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Plant endophytic fungi exhibit diverse biotransformation pathways of mogrosides and show great potential application in siamenoside Ⅰ production

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Abstract

Fungal endophytes, as an untapped resource of glycoside hydrolase biocatalysts, need to be further developed. The primary active compound in the fruit of *Momordica grosvenorii*, mogroside V, can be converted into other various bioactive mogrosides by selective hydrolysis of glucose residues at C3 and C24 positions. In present study, 20 fungal strains were randomly selected from our endophytic fungal strain library to investigate their capability for transforming mogroside V. The results revealed that relatively high rate (30%) endophytic fungal strains exhibited the ability of transformation. Further analysis indicated that endophytic fungi could produce abundant mogrosides, and the pathways for biotransforming mogroside V showed diverse. Among the given fungal endophytes, *Aspergillus* sp. S125 could almost completely transform mogroside V into the end-products mogroside II A and aglycone only after 2 days of fermentation; *Muyocopron* sp. A5 produced rich intermediate products, including siamenoside Ⅰ, and the end-product mogroside II E. Furthermore, *Aspergillus* sp. S125 and *Muyocopron* sp. A5 were selected to optimize the fermentation conditions in order to evaluate the feasibility of large-scale conversion of mogroside V. After optimization, *Aspergillus* sp. S125 could convert 10 g/L of mogroside V into 4.5 g/L of mogroside II A and 3.6 g/L of aglycone after 3 days of fermentation, while *Muyocopron* sp. A5 could selectively produce 4.88 g/L of siamenoside Ⅰ from 7.5 g/L of mogroside V after 36 hours of fermentation. This study not only provides a class of highly effective biocatalytic candidates for transform mogrosides, but also strongly indicates that plant endophytic fungi can be used as a potential resource for biocatalysis of natural compounds.

1. Introduction

Endophytic fungi are an ecological group of fungi that inhabit the tissues and organs of healthy plants, without causing any external symptoms of the host plant (Staniek et al., 2008). In last decades, endophytic fungi have been attracted more and more attention due to its excellent capacity for secreting a diverse array of bioactive compounds and enzymes (Higginbotham et al., 2013; Suryanarayanan et al., 2012; Gao et al., 2021; Xiao et al., 2022). Recently, some studies have found that plant fungal endophytes exhibit excellent properties in bioconversion of natural saponin, such as glycyrrhizic acid (Gao et al., 2021; Xiao et al., 2022), ginsenosides (Eom et al., 2018), with high activity and substrate specificity. Indeed, fungal endophytes have been suggested as high-efficient biocatalyst sources, revealing great potential application in medicine, food security, and social sustainability (Schulz et al., 2002; Suryanarayanan et al., 2012; Choudhary et al., 2021).

Siraitia grosvenorii, a perennial vine belonging to *Cucurbitaceous*, is an indigenous plant of China which primarily found in provinces of Guangxi, Guangdong and Jiangxi, China (Wang et al., 2019). For centuries, its fruits, called as luo han guo (LHG), have been used as Chinese herbal medicines for treating constipation, lung congestion, dry cough *etc.* (Takasaki et al., 2003). LHG contains a diverse array of bioactive compounds, such as flavonoids, alkaloids, polysaccharides, vitamins, aliphatic acids and triterpene saponins (Shivani et al., 2021). Of particular significance, morgrosides, a group of cucurbitane-type triterpene saponins (Fig. 1A), serve as the key pharmacological component of LHG, and also have

widely used as sugar substitute in the world due to their high sweetness and low calorie (Kathuria et al., 2019). In fact, the relative sweetness of mogroside Ⅴ, mogroside Ⅳ, siamenoside Ⅰ and mogroside Ⅱ were assayed to be 378, 300, 465 and 195 times sweetness than sucrose in water, respectively. Among these mogrosides, siamenoside Ⅰ shows the highest sweetness and more acceptable taste quality (Muñoz-Labrador et al., 2021). Currently, numerous studies have revealed a variety of biological functions of mogrosides, including anti-inflammatory (Qi et al., 2008), anti-oxidation (Chen et al., 2007), anti-tumor (Liu et al., 2015), liver protection (Shi et al., 2014) and intervening glycolipid metabolism (Liu et al., 2018). Overall, existing researches have strongly suggested that mogrosides are promising candidates for the development of novel drugs and high-sweetness natural sweeteners (Wu et al., 2022).

