A_2$ (MG-II $A_2$) | Glc6-Glc- | H | H | 800 |
| mogroside II B (MG-II B) | Glc- | H | Glc- | 800 |
| mogroside II E (MG-II E) | Glc- | Glc- | H | 800 |
| mogroside I $A_1$ (MG-I $A_1$) | H | Glc- | H | 638 |
| mogroside I $E_1$ (MG-I $E_1$) | Glc- | H | H | 638 |
| mogrol (MG) | H | H | H | 476 |

**Received:** May 12, 2013
**Revised:** June 21, 2013
**Accepted:** June 24, 2013
**Published:** June 24, 2013

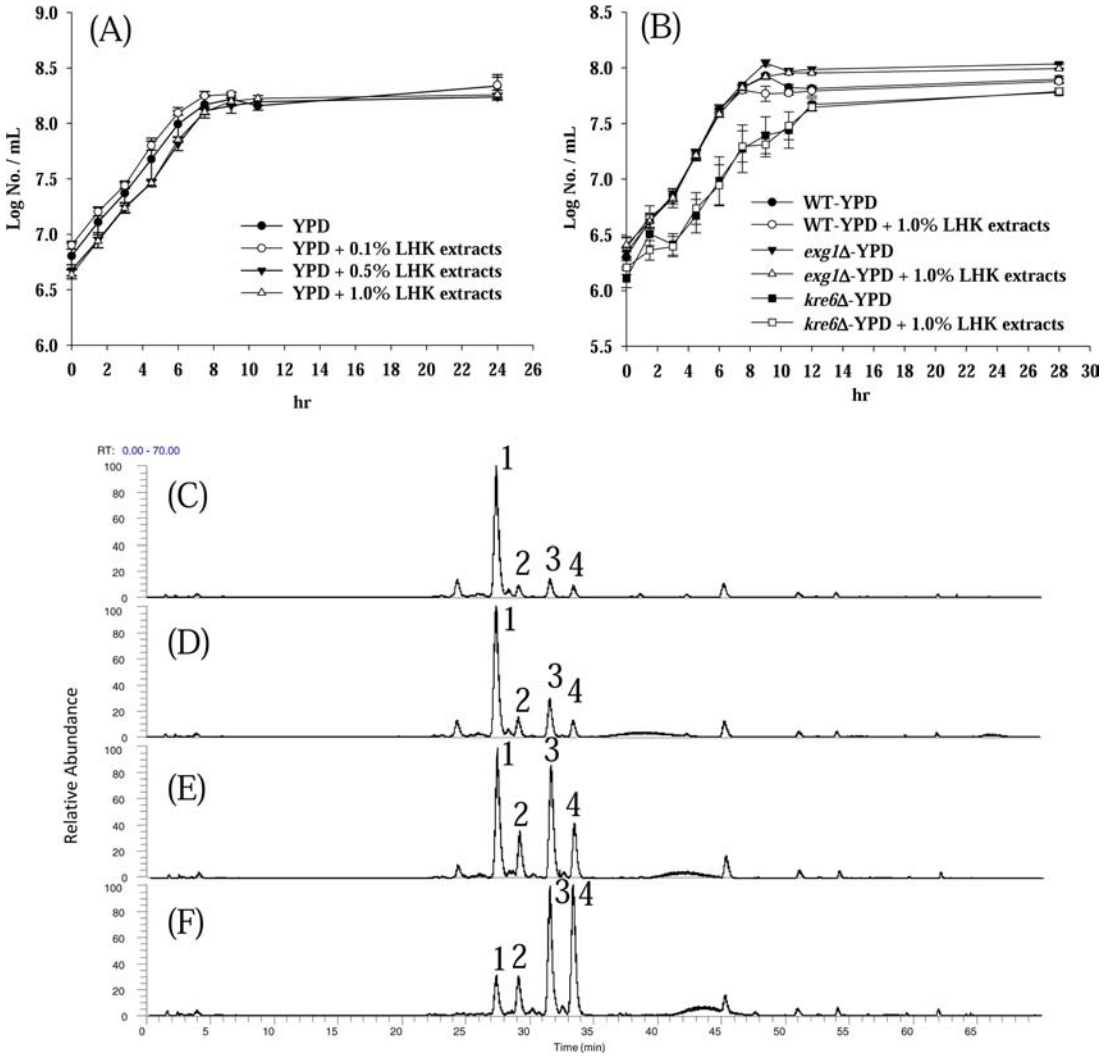



**Figure 1.** Mogrosides do not affect cell growth. (A) Wild-type cells were grown in YPD media with the indicated concentrations of LHK extract. (B) The *exg1*Δ and *kre6*Δ mutant strains were grown in YPD media with 1% LHK extracts. Cell growth was estimated based on optical density (O.D.) values at a wavelength of 600 nm. Data are presented as the mean ± SD from three independent experiments. (C–F) LC-MS chromatograms of mogrosides cultured with wild-type cells for (C) 0, (D) 2, (E) 4, and (F) 6 h. Peak 1, mogroside V (MG-V); peak 2, siamenoside I (S-I); peak 3, mogroside IV (MG-IV); and peak 4, mogroside IIIE (MG-III E).

has been applied to various food systems to obtain bioactive products; for example, *Aspergillus niger* has been used to transform ginsenoside $Rb_2$ into compound K,[14] and probiotic bacteria in combination with yeast *Saccharomyces boulardii* can greatly enrich the amount of isoflavones in fermented soymilk.[15] In yeast, glycoside hydrolases, such as *β*-glucosidase and glucanase, are often involved in cell wall synthesis and metabolism.[16] These enzymes may be able to hydrolyze glycosidic bonds in mogrosides, and as such, we investigated whether the budding yeast *S. cerevisiae* is suitable for bioconversion of mogrosides from *S. grosvenorii*. The availability of yeast deletion sets (containing more than 4600 strains with single deletions of nonessential genes) together with the gene functional annotations in the *S. cerevisiae* genome database (SGD) enabled us to identify specific genes required for mogroside conversion. This revealed that *EXG1* and *KRE6* initiate mogroside hydrolysis and promote MG-III E production in yeast, respectively.

