separations

MDPI

Article

Improvement of Cucurbitacin B Content in *Cucumis melo* Pedicel Extracts by Biotransformation Using Recombinant β-Glucosidase

Jianfeng Mei [1], Xia Wu [1], Sujing Zheng [1], Xiang Chen [2,*], Zhuliang Huang [2] and Yichun Wu [2]

[1] College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310014, China; mrion@zjut.edu.cn (J.M.); shell-wu@163.com (X.W.); 2111907121@zjut.edu.cn (S.Z.)
[2] Zhoushan Institute for Food and Drug Control, Zhoushan 316021, China; huangzhuliang@zifdc.com (Z.H.); yichunwu@zifdc.com (Y.W.)
* Correspondence: xiang_chen@zifdc.com; Tel.: +86-580-2031172

Abstract: For the efficient biotransformation of cucurbitacin B 2-O-β-D-glucoside (CuBg) to cucurbitacin B (CuB) in *Cucumis melo* pedicel extracts, the β-glucosidase gene *bglS*—consisting of 1344 bp (447 amino acids) from *Streptomyces* sp. RW-2—was cloned and expressed in *Escherichia coli* BL21(DE3). The activity of recombinant β-glucosidase with *p*-nitrophenyl-β-D-glucoside (pNPG) as a substrate was 3.48 U/mL in a culture. Using the recombinant β-glucosidase for the biotransformation of *C. melo* pedicel extracts, CuBg was converted into CuB with a conversion rate of 87.6% when the concentration of CuBg was 0.973 g/L in a reaction mixtures. The concentration of CuB in *C. melo* pedicel extracts was improved from 13.6 to 20.2 g/L after biotransformation. The present study provides high-efficiency technology for the production of CuB from its glycoside by biotransformation.

Keywords: cucurbitacin B; cucurbitacin B 2-O-β-D-glucoside; *Cucumis melo* pedicel extracts; biotransformation; recombinant β-glucosidase

Citation: Mei, J.; Wu, X.; Zheng, S.; Chen, X.; Huang, Z.; Wu, Y. Improvement of Cucurbitacin B Content in *Cucumis melo* Pedicel Extracts by Biotransformation Using Recombinant β-Glucosidase. *Separations* **2021**, *8*, 138. https://doi.org/10.3390/separations8090138

Academic Editor: Paraskevas D. Tzanavaras

Received: 22 July 2021
Accepted: 26 August 2021
Published: 1 September 2021

1. Introduction

Cucurbitacin B (CuB) has been reported to show various biological activities, such as anti-inflammatory, anticancer, antimicrobial, antidiabetic, antiobesity, and antihyperglycemic effects [1–5]. In particular, the anticancer activity of CuB has attracted much attention because of its significant cytotoxic effects in more than 100 cancer cell lines [6]. Though CuB is not used worldwide as a drug at present, it has been considered to be the main active ingredient in extracts of *Cucumis melo* L. pedicel, which have been used as a traditional herbal medicine (*HuLuSu Pian*) for the digestive system and hepatic diseases for more than 20 years in China [7]. The content of CuB in *C. melo* pedicels ranges from 8 to 14 mg/g [8,9]. In fact, the glycoside of CuB, cucurbitacin B 2-O-β-D-glucoside (CuBg), is also abundant in *C. melo* pedicels. Our analysis showed that the contents of CuB and CuBg in *C. melo* pedicels from Bozhou (Anhui, China) were 13.6 and 9.73 mg/g, respectively. Due to the relatively low bioavailability of glycosides [10], CuBg is not considered to be an active ingredient in extracts of *C. melo* pedicel. If CuBg in *C. melo* pedicel is converted into CuB, the content of CuB can be markedly increased, which will then greatly increase the extraction yield of CuB. The conversion of CuBg to CuB can be accomplished through acid hydrolysis, but this method is not efficient. For example, when extracts from *C. melo* pedicels were treated by 0.1 mol/L acetic acid, the content of CuB was only increased by 7.46% [11].

Biotransformation by a microbial enzyme has high specificity and efficiency; it has been applied in the production of many natural drugs. In our previous study, a process for the biotransformation of CuBg to CuB using crude enzyme from *Streptomyces* sp. RW-2 was developed [12]. When *C. melo* pedicel extracts were used as the substrate, CuBg was specifically converted into CuB with an almost 100% conversion rate, and the concentration of CuB in *C. melo* pedicel extracts doubled after biotransformation. However,

with this method, the concentration of substrate is low, resulting in a low productivity. Using purified β-glucosidase from *Streptomyces* sp. RW-2 may improve biotransformation efficiency. However, the low production of β-glucosidase from *Streptomyces* may impede the application of this method. Using recombinant *Escherichia coli* to express heterologous enzymes has numerous advantages; in particular, it can increase enzyme production [13]. This method has been applied in the biotransformation of many natural products [14–18], resulting in improved biotransformation efficiency.