Mogrosides are a variety of diverse derivatives with complex stereoconfiguration consisting of both aglyone (cucurbitane-type triterpene) and 1 to 5 glucose moieties (Fig. 1A) (Chiu et al., 2013). These derivatives are extracted from LHG, mainly include mogroside Ⅴ, mogroside Ⅳ, siamenoside Ⅰ, mogroside Ⅲ, mogroside Ⅱ and mogroside Ⅱ (Fig. 1A) (Gong et al., 2019). Among them, mogroside Ⅴ is the main component (~ 60%, w/w) of total saponins in LHG (Pawar et al., 2013), the contents of other mogrosides, however, are considerably low. Therefore, other mogrosides are produced mainly by hydrolysis of mogroside Ⅴ. Unfortunately, due to the intricate sugar moieties in mogroside Ⅴ, producing the desired products by the traditional chemical hydrolysis methods, such as acid hydrolysis, proves to be challenging (Bin et al., 2020). Compared to chemical hydrolysis, biotransformation approach has significant advantages in spatial stereoselectivity, sustainability, and environmental friendliness, and has been considered as more potential strategy for producing derivatives of mogroside (Li et al., 2022). Given the excellent sweetness intensity and good taste of siamenoside Ⅰ, its bioconversion has recently been investigated (Xu et al., 2021; Duan et al., 2023). However, few biocatalytic candidates are screened to convert mogroside Ⅱ into siamenoside Ⅰ, and even fewer are highly selective and efficient in this transformation. In addition, there are also few reports on the biotransformation of other rare mogrosides, including mogroside Ⅲ, mogroside Ⅳ, and mogroside Ⅵ, leading to hinder further investigation of these mogrosides. Indeed, existing studies predominantly focus on catalytic enzymes, while ignore the screening of strains, which is the source of efficient and specific enzyme. Furthermore, in some aspects, biotransformation using whole-cell catalysis maybe exhibit advantages over enzyme catalysis because of its lower cost and higher stability (Xu et al., 2022).

In our previous study, a total of 229 strains of endophytic fungi belonging to 19 genera were isolated from Dongxiang wild rice (*Oryza rufipogon* Griff.) (Wang et al., 2015). Further investigation revealed that these endophytic fungi could secreted abundant glycoside hydrolase, suggesting great potential application in biotransformation of the natural products, especially glycosides (Gao et al., 2021; Gao et al., 2023). In the present study, we randomly selected 20 strains from our fungal endophytes library for screening the ability of mogrosides transformation. Our results revealed that a high proportion of strains showed the capacity for converting mogroside Ⅱ to other glycosides. Furthermore, two strains, A5 and S125, which showed high conversion ability and high β-glucosidase activity, were selected to produce different mogroside derivatives. This study not only provides diverse biocatalytic candidates for

mogroside bioconversion which significantly facilitate the development of mogroside industry, but also demonstrates that endophytic fungi are a great potential biocatalytic resource for natural products.

2 Materials and methods

2.1 Medium and chemicals

Medium for the primary culture was potato dextrose agar (PDA) containing 200 g/L of potato, 20 g/L of glucose, and 20 g/L of agar powder. The culture medium for screening strain contained the following components: 5.0 g LHG extract; 2.0 g KH_2PO_4; 5.0 g NH_4NO_3; 0.5 g NaCl; 0.05 g yeast extract; 0.4 g $MgSO_4 \cdot 7H_2O$; 0.04 g $ZnSO_4 \cdot 7H_2O$; 1 L of sterile water; pH 6.0. The LHG extract (50% of mogroside V) purchased from Luoyang Tianluo Biological Co., LTD (China). The standard products of mogroside V, IV E, IV A, III A, III E, II A, II E, I A, I E, siamenoside I and aglycone (purity, 98%) were purchased from Chengdou Munster Biotechnology Co., LTD (China). All other chemicals employed in this study adhered to analytical grade standards.

2.2 Strain screening

A total of 229 strains of endophytic fungi involving 19 genera were previously isolated from Dongxiang wild rice (Wang et al., 2015), with a subsequent random selection of 20 strains from the endophytic fungal library for mogaloside V transformation. Verification of mogaloside V hydrolyzing ability was carried out using extracellular enzymes produced through the submerged fermentation of these 20 fungi. Activation of the fungi transpired in either PDA or PDB, and the fungal mycelia of the selected 20 strains were inoculated at a 2% (v/w) concentration into an inorganic medium featuring 5% (w/v) LHG extract as the exclusive carbon source. Aerobic fermentation performed at 160 rpm in a shaker at 28℃ over a period of 7 days. Broth samples were collected every 24 hours during the fermentation, and the supernatant was acquired by centrifugation at 4 ℃, 7000 rpm. Products analysis of the supernatant was performed using a thin-layer chromatographic plate (TLC). The developing agent consisted of dichloromethane: methanol in a 5:3 (v/v) ratio, and the color developer was a 10% ethanol sulfate solution. High-performance liquid chromatography (HPLC, Agilent, USA) was employed to further identify the hydrolysis products of mogroside V produced by these strains. Strains exhibiting conversion ability were subsequently selected for further investigation.