## MATERIALS AND METHODS

**Yeast Strains, Media, and Cell Growth.** The *Saccharomyces cerevisiae* EUROSCARF strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) was used as a background strain in this study. Wild-type cells and mutants (including *exg1*Δ, *kre6*Δ, and *skn1*Δ) were cultured in yeast extract–peptone–dextrose (YPD) media (1% yeast extract, 2% peptone, and 2% glucose), with or without LHK extracts containing 25.9% mogroside, at 30 °C in an orbital shaker at a speed of 200 rpm. Cell growth was determined every 2 h based on $OD_{600}$ values and is presented as log no. cells/mL. Media were collected during cell fermentation and analyzed by HPLC-ESI-MS.

**Complementation Assay.** Plasmids carrying the ORF of the *EXG1* (pGal-*EXG1*-HA) or *KRE6* (pGal-*KRE6*-HA) gene, a *URA3* selection marker, and *GAL1* promoter sequences were obtained from the Thermo Scientific Open Biosystems Yeast ORF Collection (Waltham, MA, USA). The plasmids were amplified and purified from *E. coli* using a High-Speed Plasmid Mini Kit (Geneaid, New Taipei City, Taiwan). Plasmids pGal-*EXG1*-HA or pGal-*KRE6*-HA were used to complement the corresponding *exg1*Δ or *kre6*Δ mutant, enabling overexpression of EXG1 or KRE6 in mutants by galactose induction. Induction was performed in the presence or absence of LHK extracts. Cells were harvested at various time points during galactose induction, and protein expression was analyzed by Western blot.

**Western Blot Analysis.** Cells were cultured in yeast media with or without LHK extracts and were then collected for Western blot analysis. Total proteins were extracted using SUMEB (1% SDS, 4 M urea,

0.1 M MOPS, and 0.1 M EDTA). Total cellular proteins were resolved by electrophoresis on a 15% SDS-polyacrylamide gel. Resolved proteins were transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, MA, USA) and then probed first with primary antibodies (anti-HA or antiglucose-6-phosphate dehydrogenase (G6PDH) as a loading control), and then with secondary antibodies (antirat for HA and antirabbit for G6PDH). The proteins were visualized using a chemiluminescent kit (Immobilon Western, Millipore, MA, USA) and imaged using the BioSpectrum Imaging System (UVP, CA, USA). Band intensities were analyzed using VisionWorksLS (UVP, CA, USA) software.

**Table 2. Major Functions of Genes Selected for Deletion**

| gene name | systematic name | function[a] |
|---|---|---|
| ATG26 | YLR189C | UDP-glucose/sterol glucosyltransferase |
| BGL2 | YGR282C | endo-$\beta$-1,3-glucanase |
| CRR1 | YLR213C | putative glycoside hydrolase of the spore wall envelope |
| CWH41 | YGL027C | glucosidase I |
| DSE2 | YHR143W | daughter cell-specific secreted protein with similarity to glucanases |
| EXG1 | YLR300W | major exo-1,3-$\beta$-glucanase of the cell wall |
| HPF1 | YOL155C | haze-protective mannoprotein with glucosidase activity |
| IMA1 | YGR287C | major isomaltase ($\alpha$-1,6-glucosidase/ $\alpha$-methylglucosidase) |
| IMA5 | YJL216C | $\alpha$-glucosidase |
| KRE6 | YPR159W | type II integral membrane protein required for $\beta$-1,6 glucan biosynthesis; putative $\beta$-glucan synthase |
| SKN1 | YGR143W | type II membrane protein with similarity to Kre6p |
| SPR1 | YOR190W | sporulation-specific exo-1,3-$\beta$-glucanase |
| SUC2 | YIL162W | invertase, sucrose-hydrolyzing enzyme |
| SUN4 | YNL066W | cell wall protein related to glucanases |
| SCW4 | YGR279C | cell wall protein with similarity to glucanases |
| YIR007W | YIR007W | putative glycosidase |

[a]Gene functions were annotated according to SGD.

**Mogroside Analysis by HPLC-ESI-MS.** Mogrosides from culture media were purified by taking the fraction eluted out with 45−80% of methanol and performing separation on a reversed-phase C-18 solid-phase-extraction cartridges (500 mg/3 mL, Chrome expert, Sacramento, CA, USA). Mogrosides were separated and identified by HPLC-DAD-ESI-MS. The system included a HPLC equipped with a quaternary pump, autosampler (25 $\mu$L), a photodiode array detector (200−600 nm) (Surveyor, San Jose, CA, USA), and a Thermo Finnigan model LXQ linear ion trap mass spectrometer (San Jose, CA, USA) operated at negative ion electrospray mode. The YMC Hydrosphere C18 analytical column (2.0 × 150 mm, 5 $\mu$m, YMC, Kyoto, Japan) was used and maintained at room temperature with a flow-rate set at 0.2 mL/min. The mobile phase consisted of water (Milli-Q, Millipore, Billerica, MA) and methanol (HPLC grade, Mallinckrodt Baker, Philipsburg, NJ, USA), both containing 0.01% formic acid (98−100% analytical grade, RdH, Seelze, Germany). Mogrosides were eluted with gradient methanol−water from 45:55 (v/v) to 100% methanol, sequentially. The ESI parameters were set as follows: spray voltage was −6.0 kV, capillary temperature was 400 °C, sheath gas was 21 arb, auxiliary gas was 10 arb, sweep gas was 5 arb, collision energy was 20%, isolation width was 2.0 Da, and scan range was 50−2000 $m/z$. Xcalibur 2.0.7 software was used to perform data analyses (San Jose, CA, USA). Standard curves were established and quantified by HPLC-ESI-MS using MG-V (98%, CHEMOS GmbH Co, Regenstauf, Germany), MG-IV, S-I, MG-III E, and MG-II A purified and collected from culture media containing LHK extracts. The purities of the isolated MG-IV, S-I, MG-III E, and MG-II A were 98.01%, 98.29%, 98.96%, and 80.54%, respectively, based on peak area in each HPLC chromatogram. The predominant molecular ion in mogroside ESI/MS$^n$ spectra was $[M - H]^-$, and sequential glucose loss (−162) was observed in the fragmentation pattern in