In the present study, the β-glucosidase gene *bglS* from *Streptomyces* sp. RW-2 was cloned and expressed in *Escherichia coli* BL21(DE3). Using the recombinant β-glucosidase for the biotransformation of *C. melo* pedicel extracts, the productivity of CuB was significantly improved. The biotransformation scheme of CuBg to CuB by β-glucosidase is illustrated in Figure 1.

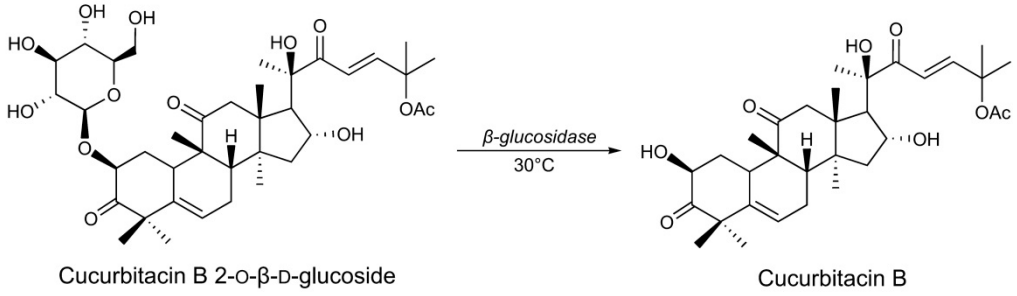

Figure 1. Biotransformation of CuBg to CuB by β-glucosidase.

2. Materials and Methods

2.1. Chemicals and Enzymes

Standard CuB was purchased from Sigma-Aldrich (St. Louis, MO, USA). CuBg (97.4% purity) was separated from *C. melo* pedicels by HPLC. DNA isolation, PCR, and plasmid isolation kits, as well as restriction enzymes, were obtained from Sangon Biotech (Shanghai, China). All other chemicals were of HPLC, analytical, or biochemical grade and obtained from local suppliers.

2.2. Strains and Plasmids

Streptomyces sp. RW-2, which was isolated from an enrichment culture of *C. melo* pedicels, was deposited at the China Center for Type Culture Collection (CCTCC, no. M2013330). *E. coli* BL21(DE3) cells from Tsingke Biological Technology (Beijing, China) were used for enzyme expression. The pET-28a (+) plasmid (Sangon Biotech, Shanghai, China) was used for cloning.

2.3. Construction of Recombinant E. coli

The degenerate primers (5′-AC(G/C)CT(G/C)T(A/T)CCACTGGGACCT-3′ and 5′-(G/C)GCCCA(C/T)TCGAAGTTGTC-3′) were designed based on the conserved sequences of β-glucosidase genes from known *Streptomyces* strains retrieved from NCBI (http://www.ncbi.nlm.nih.gov). Using the degenerate primers, a partial sequence of the β-glucosidase gene was amplified from the genomic DNA of *Streptomyces* sp. RW-2 via polymerase chain reaction (PCR), and compared by NCBI BLAST to obtain a known β-glucosidase gene sequence from a *Streptomyces* strain. According to this sequence, two primers (5′-tgggtcgcggatccGAATTCATGGCGATCGACGAGCGCGGC-3′ and 5′-tggtggtggtggtggtgCTCGAGTCAGCCGGTCCGGGGC-3′), which included *EcoR*I and *Xho*I restriction sites (underlined) and homologous arm sequences (lower case), were designed. The full-length β-glucosidase gene was amplified from genomic DNA of *Streptomyces* sp. RW-2, ligated into the pET-28a (+) expression vector, sequenced, and introduced into *E. coli* BL21(DE3) by the standard method, thus generating recombinant *E. coli* BL21-bglS.

2.4. Expression of Recombinant β-Glucosidase

Pre-cultures of *E. coli* BL21-bglS were prepared in a Luria-Bertani (LB) medium containing 50 μg/mL of kanamycin and cultured overnight in a shaking incubator at 37 °C and 200 r/min. Aliquots (2.5 mL) of the pre-culture were inoculated into fresh LB containing kanamycin. β-Glucosidase expression was evaluated by culturing at 37 °C and 200 r/min until an OD_{600} of 0.6 was reached; then, 0.5 mmol/L IPTG was added to induce enzyme expression at 25 °C and 200 r/min for a further 10 h.

2.5. Enzyme Essay

BL21-bglS cells were harvested from 50 mL cultures by centrifugation at 8000× *g* for 5 min, washed twice with deionized water, resuspended in 5 mL phosphate-buffered saline (PBS; 0.2 mol/L, pH 6.0), and then disrupted by sonication. The cell lysates were centrifuged at 8000× *g* for 10 min. Then, the supernatant fraction was used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and a β-glucosidase activity assay.