2.3 Morphological and phylogenetic analysis of strains

The colony morphology of endophytic fungi was observed on PDA medium at 28℃, while the morphology of spore, sporangium, and spore chain were examined using VEGA3 scanning electron microscopy (Tescan, USA). Genomic DNA extraction from the given fungal strains employed the cetyltrimethylammonium bromide (CTAB) method (Van burik et al., 1998). Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were utilized for amplifying internal transcriptional spacers. PCR was employed to obtain the amplified DNA fragments, and the sequencing was carried out by Qingke Biological Company (China). Phylogenetic analysis of the ITS-

rDNA sequences of these six endophytic fungi was carried out. The ITS sequences of endophytic fungi were compared online with NCBI (http://www.ncbi.nlm.nih.gov/BLAST) using the BLAST (Basic Local Alignment Search Tool) to ascertain the genetic relationships and classification status of the endophytic fungi. Sequence data for relevant species were downloaded based on GenBank results. MEGA software (6.0.6) was utilized for comparing multiple sequences and constructing a phylogenetic tree based on evolutionary distance data. The tree figure was manually edited using iTOL version 4.

2.4 Optimization of fermentation conditions for mogrisdes bioconversion

Two endophyte strains, S125 and A5, were selected for further exploration. The optimization of substrate concentration, nitrogen source type, and nitrogen source concentration for β-glucosidase production by strains S125 and A5 was carried out using a one-by-one method. The fundamental fermentation conditions were as follows: 5 g/L LHG extract (w/v), 5 g/L NH_4NO_3 (w/v), 2.0 g/L KH_2PO_4, 0.5 g/L NaCl, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 0.04 g/L $ZnSO_4 \cdot 7H_2O$, and a natural pH. The seed broth was inoculated into the basic fermentation medium at a 2% inoculation rate, with a loading of 50 mL/250 mL, and fermented at 28℃, 160 r/min for 7 days. Subsequently, enzyme activity was determined using the method described by Javed et al. (2018). The effect of carbon and nitrogen source concentrations on β-glucosidase activity was investigated through a single-factor test in the basic medium with LHG extract as the sole carbon source, respectively. Under the basic fermentation conditions, the concentration gradient was set from 1 g/L to 30 g/L, exploring the effects of different substrate concentrations on β-glucosidase activity. Considering the fermentation conditions for basic enzyme production and the study of optimal substrate concentration, variations such as 0.5% (w/v) of $NaNO_3$, NH_4NO_3, NH_4Cl, beef extract, peptone, urea, and yeast powder were employed to replace the nitrogen source in the basic fermentation medium, respectively. The influence of different nitrogen sources on β-glucosidase activity was then examined. Subsequently, the optimal nitrogen source was selected, and its concentration was set at 1 g/L, 3 g/L, 5 g/L, 7 g/L, 9 g/L, and 11 g/L, respectively, to investigate the effects of varying nitrogen source dosages on β-glucosidase activity. Finally, the production of mogrosides by the fermentation of strains S125 and A5 was carried out under optimal conditions, respectively.

2.5 Mogroside analysis by HPLC

The fermentable broth from the given endophytic fungi was collected for further analysis. Initially, the fermentative supernatant was separated through filtration, followed by the addition of an equal volume of n-butanol for extraction. After thorough oscillation and mixing, the organic residue was evaporated and dried using a rotary evaporator, then dissolved in methanol and filtered through a 0.22 μm membrane for HPLC analysis. Standard products of mogroside V, IV E, III E, III, II A, II E, I A, I E, siamenoside I, and aglycone were dissolved in 10 mL of chromatography-grade methanol to create a 1 mg/mL standard solution. The contrast solution of mogrosides with different concentration gradients was prepared from the mother liquor and analyzed by HPLC to investigate its linear relationship and range. The standard curve was constructed with the mass concentration (X, μg/mL) of the control

solution as the X coordinate and the peak area (Y, AU*s) as the Y coordinate, the standard curve was drawn and the regression. Chromatographic conditions included an SB-C18 column (150 mm×4.6 mm, 5 μm) with a wavelength of 203 nm, a flow rate of 0.8 mL/min, a sample volume of 10 μL, and acetonitrile:water as the mobile phase in a gradient elution ranging from 2.8:7.2 to 6.5:3.5. By comparing the retention time of each standard with the samples, the presences of different types of mogrosides in the fermentation broth were determined. The content of mogrosides in the sample was calculated by regression curve of standard mogrosides.