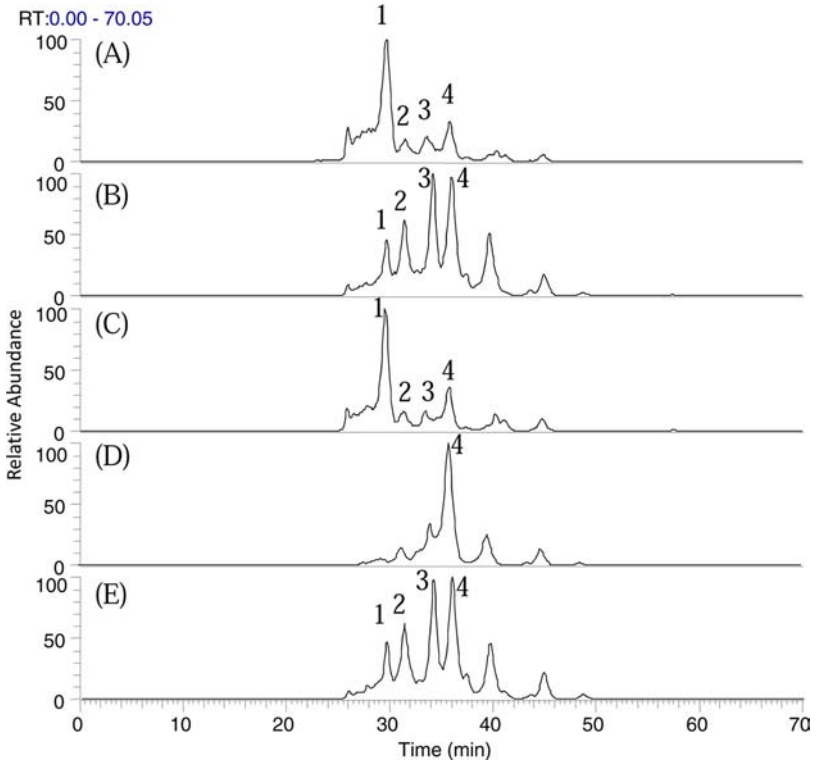



**Figure 2.** LC-MS chromatograms of mogrosides. (A) Mogroside profiles from LHK water-extract solutions. Representative LC-MS chromatograms from (B) wild-type cells, (C) *exg1*Δ mutants, (D) *kre6*Δ mutants, and (E) *skn1*Δ mutants fermented with LHK extracts after 24 h of incubation. Peak 1, mogroside V (MG-V); peak 2, siamenoside I (S-I); peak 3, mogroside IV (MG-IV); and peak 4, mogroside IIIE (MG-III E).