The expression of the recombinant β-glucosidase was confirmed by SDS-PAGE using 12% (*w/v*) polyacrylamide gels [19].

The β-glucosidase activity was assayed using *p*-nitrophenyl-β-D-glucoside (pNPG) as a substrate [20]. The enzymatic reaction mixtures (100 μL) containing 50 μL of the enzyme preparation and 50 μL of pNPG (5 mmol/L in PBS) were incubated for 15 min at 30 °C in a 96-well microtiter plate. After the addition of 50 μL of 0.4 mol/L Na_2CO_3 to the reaction, the amount of *p*-nitrophenol (pNP) released was measured at 410 nm on a microplate reader. One enzyme unit (U) was defined as the amount of enzyme that released 1 μmol pNP per minute in the abovementioned conditions.

2.6. Biotransformation Process

CuBg, dissolved in 1 mL of methanol or 1 mL *C. melo* pedicel extracts, was mixed with 1 mL of cell lysates and 8 mL of PBS in a 50 mL flask. Biotransformation was performed at 30 °C and 200 r/min for 16 h. After biotransformation, the mixtures were extracted twice with 10 mL of ethyl acetate. After the evaporation of the solvent, the residue was dissolved in 1 mL of methanol, filtered, and analyzed by high performance liquid chromatography (HPLC).

The preparation of *C. melo* pedicel extracts has previously been described [12]. Extracts from *C. melo* pedicels (10 g) were dissolved in 10 mL of ethanol, resulting in concentrations of CuBg and CuB of 9.73 and 13.6 g/L, respectively.

2.7. Analysis of Biotransformation Products

The quantification of CuB and CuBg was performed by HPLC on a Shimadzu SPD-20A (Kyoto, Japan) equipped with a Zorbax Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm). The mobile phase was methanol-water solution (60:40, *v/v*), at a flow rate of 1 mL/min. The constituents were detected at 228 nm.

The presence of CuBg and CuB in *C. melo* pedicel extracts after biotransformation was confirmed by liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) on an LCQTM Deca XP plus mass spectrometer (Thermo-Fisher, Waltham, MA, USA). The capillary temperature was 350 °C, and the capillary voltage was 4.0 kV.

3. Results and Discussion

3.1. Cloning of bglS and Sequence Analysis

An 836 bp nucleotide sequence was cloned from genomic DNA of *Streptomyces* RW-2 by PCR with degenerate primers. The NCBI BLAST analysis of this sequence showed that it had 99.4% similarity to a β-glucosidase gene from *Streptomyces nigra* strain 452 (GenBank accession no. AWE50172). According to this β-glucosidase gene sequence, two primers were designed to clone the full-length β-glucosidase gene from the genomic DNA of *Streptomyces* sp. RW-2, and a sequence of approximately 1400 bp was obtained by PCR (Figure 2). An analysis of the sequenced DNA by DNAMAN 10.0 (http://dnaman.software.

informer.com/6.0) showed that it contained a 1344 bp open reading frame encoding a protein of 447 amino acids. The nucleotide sequence of *bglS* was submitted to GeneBank (accession no. MW916284).

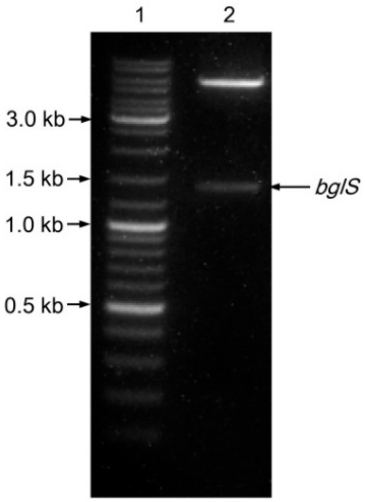

Figure 2. Electrophoretogram of PCR product corresponding to a β-glucosidase gene from *Streptomyces* sp. RW-2. 1, DNA marker; 2, PCR product.

The NCBI BLAST analysis of *bglS* showed the highest nucleotide sequence similarity (99.63%) to a β-glucosidase gene from *S. nigra* strain 452 but less than 87.35% similarity to that from other *Streptomyces* strains. The similarity of the 16S rRNA sequences between the *Streptomyces* sp. RW-2 strain (GenBank accession no. KJ472430.1) and the *S. nigra* strain 452 (GenBank accession no. CP029043) was 99.72%. The high similarity of the 16S rRNA sequences and β-glucosidase genes between the two strains suggested that strain RW-2 may be *S. nigra*.