2.6 Statistical analysis

All experiments were conducted in triplicate with the average value being reported on the dry basis. The differences between variables were tested for significance using ANOVA and Duncan's multiple range test. Differences between means were considered significantly different at $P < 0.05$.

3. Results and discussions

3.1 Screening fungal endophytes for bioconversion of mogrisdes

Recently, some microorganism, mainly yeast, have been employed to transform mogrisdes due to their glucosidase activity, and showing the ability for converting mogriside □ into a mixture of groside IV, siamenoside I and mogroside III E (Chiu et al., 2013; Wang et al., 2019). According to our previous studies, fungal endophytes could secret rich glycosidases and exhibited the ability of transforming terpenoid saponins, such as glycyrrhizic acid (Xiao et al., 2022). Therefore, we supposed that the fungal endophytes maybe have the capacity for transforming mogrosides. In order to reveal our hypothesis, 20 strains from our endophytic fungi library were randomly selected to transform the LHG extracts (contains 50% mogriside □). After screening test, a total 6 of endophytic fungi which could utilize LHG extracts for growth was found, suggesting their potential capacity for converting mogrisdes. Subsequently, the fermentation of the 6 strains using LHG extracts was carried out, respectively, and fermentable supernatant were sampled per one day for further analyzing by TLC method. As shown in **S** Fig. 1, 6 given strains exhibited diverse mogriside profiles, revealing diverse and excellent ability of mogriside bioconversion. Notably, strain S125 exhibited excellent ability for selective efficient bioconversion of mogriside □ into two products, meaning great potential for specific production of rare mogrisdes. Meanwhile, diverse intermediate products were produced during fermentation of strain FL7, L55, A5, L99 and J5, but only one kind of mogriside derivate was mainly obtained in the middle or late fermentation period. Previously, some microorganisms, including yeast, lactic acid bacteria, were selected for mogrosides conversion testing, but only several yeasts showed the mogroside converting ability (Wang et al., 2018; Yang et al., 2007). Thus, screening of novel biocatalytic candidates for efficient conversion of mogroside needs to be imminently carried out. When comparing our results to those previous studies, it must be pointed out that relatively high ratio (about 30%) of fungal endophytes with the capability of mogroside conversion were screened, and these given fungal endophytes also exhibited excellent

properties for producing diverse mogroside derivatives. Indeed, our results also strongly suggest plant endophytic fungi as potential biocatalytic candidates for mogroside conversion.

3.2 Diverse pathways for biotransformation of mogrosides by fungal endophytes

To further identify the intermediate or end products, HPLC analysis was employed for products identification. Our results showed that most of the given fungal endophytes, except strain A5, were able to completely convert mogroside Ⅴ to be aglycone as the end-product (Fig. 1B). Currently, the reported biocatalytic strains transform mogroside Ⅴ to almost produce mogroside Ⅴ E as the end-product (Table 1). Similarly, mogroside Ⅴ E still was the main component of products which generated in middle fermentation period of fungal endophytes, such as strain L55, A5, J5 and L99. Meanwhile, we also found the production of siamenoside Ⅰ in the early fermentation period of strain A5, reveling a potential for production of high sweetness natural sweetener namely siamenoside Ⅰ. In the middle fermentation period (2 ~ 4 days) of strain L55 and FL7, relatively high content of mogroside Ⅴ A was produced. In addition, strain J5 exhibited quite specific property which could produced a kind of rare mogroside namely mogroside Ⅴ A in the middle fermentation period. To our knowledge, there are no reports of strain or enzyme that can convert mogroside Ⅴ into mogroside Ⅴ E. Surprisingly, strain S125 exhibited high conversion efficiency, and almost all of substrates were utilized for producing end-products (mogroside Ⅴ A, and aglycone) after only one day of fermentation, demonstrating great potential application to produce rare mogrosides.

Table 1
Bioconversion of mogrisdes by biocatalytic candidates in 7 days.