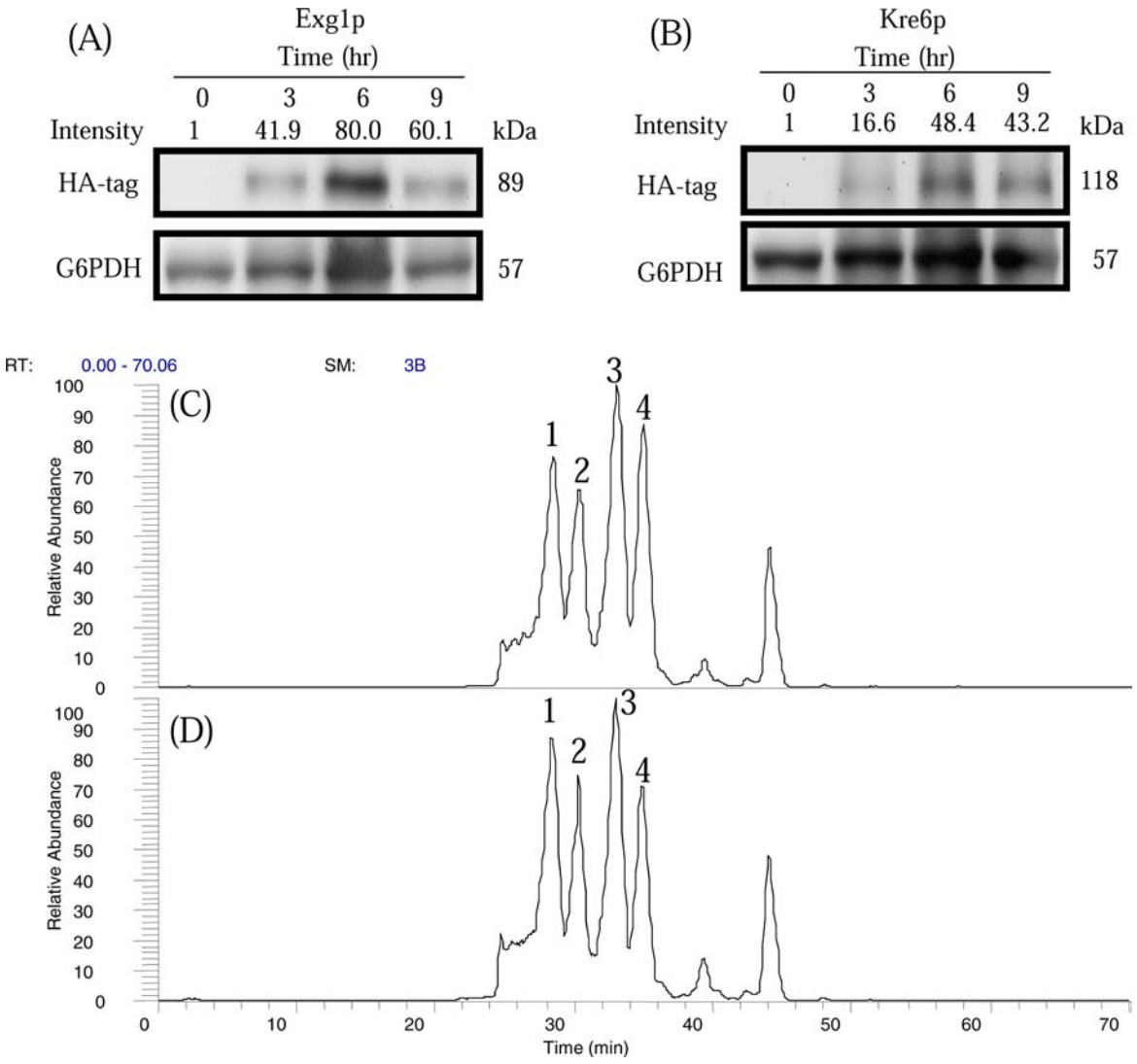



**Figure 3.** Overexpression of Exg1p and Kre6p rescues the mutant phenotypes. Western blots results revealing that transformation with (A) pGal-*EXG1*-HA and (B) pGal-*KRE6*-HA plasmids restored the levels of EXG1p and KRE6p after 6 h of galactose induction in *exg1*Δ and *kre6*Δ mutants, respectively. LC-MS chromatograms of mogrosides in complemented (C) *exg1*Δ and (D) *kre6*Δ cells. Peak 1, mogroside V (MG-V); peak 2, siamenoside I (S-I); peak 3, mogroside IV (MG-IV); and peak 4, mogroside III E (MG-III E).

collision-induced dissociation mode. MG-V, S-I, MG-IV, MG-III E, MG-II A, MG-I, and MG were identified based on the presence of ions with $m/z$ of 1285, 1123, 1123, 961, 799, 637, and 475, respectively. MG-V was assigned as the 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside]-24-*O*-[*β*-D-glucopyranosyl-(1→2)]-[*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranoside] of MG[1,17,18]

**$^{1}$H and $^{13}$C NMR Spectroscopy.** Mogrosides were dissolved in pyridine-d5 (99.8%, Merck, Darmstadt, Germany). $^{1}$H and $^{13}$C NMR spectra and COSY, NOSEY, HSQC, and HMBC were recorded using a 600 MHz spectrometer (Bruker DMX-600 NMR, Fallanden, Switzerland). Mogroside chemical structures were confirmed by NMR analysis, as described previously.[1,19]

**Statistical Analysis.** Data are presented as the mean ± standard error. Statistical analysis was performed using one-way ANOVA followed by Duncan's new multiple range test (SAS 9.3 software). A $p$ value of <0.05 was considered to be statistically significant.

## RESULTS

**LHK Extracts Have No Detectable Toxic Effect on Cell Growth.** In order to investigate the mechanisms of mogroside bioconversion by yeast, we first evaluated the effects of LHK extracts on cell growth. We compared the growth rates of yeast cells in YPD media containing 0.1, 0.5, or 1.0% of LHK extracts to that of control cells grown in YPD without the extract. We found that the growth of wild-type cells was not affected by the tested concentrations of LHK extract (Figure 1A). In addition, 1.0% of LHK extract did not affect the growth of *exg1*Δ or *kre6*Δ cells, although *kre6*Δ cells exhibit a slow growth phenotype under all conditions tested (Figure 1B). Therefore, LHK extracts have no detectable toxic effects on cell growth. The decreased growth rate of *kre6*Δ may be due to disruptions in *β*-glucan cell wall synthesis and daughter cell formation in the absence of Kre6p.[20]

**Bioconversion of Mogrosides.** Glycoside hydrolases and in particular glucosidases are widespread in eukaryotic organisms and play a pivotal role in many biological processes, including cell wall synthesis and degradation.[21] We hypothesized that mogrosides may undergo bioconversion by yeast through one or more of these enzymes. To test this, we used LC-MS to detect mogroside metabolites in the culture media of wild-type cells incubated for 2, 4, or 6 h. This revealed that MG-V is the major compound in LHK extracts (Figure 1C).