3.2. Expression of bglS in E. coli

The β-glucosidase encoded by *bglS* was successfully expressed in *E. coli* BL21(DE3) following induction at 25 °C for 10 h with 0.5 mmol/L IPTG. SDS-PAGE analysis revealed that the expressed protein appeared as a single band with a molecular mass of approximately 50 kDa (Figure 3), consistent with the predicted value of 49.5 kDa based on its 447 amino acids (http://web.expasy.org/compute_pi/). An analysis of the amino acid sequence of this β-glucosidase indicated that it was most similar (99.55%) to the β-glucosidase of two *S. nigra* strains (GenBank accession nos. WP_164492287 and AWE50172), followed by that of *Streptomyces* sp. JHA19 (97.32%, GenBank accession no. WP_199835400) and *Streptomyces* sp. M7 (96.88%, GenBank accession no. WP_181890503.1); additionally, it had less than 93.74% similarity with other *Streptomyces* strains. An analysis of the amino acid sequence using the SMART program (http://smart.embl.de/) showed that it contained a typical glycoside hydrolase 1 family (GH1) domain, indicating it belongs to the GH1 family.

SDS-PAGE analysis indicated that most of the β-glucosidase was in the soluble fraction, so the crude cell extracts were expected to exhibit enzymatic activity. The activity of recombinant β-glucosidase against pNPG was 3.48 U/mL in the culture.

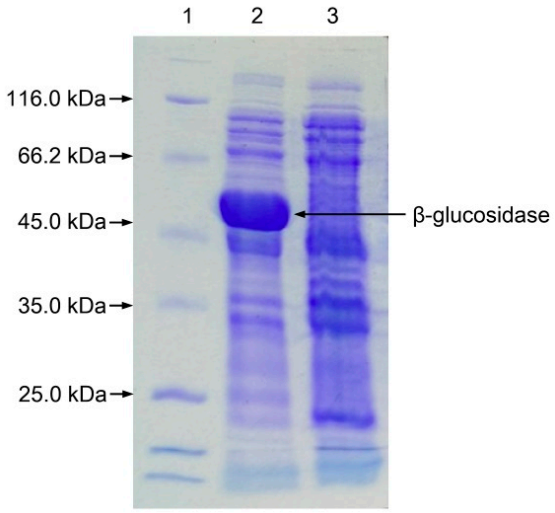

Figure 3. SDS-PAGE analysis of the recombinant β-glucosidase expressed in *E. coli* BL21(DE3). Lane 1, molecular weight marker; lane 2, total protein of *E. coli* BL21(DE3) harboring the recombinant *blgS* in pET28a (+); lane 3, total protein of *E. coli* BL21(DE3) harboring empty pET28a (+) as a negative control.

3.3. Biotranformation of CuBg by Recombinant β-glucosidase

The biotransformation of CuBg to CuB using the recombinant β-glucosidase was carried out with an enzymatic activity of 3.48 U/mL in the reaction system. Conversion rates at different CuBg concentrations are shown in Figure 4. At 0.25 g/L of CuBg in the reaction mixture, the conversion rate was as high as 98.1%, indicating that CuBg was specifically converted into CuB. Indeed, no other product was detected at 228 nm by HPLC. During biotransformation by the crude enzyme from *Streptomyces* sp. RW-2, 0.25 g/L of CuBg in the reaction system resulted in a conversion rate of 92.6% [12]. However, during biotransformation with the recombinant β-glucosidase, 0.75 g/L of CuBg resulted in a conversion rate of 93.2%. These conversion rates were nearly equal, but the substrate concentration was increased three-fold, resulting in a three-fold increase in productivity. These results indicate that using recombinant β-glucosidase from *Streptomyces* sp. RW-2 significantly improved biotransformation efficiency.

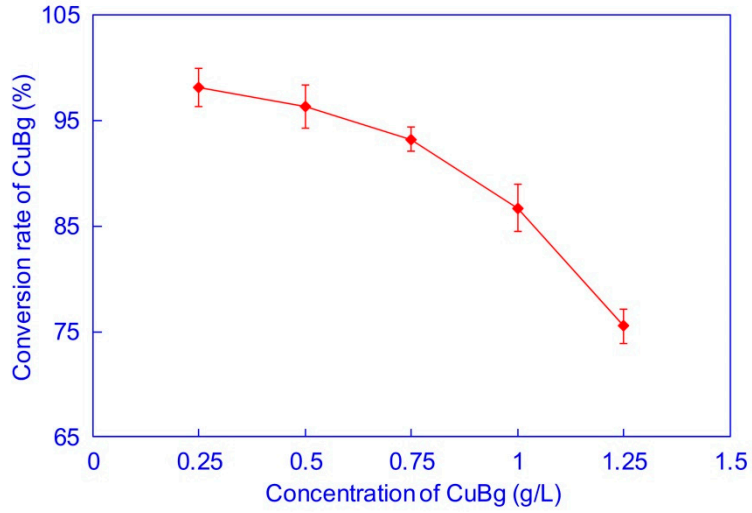

Figure 4. Conversion rate of CuBg to CuB by biotransformation using the recombinant β-glucosidase at different substrate concentrations.