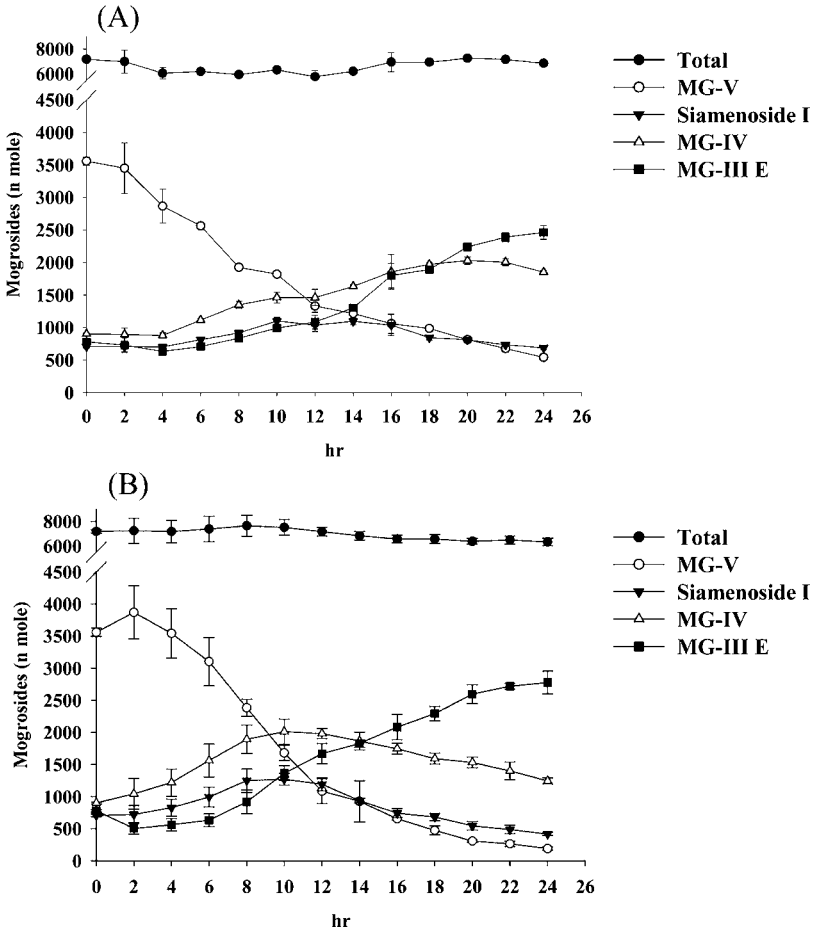



**Figure 4.** Mogroside content in cells during 24 h of culture with LHK extracts. Total and individual mogrosides in (A) wild-type cells and (B) *kre6*Δ mutants.

After incubating cells with LHK extracts for 6 h, mogroside bioconversion was observed (Figure 1D−F). MG-V was converted to S-I through the removal of one glucose moiety from MG-V's C-3 position, MG-IV through the removal of one glucose from MG-V's C-24 position, and MG-III E through the removal of glucose residues from MG-V's C-3 and C-24 positions (Figures 1D−F and 5). Although trace amounts of MG-II A, MG-I, and mogrol aglycone were detected in LHK extracts, concentrations of these mogrosides were not altered by bioconversion. These results demonstrate that the major mogroside in LHK extracts, MG-V, can be modified through whole-cell bioconversion by yeast.

**Effect of β-Glucosidase Knockout on the Bioconversion Patterns of Mogrosides.** The fermentation products of MG-V, including S-I, MG-IV, and MG-III E, differ in their pattern of glucosyl links to aglycone. The glucosyl residues are linked through glycosidic bonds, and thus, their removal from MG-V may occur through hydrolysis by yeast glucosidases or glucanases during fermentation. As such, we hypothesized that the deletion of specific genes encoding glucosidases or glucanases may alter the metabolic process, resulting in the production of a specific mogroside. Taking advantage of the complete *Saccharomyces cerevisiae* genome database, we searched for glucosidase or glucanase genes that may affect the biotransformation patterns of MG-V. We selected 16 mutants (Table 2), which bear a single glucosidase or glucanase gene deletion, and examined whether MG-V bioconversion was altered in these strains. Remarkably, we found that *exg1*Δ mutants lost the ability to convert MG-V into other glycosylated compounds. Thus, the LC-MS chromatograms of mogrosides in *exg1*Δ mutants were similar to those of LHK extracts (Figure 2A and C). However, MG-III E was the main bioconverted compound in *kre6*Δ mutants (Figure 2D), while *SKN1* (*SKN1*, a homologue of *KRE6*) deletion did not affect the wild-type pattern (Figure 2B and E).

To further verify the functions of *EXG1* and *KRE6* in the LHK fermentation process, we introduced pGal-*EXG1*-HA and pGal-*KRE6*-HA plasmids (encoding the full-length DNA sequences of *EXG1* and *KRE6*, respectively) into *exg1*Δ and *kre6*Δ mutants, respectively. Expression of the recombinant proteins was confirmed by Western blot, using G6PDH as a loading control. Expression of Exg1-HA and Kre6-HA peaked at 6 h after galactose induction (Figure 3A and B). Cells expressing these recombinant proteins were then incubated with LHK extracts to determine whether these cells could process mogrosides in a manner similar to that of wild-type cells. Indeed, both complemented mutant strains regained their ability to convert MG in a wild-type pattern (Figure 3C and D).

**Efficiency of Mogroside Bioconversion.** To examine the degree and efficiency of mogroside biotransformation, we determined the levels of each mogroside and total mogroside content in wild-type and *kre6*Δ cells over a 24 h incubation period with LHK extract. We found that total mogroside contents remained constant during this period in both wild-type and *kre6*Δ cells (Figure 4A and B). However, a nonlinear response was observed for various types of mogroside.