3.4. Biotransformation of C. melo Pedicel Extracts by Recombinant β-Glucosidase

Extracts (1 mL) of *C. melo* pedicels in a 10 mL reaction system—with concentrations of CuBg and CuB of 0.973 and 1.36 g/L, respectively—were treated with the recombinant β-glucosidase with an activity of 3.48 U/mL. Biotransformation was carried out at 30 °C and monitored for 20 h by HPLC (Figure 5). The enzyme hydrolyzed the greatest amount of CuBg to CuB within 12 h. After biotransformation, the concentration of CuB in the reaction mixture was improved to 2.02 g/L, which represented an increase of 48.5% compared to the absence of biotransformation. The conversion rate of CuBg reached 87.6%. When the concentration of CuBg from *C. melo* pedicel extracts added to the reaction mixture was 0.973 g/L, the conversion rate was nearly equal to that of the biotransformation of pure CuBg at a concentration of 1.0 g/L. This indicated that the existing CuB from *C. melo* pedicel extracts did not inhibit the enzymatic reaction.

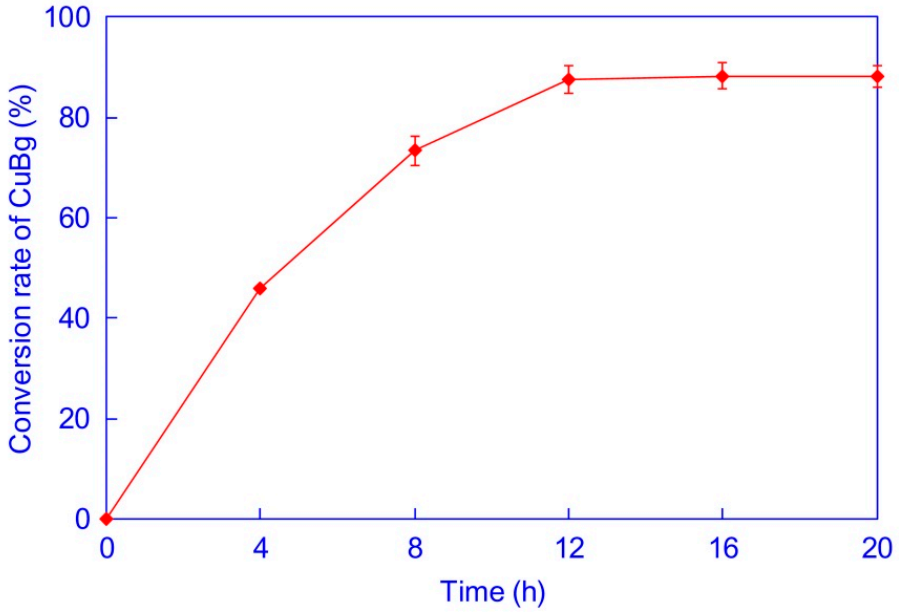

Figure 5. Time course of biotransformation of *C. melo* pedicel extracts using recombinant β-glucosidase.

After biotransformation, the concentration of CuB in *C. melo* pedicel extracts improved from 13.6 to 20.2 g/L. The HPLC analysis of *C. melo* pedicel extracts after biotransformation for 12 h using the recombinant β-glucosidase is shown in Figure 6. ESI-MS spectra of CuBg and CuB in *C. melo* pedicel extracts after biotransformation are shown in Figure 7.