dx.doi.org/10.1021/jf402058p | J. Agric. Food Chem. 2013, 61, 7127−7134

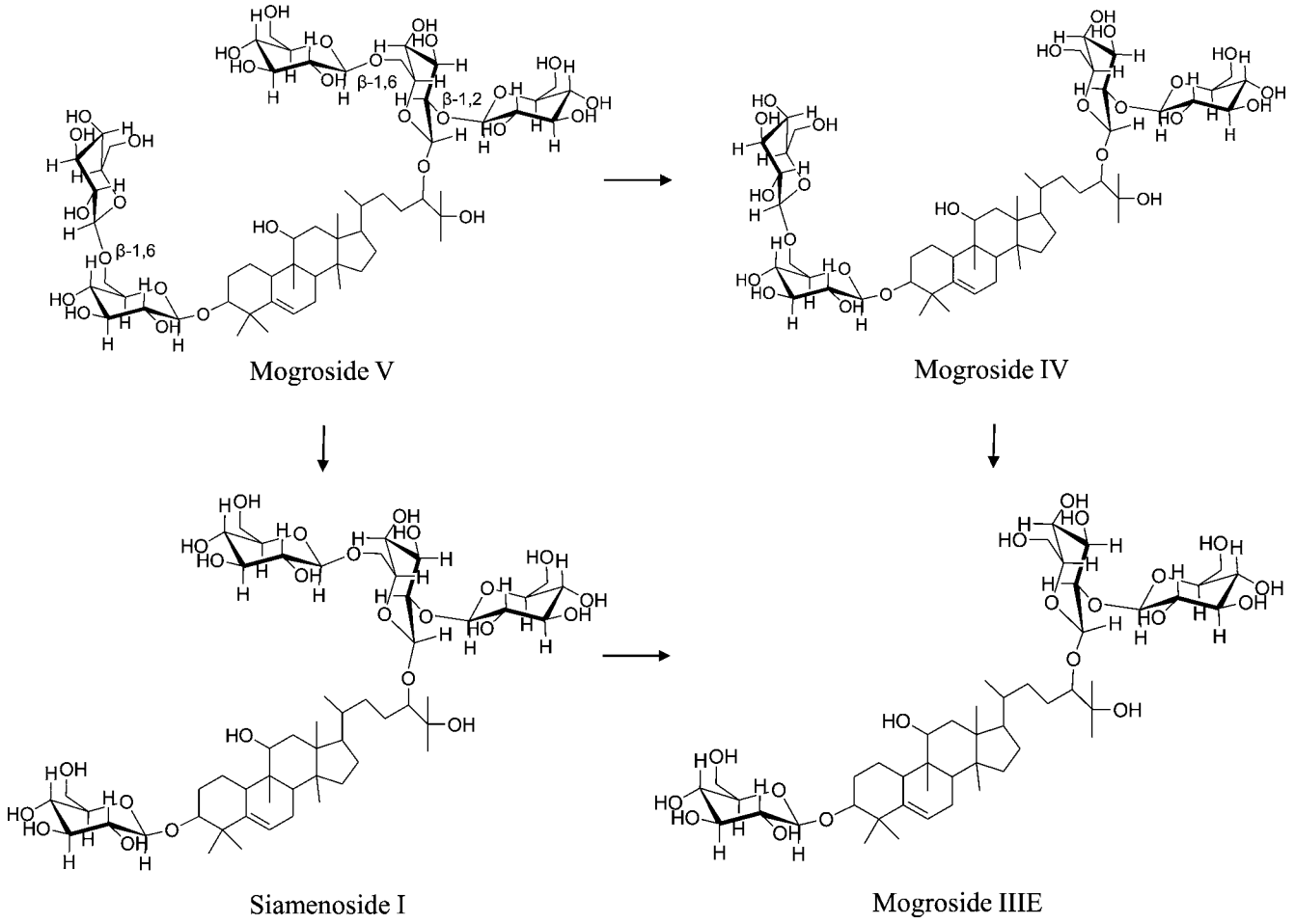



**Figure 5.** Presumed biotransformation pathway of mogroside V during the fermentation process. Mogroside V is transformed to mogroside III E via siamenoside I and mogroside IV.

Therefore, a nonlinear model was applied to describe the data, which were fitted to the equation $y = y_{min} + (y_{max} - y_{min})/(1 + (x/EC_{50})^{Hill\ slope})$[22,23] using Sigmaplot 9.0 software. The Hill slope represents how rapidly the response moves from the minimum to the maximum levels. The minimum and maximum asymptotes are abbreviated as min and max. $x$ represents the time required for 50% mogroside conversion.

We report that mogroside V steadily degraded into siamenoside I and mogroside IV through the hydrolysis of peripheral (1,6)-$\beta$-D-glucosyl during yeast growth. The reduction rate of MG-V was significantly lower ($p < 0.05$) in wild-type cells (Hill slope = −1.75 ± 0.23) than in *kre6*Δ mutants (Hill slope = −3.42 ± 0.31) (Figure 4B). MG-III E accumulated dramatically ($p < 0.05$) during the degradation of MG-V; furthermore, the half-maximal amounts of MG-III E were significantly increased in *kre6*Δ mutants (1.31 ± 0.09 $\mu$ moles) as compared to wild-type cells (0.98 ± 0.06 $\mu$mol). Moreover, *kre6*Δ mutants required less time (14.05 ± 0.44 h) to reach a 50% increase in MG-III E as compared to wild-type cells (16.54 ± 0.95 h). Wild-type cells exhibited a mild increase of MG-IV and S-I. In *kre6*Δ mutants, however, MG-IV markedly increased during the early stages of fermentation but declined after 10 h of incubation, concomitant with the increase of MG-III E.

## DISCUSSION

In the present study, we have identified that Exg1 is a major enzyme involved in the initiation of mogroside bioconversion and that deletion of *KRE6* facilitates the production of MG-IIIE from LHK extracts in yeast. On the basis of these findings, we have proposed a possible MG-V biotransformation pathway in *Saccharomyces cerevisiae*, as shown in Figure 5. MG-V is transformed to MG-III E via S-I and MG-IV. The biotransformation of mogrosides primarily involves deglucosylation at the peripheral (1→6)-$\beta$-D-glucosyl linkages at the C-3 and C-24 positions of mogrol. We have also demonstrated that yeast knockout mutant sets are a valuable tool for identifying the functions of specific enzymes in food bioconversion.

Exg1p is the major exo-(1,3)-$\beta$-glucanase of the cell wall, and it is involved in cell wall $\beta$-D-glucan assembly.[24,25] Exg1p is known to hydrolyze *O*-$\beta$-D-glycosidic linkages at the nonreducing end of polymer chains, resulting in the release of glucose.[26] In vitro studies have demonstrated that recombinant yeast Exg1p has a broad specificity for (1→3)-$\beta$- and (1→6)-$\beta$-linkages but much stronger activity with (1→3)-$\beta$-D-glucans, such as laminaribiose.[27] However, the ability of purified yeast Exg1 to hydrolyze (1→6)-$\beta$-D-glucose-linked substrates, such as pustulan and amygdalin, was compromised.[27,28] Schmidt et al. reported that *EXG1* knockout yeast were unable to hydrolyze flavonoid glucosides.[21] In agreement with this finding, we demonstrated that wild-type *Saccharomyces cerevisiae* can hydrolyze mogrosides by degrading (1→6)-$\beta$-D-glucose linkages on MG-V and that deletion of the *EXG1* gene prevents MG-V conversion. Additionally, our results indicate that Exg1p cannot hydrolyze the $\beta$-glucopyranosyl and $\beta$-glucopyranosyl-(1→2)-$\beta$-D-glucopyranosyl attached on

C-3 and -24 of mogrol, respectively; earlier studies, however, have reported that purified Exg1 can hydrolyze 7- and 4-*O*-glucosides of isoflavones, flavonols, flavones, and flavanones in vitro.[21] Thus, our results suggest that Exg1p may recognize specific structures of aglycone and glucose.