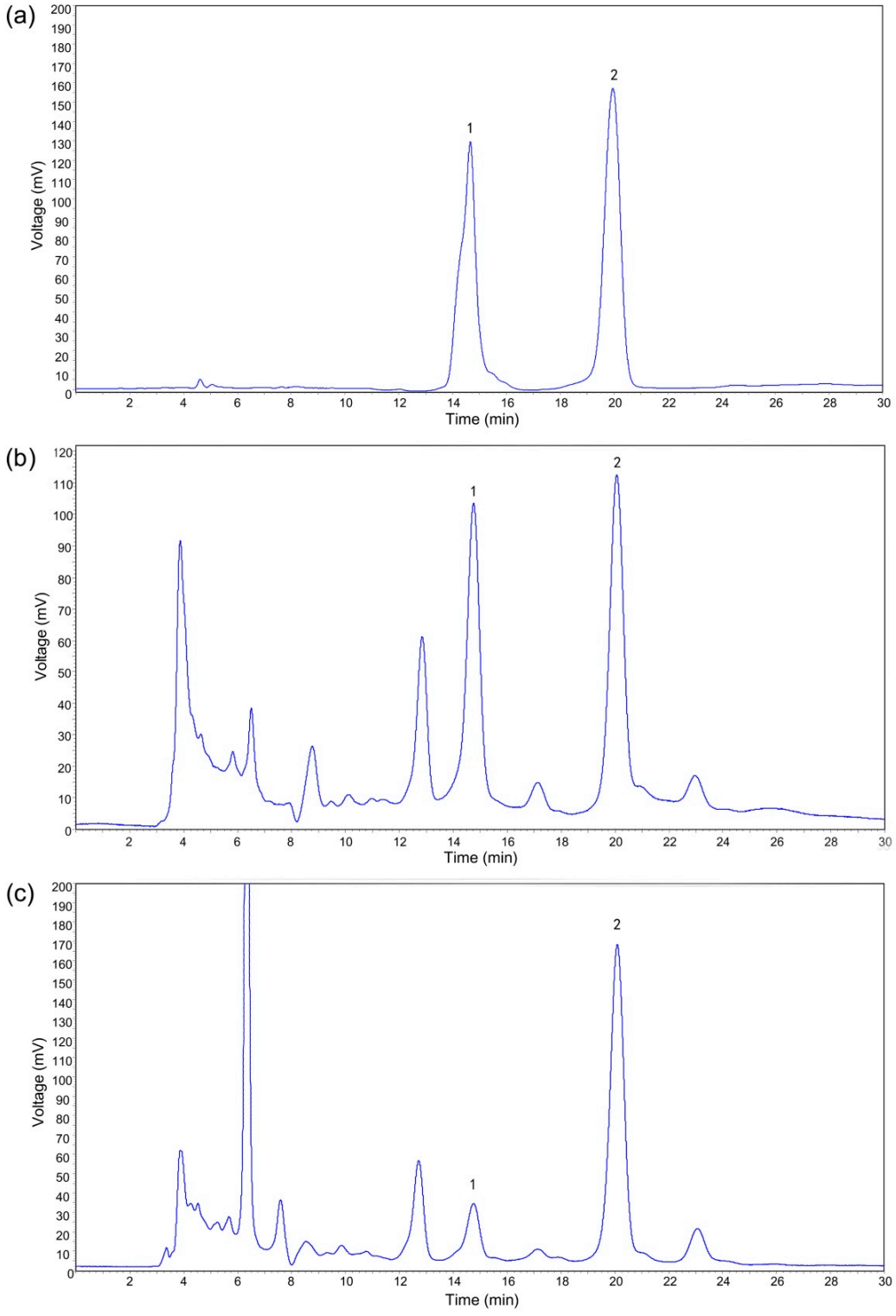

Figure 6. HPLC–UV chromatograms of cucurbitacin B 2-O-β-D-glucoside (CuBg) and cucurbitacin B (CuB). (**a**) Standard CuBg and CuB dissolved in methanol. (**b**) *C. melo* pedicel extracts without biotransformation. (**c**) *C. melo* pedicel extracts after biotransformation by the recombinant β-glucosidase. 1, CuBg; 2, CuB.

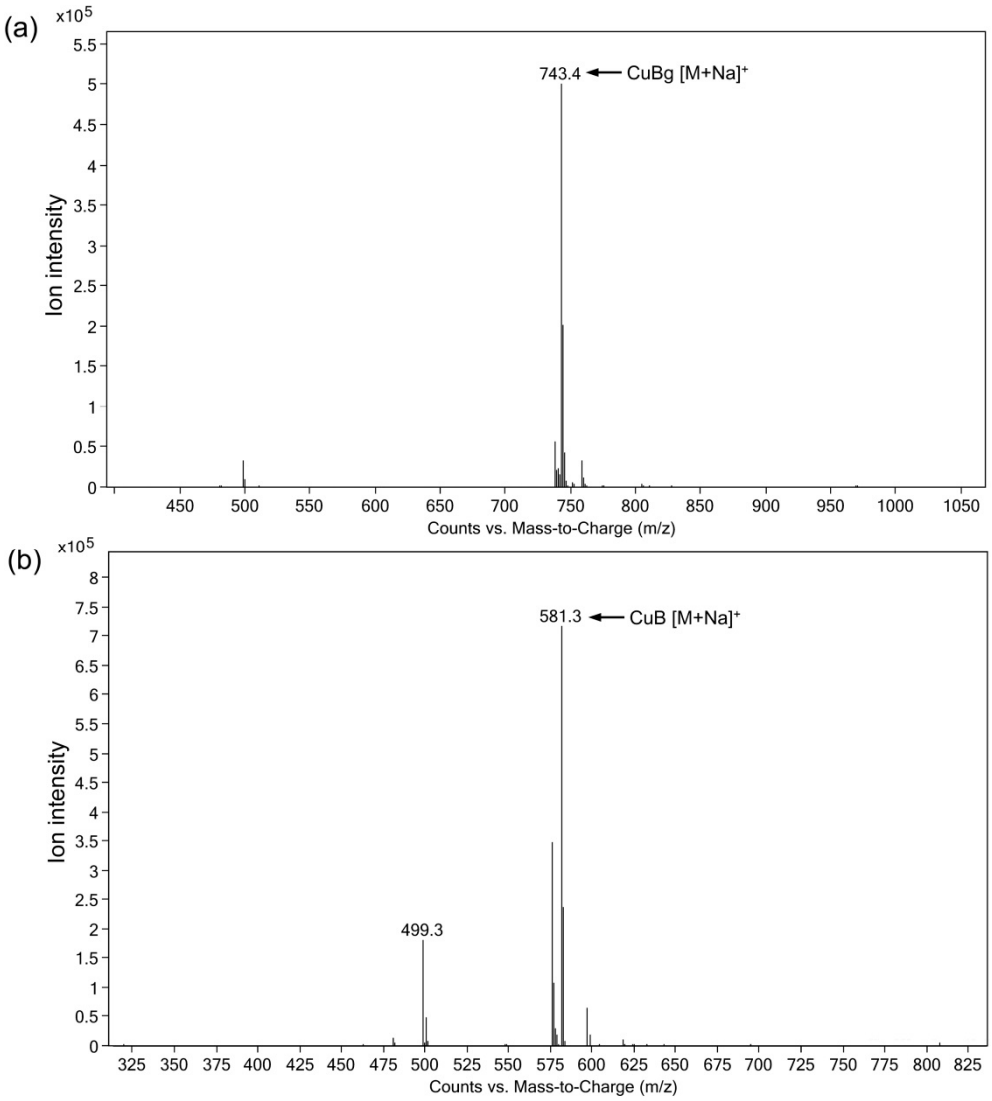

Figure 7. LC–ESI-MS spectra of CuBg and CuB in *C. melo* pedicel extracts after biotransformation. (**a**) Peak 1 in Figure 6, corresponding to [M + Na] $^{+}$ at *m*/*z* 743.4, indicates that the molecular weight of the compound is 720.4 Da (CuBg). (**b**) Peak 2 in Figure 6, corresponding to [M + Na] $^{+}$ at m/z 581.3, indicates that the molecular weight of the compound is 558.3 Da (CuB).

4. Conclusions

A gene encoding β-glucosidase for the biotransformation of CuBg into CuB was cloned from *Streptomyces* sp. RW-2. This enzyme was expressed in *E. coli* BL21(DE3) in a soluble form with an activity of 3.48 U/mL with pNPG as substrate in a culture. The recombinant β-glucosidase efficiently converted CuBg into CuB in a PBS system. When using the recombinant β-glucosidase for the biotransformation of *C. melo* pedicel extracts, CuBg was converted into CuB with a conversion rate of 87.6% at a concentration of 0.973 g/L of CuBg in the reaction mixture. The concentration of CuB in *C. melo* pedicel extracts was improved from 13.6 to 20.2 g/L after biotransformation. The present study provides high-efficiency technology for the production of CuB from its glycoside by biotransformation that also has potential applications in the pharmaceutical industry for increasing the productivity of CuB from *C. melo* pedicels.

5. Patents

Chinese Patent CN202110607500.1 resulted from the work reported in this manuscript.