We have also identified that cells lacking functional *KRE6* (killer toxin resistant 6) produce more MG-III E than wild-type cells. Several studies have indicated that Kre6p is involved in (1→6)-*β*-D-glucan biosynthesis and cell wall integrity in yeast.[20,29] However, it is unlikely that Kre6p directly inhibits the production of MG-III E. It is more likely that reduced cell wall synthesis in *kre6*Δ cells results in increased levels of Exg1 in the culture medium, thereby increasing MG-III E production. Indeed, deletion of *KRE6* alters cell wall ultrastructure, increasing the amount of amorphous regions.[30] Alterations in cell wall structure were previously associated with the hyper-production of extracellular enzymes in fungus.[31] However, it is possible that deletion of *KRE6* may enhance MG-III E production by facilitating the activity of other enzymes. Kurita et al. reported that Skn1p, a homologue of Kre6p in yeast, was detectable in the cell wall of *kre6*Δ cells but not of wild-type cells.[32] This implies that Skn1p may partially compensate for the loss of Kre6p. Therefore, we analyzed mogroside profiles in *skn1*Δ cells incubated with LHK extract. Interestingly, *skn1*Δ cells had mogroside conversion patterns similar to those of wild-type cells. This suggests that the enhanced and efficient production of MG-III E in *kre6*Δ cells may be due to compensatory Skn1p function. In conclusion, we have identified two gene (*EXG1* and *KRE6*) products that are involved in mogroside metabolism in *Saccharomyces cerevisiae*. The presence of Exg1p and the absence of Kre6p may contribute to the hydrolysis of natural glycosylated aglycone.

## AUTHOR INFORMATION


### Corresponding Author

*(Y.-C.L.) Tel: 886-2-3366-4123. Fax: 886-2-2362-0849. E-mail: loyichen@ntu.edu.tw. (T.-J.L.) Tel: 886-2-3366-4131. Fax: 886-2-2362-0849. E-mail: tjlu@ntu.edu.tw.



### Funding

This research was financially supported by National Science Council, Taiwan, and Internal Supporting Fund from the College of Bioresources and Agriculture, National Taiwan University, Taiwan.


### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS


We thank Dr. Tien-Hsien Chang (Academia Sinica, Taiwan) for providing pGal-*EXG1*-HA and pGal-*KRE6*-HA.


## REFERENCES


(1) Takemoto, T.; Arihara, S.; Nakajima, T.; Okuhira, M. Studies on the constituents of fructus Momordicae. III. Structure of mogrosides. *Yakugaku Zasshi* **1983**, *103*, 1167−1173.

(2) Pawar, R. S.; Krynitsky, A. J.; Rader, J. I. Sweeteners from plants-with emphasis on *Stevia rebaudiana* (Bertoni) and *Siraitia grosvenorii* (Swingle). *Anal. Bioanal. Chem.* **2013**, *405*, 4397−4407.

(3) FDA. U. S. GRAS Notice 000301: Luo Han Fruit 2010. U.S. Patent.

(4) Qi, X. Y.; Chen, W. J.; Zhang, L. Q.; Xie, B. J. Mogrosides extract from *Siraitia grosvenori* scavenges free radicals in vitro and lowers oxidative stress, serum glucose, and lipid levels in alloxan-induced diabetic mice. *Nutr. Res.* **2008**, *28*, 278−284.

(5) Di, R.; Huang, M. T.; Ho, C. T. Anti-inflammatory activities of mogrosides from *Momordica grosvenori* in murine macrophages and a murine ear edema model. *J. Agric. Food.Chem.* **2011**, *59*, 7474−7481.

(6) Mizushina, Y.; Akihisa, T.; Hayakawa, Y.; Takeuchi, T.; Kuriyama, I.; Yonezawa, Y.; Takemura, M.; Kato, I.; Sugawara, F.; Yoshida, H. Structural analysis of mogrol and its glycosidcs as inhibitors of animal DNA polymerase and human cancer cell growth. *Lett. Drug Des. Discovery* **2006**, *3*, 253−260.

(7) Ukiya, M.; Akihisa, T.; Tokuda, H.; Toriumi, M.; Mukainaka, T.; Banno, N.; Kimura, Y.; Hasegawa, J.; Nishino, H. Inhibitory effects of cucurbitane glycosides and other triterpenoids from the fruit of *Momordica grosvenori* on epstein-barr virus early antigen induced by tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate. *J. Agric. Food Chem.* **2002**, *50*, 6710−6715.

(8) Zhou, Y.; Zheng, Y.; Ebersole, J.; Huang, C. F. Insulin secretion stimulating effects of mogroside V and fruit extract of luo han kuo (*Siraitia grosvenori* Swingle) fruit extract. *Yao Xue Xue Bao* **2009**, *44*, 1252−1257.

(9) Suzuki, Y. A.; Murata, Y.; Inui, H.; Sugiura, M.; Nakano, Y. Triterpene glycosides of *Siraitia grosvenori* inhibit rat intestinal maltase and suppress the rise in blood glucose level after a single oral administration of maltose in rats. *J. Agric. Food Chem.* **2005**, *53*, 2941−2946.