Author Contributions: Conceptualization, J.M.; methodology, J.M. and X.W.; validation, X.C. and Y.W.; formal analysis, S.Z. and Z.H.; investigation, X.W. and S.Z.; resources, Z.H. and Y.W.; writing—original draft preparation, X.W.; writing—review and editing, J.M.; visualization, X.W. and S.Z.; supervision, J.M. and X.C.; project administration, X.C.; funding acquisition, X.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Zhejiang Province Public Welfare Technology Application Research Project (Grant No. LGN19H300001).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data within the article are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chen, J.C.; Chiu, M.H.; Nie, R.L.; Cordell, G.A.; Qiu, S.X. Cucurbitacins and cucurbitane glycosides: Structures and biological activities. *Nat. Prod. Rep.* **2005**, *22*, 386–399. [CrossRef] [PubMed]
2. Miró, M. Cucurbitacins and their pharmacological effects. *Phytother. Res.* **1995**, *9*, 159–168. [CrossRef]
3. Aeri, V.; Kaushik, U.; Mir, S. Cucurbitacins—An insight into medicinal leads from nature. *Pharmacogn. Rev.* **2015**, *9*, 12–18. [CrossRef] [PubMed]
4. Cai, Y.; Fang, X.; He, C.; Li, P.; Xiao, F.; Wang, Y.; Chen, M. Cucurbitacins: A systematic review of the phytochemistry and anticancer activity. *Am. J. Chin. Med.* **2015**, *43*, 1331–1350. [CrossRef] [PubMed]
5. Garg, S.; Kaul, S.; Wadhwa, R. Cucurbitacin B and cancer intervention: Chemistry, biology and mechanisms (Review). *Int. J. Oncol.* **2017**, *52*, 19–37. [CrossRef]
6. Hussain, H.; Green, I.R.; Saleem, M.; Khattak, K.F.; Irshad, M.; Ali, M. Cucurbitacins as anticancer agents: A patent review. *Recent Pat. Anti-Cancer Drug Discov.* **2019**, *14*, 133–143. [CrossRef]
7. Piao, X.-M.; Gao, F.; Zhu, J.-X.; Wang, L.-J.; Zhao, X.; Li, X.; Sheng, M.M.; Zhang, Y. Cucurbitacin B inhibits tumor angiogenesis by triggering the mitochondrial signaling pathway in endothelial cells. *Int. J. Mol. Med.* **2018**, *42*, 1018–1025. [CrossRef] [PubMed]
8. Shan, W.G.; Tang, L.; Shan, H.F.; Zhan, Z.J. A Method for Extraction and Separation of Cucurbitacin B. CN101759746A, 30 June 2010.
9. Su, L.H. A Method for Extraction of Cucurbitacin B. CN101974055A, 16 February 2011.
10. Chao, P.-D.L.; Hsiu, S.-L.; Hou, Y.-C. Bioavailability, metabolism, and pharmacokinetics of glycosides in Chinese herbs. *ACS Symp. Ser.* **2006**, *925*, 212–223. [CrossRef]
11. Xu, X.; Tang, L.; Shan, H.F.; Wang, Z.Q.; Shan, W.G. Study on extraction of cucurbitacin B from the pedicel of *Cucumis melo* L. by acid hydrolysis. *Adv. Mater. Res.* **2013**, *704*, 61–65. [CrossRef]
12. Mei, J.; Li, S.; Jin, H.; Tang, L.; Yi, Y.; Wang, H.; Ying, G. A biotransformation process for the production of cucurbitacin B from its glycoside using a selected *Streptomyces* sp. *Bioprocess. Biosyst. Eng.* **2016**, *39*, 1435–1440. [CrossRef] [PubMed]
13. Rosano, G.L.; Ceccarelli, E.A. Recombinant protein expression in *Escherichia coli*: Advances and challenges. *Front. Microbiol.* **2014**, *5*, 172. [CrossRef] [PubMed]
14. Ruan, C.-C.; Zhang, H.; Zhang, L.-X.; Liu, Z.; Sun, G.-Z.; Lei, J.; Qin, Y.-X.; Zheng, Y.-N.; Li, X.; Pan, H.-Y. Biotransformation of ginsenoside Rf to Rh1 by recombinant β-glucosidase. *Molecules* **2009**, *14*, 2043–2048. [CrossRef] [PubMed]
15. Cardillo, A.B.; Perassolo, M.; Sartuqui, M.; Talou, J.R.; Giulietti, A.M. Production of tropane alkaloids by biotransformation using recombinant *Escherichia coli* whole cells. *Biochem. Eng. J.* **2017**, *125*, 180–189. [CrossRef]
16. Park, H.L.; Lee, J.C.; Lee, K.; Lee, J.M.; Nam, H.J.; Bhoo, S.H.; Lee, T.H.; Lee, S.-W.; Cho, M.-H. Biochemical characterization of a flavonoid O-methyltransferase from *Perilla* leaves and its application in 7-methoxyflavonoid production. *Molecules* **2020**, *25*, 4455. [CrossRef] [PubMed]
17. Siddiqi, M.Z.; Medjebouri, S.; Liu, Q.; Park, H.Y.; Kim, G.-R.; Im, W.-T. Efficient production of various minor ginsenosides from PPD- and PPT-type major ginsenosides using a single recombinant BglFc isolated from *Flavobacterium chilense*. *Biotechnol. Bioprocess. Eng.* **2021**, *26*, 232–246. [CrossRef]
18. Koko, M.; Sami, R.; Muhoza, B.; Khojah, E.; Mansour, A. Promising pathway of thermostable mannitol dehydrogenase (MtDH) from *Caldicellulosiruptor hydrothermalis* 108 for D-mannitol synthesis. *Separations* **2021**, *8*, 76. [CrossRef]
19. Brunelle, J.L.; Green, R. One-dimensional SDS-polyacrylamide gel electrophoresis (1D SDS-PAGE). *Methods Enzymol.* **2014**, *541*, 151–159. [CrossRef] [PubMed]
20. Cruz Rodríguez, A.; Sánchez Esperanza, F.A.; Pérez-Campos, E.; Hernández-Huerta, M.T.; Pérez-Campos Mayoral, L.; Matias-Cervantes, C.A.; Martínez Barras, A.; Mayoral-Andrade, G.; Santos Pineda, L.Á.; Díaz Barrita, A.J.; et al. Aggregation and molecular properties of β-glucosidase isoform II in chayote (*Sechium edule*). *Molecules* **2020**, *25*, 1699. [CrossRef] [PubMed]