(10) Chen, X. B.; Zhuang, J. J.; Liu, J. H.; Lei, M.; Ma, L.; Chen, J.; Shen, X.; Hu, L. H. Potential AMPK activators of cucurbitane triterpenoids from *Siraitia grosvenorii* Swingle. *Bioorg. Med. Chem.* **2011**, *19*, 5776−5781.

(11) Yang, X. W.; Zhang, J. Y.; Xu, W. Biotransformation of mogroside III by human intestinal bacteria. *Beijing Da Xue Xue Bao* **2007**, *39*, 657−662.

(12) Murata, Y.; Ogawa, T.; Suzuki, Y. A.; Yoshikawa, S.; Inui, H.; Sugiura, M.; Nakano, Y. Digestion and absorption of *Siraitia grosvenori* triterpenoids in the rat. *Biosci., Biotechnol., Biochem.* **2010**, *74*, 673−676.

(13) Park, C. S.; Yoo, M. H.; Noh, K. H.; Oh, D. K. Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 9−19.

(14) Chi, H.; Kim, D. H.; Ji, G. E. Transformation of ginsenosides Rb2 and Rc from Panax ginseng by food microorganisms. *Biol. Pharm. Bull.* **2005**, *28*, 2102−2105.

(15) Rekha, C. R.; Vijayalakshmi, G. Bioconversion of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soymilk by probiotic bacteria and yeast. *J. Appl. Microbiol.* **2010**, *109*, 1198−1208.

(16) Lante, F.; Cavalier, M.; Cohen-Solal, C.; Guiramand, J.; Vignes, M. Developmental switch from LTD to LTP in low frequency-induced plasticity. *Hippocampus* **2006**, *16*, 981−989.

(17) Takemoto, T.; Arihara, S.; Nakajima, T.; Okuhira, M. Studies on the constituents of fructus Momordicae. I. On the sweet principle. *Yakugaku Zasshi* **1983**, *103*, 1151−1154.

(18) Takemoto, T.; Arihara, S.; Nakajima, T.; Okuhira, M. Studies on the constituents of fructus Momordicae. II. Structure of sapogenin. *Yakugaku Zasshi* **1983**, *103*, 1155−1166.

(19) Matsumoto, K.; Kadai, R.; Ohtani, K.; TAnaka, O. Minor Cucurbitane-glycosides from fruits of *Siraitia grosvenori* (Cucurbitaceae). *Chem. Pharm. Bull.* **1990**, *38*, 2030−2032.

(20) Roemer, T.; Bussey, H. Yeast beta-glucan synthesis: KRE6 encodes a predicted type II membrane protein required for glucan synthesis in vivo and for glucan synthase activity in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11295−11299.

(21) Schmidt, S.; Rainieri, S.; Witte, S.; Matern, U.; Martens, S. Identification of a *Saccharomyces cerevisiae* glucosidase that hydrolyzes flavonoid glucosides. *Appl. Environ. Microb.* **2011**, *77*, 1751−1757.

(22) Peoples, M. C.; Karnes, H. T. Microfluidic capillary system for immunoaffinity separations of C-reactive protein in human serum and cerebrospinal fluid. *Anal. Chem.* **2008**, *80*, 3853−3858.

(23) Vignes, M.; Maurice, T.; Lante, F.; Nedjar, M.; Thethi, K.; Guiramand, J.; Recasens, M. Anxiolytic properties of green tea

polyphenol (-)-epigallocatechin gallate (EGCG). *Brain Res.* **2006**, *1110*, 102−15.
(24) Stubbs, H. J.; Brasch, D. J.; Emerson, G. W.; Sullivan, P. A. Hydrolase and transferase activities of the *β*-1,3-exoglucanase of *Candida albicans*. *Eur. J. Biochem.* **1999**, *263*, 889−95.
(25) Ramírez, M.; Hernández, L.; Larriba, G. A similar protein portion for two exoglucanases secreted by *Saccharomyces cerevisiae*. *Arch. Microbiol.* **1989**, *151*, 391−398.
(26) Dealdana, C. R. V.; Correa, J.; Sansegundo, P.; Bueno, A.; Nebreda, A. R.; Mendez, E.; Delrey, F. Nucleotide sequence of the exo-1,3-beta-glucanase-encoding gene, Exg1, of the yeast *Saccharomyces cerevisiae*. *Gene* **1991**, *97*, 173−182.
(27) Suzuki, K.; Yabe, T.; Maruyama, Y.; Abe, K.; Nakajima, T. Characterization of recombinant yeast exo-*β*-1,3-glucanase (Exg 1p) expressed in *Escherichia coli* cells. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 1310−1314.
(28) Tingle, M. A.; Halvorson, H. O. A comparison of beta-glucanase and beta-glucosidase in *Saccharomyces lactis*. *Biochim. Biophys. Acta* **1971**, *250*, 165−171.
(29) Roemer, T.; Delaney, S.; Bussey, H. Skn1 and Kre6 define a pair of functional homologs encoding putative membrane-proteins involved in beta-glucan synthesis. *Mol. Cell. Biol.* **1993**, *13*, 4039−4048.
(30) Roemer, T.; Paravicini, G.; Payton, M. A.; Bussey, H. Characterization of the yeast (1→6)-beta-glucan biosynthetic components, Kre6p and Skn1p, and genetic interactions between the PKC1 pathway and extracellular-matrix assembly. *J. Cell Biol.* **1994**, *127*, 567−579.
(31) Gratzner, H. G. Cell wall alterations associated with the hyperproduction of extracellular enzymes in *Neurospora crassa*. *J. Bacteriol.* **1972**, *111*, 443−446.
(32) Kurita, T.; Noda, Y.; Yoda, K. Action of multiple endoplasmic reticulum chaperon-like proteins is required for proper folding and polarized localization of Kre6 protein essential in yeast cell wall beta-1,6-glucan synthesis. *J. Biol. Chem.* **2012**, *287*, 17415−17424.