Strain	Substrate	Intermediate products	End-products	Reference
Ganoderma lucidum mycelium	LHG extracts	M III E, M II A	M III E, M II A	Chiu et al., 2019
Kluyveromyces marxianus	LHG extracts	S I, M III E	S I, M III E	Wang et al, 2018
Saccharomyces pastorianus	LHG extracts	M III E	M III E	Wang et al, 2018
Candida kefyr	LHG extracts	S I, M III E	S I, M III E	Wang et al, 2018
Candida utilis	LHG extracts	S I, M III E	S I, M III E	Wang et al, 2018
Yarrowia lipolytica	LHG extracts	S I, M III E	S I, M III E	Wang et al, 2018
Debaryomyces hansenii	LHG extracts	S I, M III E	S I, M III E	Wang et al, 2018
Dekkera bruxellensis	LHG extracts	S I, M III E	S I, M III E	Wang et al, 2018
Muyocopron sp. A5	LHG extracts	S I, M □ E, M III E, M II A	M II A	This study
Alternaria sp. FL7	LHG extracts	M II A, aglycone	Aglycone	This study
Sarocladium oryzae L99	LHG extracts	M III E, M II A, M □ E	Aglycone	This study
P. meleagrinum J5	LHG extracts	M III E, M III, M □ E	Aglycone	This study
Aspergillus sp. L55	LHG extracts	M □ E, M III E, M II A	Aglycone	This study
Aspergillus sp. S125	LHG extracts	M II A, aglycone	M II A, aglycone	This study

Evidences indicate that various bioactive compounds can be obtained by selectively removing glucose moieties from mogroside V at the C3 or C24 positions. These compounds exhibit diverse biological activities, such as tumor inhibition and high sweetness as a sweetener (Liu et al., 2015; Wu et al., 2022). However, mogroside V contains relatively complex glucose moieties, resulting in challenges of the targeted preparation of certain LGH glycosides. As a result, bioconversion strategies with high selectivity are preferred. There are few studies on the biotransformation of mogrosides, with most studies focusing

on using yeast for the bioconversion of mogroside V. For example, Wang et al. (2018) screened microorganisms capable of converting mogroside V, identifying eight different yeast strains with transformative abilities. Their results revealed that the main products produced by yeasts were siamenoside I and mogroside III E. It seems to be suggested a consistent pathway for yeast-mediated mogroside V conversion, namely M V → S I + M III E (herein, mogroside and siamenoside were abbreviated as M and S, respectively). Comparing to yeast, we found that utilizing endophytic fungi as catalysts for the biotransformation of mogroside V results in a more diverse conversion pathway (Fig. 2), including i) M V → M □ A → M A (FL7); ii) M V→ M □ A + M A (S125); iii) M V → M III E + M III → M □ A + M □ E → M A (J5); iv) M V → M III E + M □ A → M A (L55); v) M V → S I + M III E → M □ A + M □ E → M A (L99); vi) M V → S I + M □ E → M III E → M □ E (A5), and a richer array of products (Fig. 1 and Fig. 2). Not only similar products can be generated, but also rare products, such as mogroside I A and aglycone, which are unattainable by yeast conversion, can also be obtained by plant endophytes. From the above mentioned results, endophytic fungi can be considered as superior biocatalysts for the bioconversion of mogrosides.

3.3 Phylogenetic analysis of endophytic fungi with capacity of mogrosides biotransformation

Microorganism identification is considered necessary for further investigations of transformation characteristics, catalytic enzymes, and strain screening. Therefore, the 6 strains exhibiting the mogroside bioconversion capabilities were further identified. According to morphological characteristics, the strain S125 and L55 could be inferred as genus *Aspergillus*, while the strain J5 potentially belongs to genus *Penicllium* (Fig. 3A). To further identify these filamentous fungi, molecular taxonomy, a rapid and reliable method, was employed. The molecular phylogenetic tree was constructed based on ITS regions (Fig. 3B). All the members of *Muyocopron* species were grouped in a single cluster, and the Fungus A5 was placed on a single clade, revealing a potential novel species of genus *Muyocopron.* Although fungus FL7 belongs to genus *Alternaria* which showed the highest similarity with *Alternaria alstroemeriae* CBS 118809 ITS region (Genebank accession no. NR_163686.1), fungus FL7 gathered a single clade, demonstrating a novel species of *Alternaria.* Additionally, fungus L99 and J5 were designated as *Sarocladium oryzae* L99 and *P. meleagrinum* J5, respectively, due to high sequences identity (≥ 99%) of sequenced ITS with those from *Sarocladium oryzae* CBS 180.74 (Genebank accession no. NR_145045.1), and *P. meleagrinum* var. viridiflavum CBS 335.59 (Genebank accession no. NR_153214.1), respectively. Importantly, two strains, namely fungal strains L55 and S125 were grouped in a cluster with numbers of genus *Aspergillus*, which revealed two strains all belonging to *Aspergillus* species. Our results showed that three strains capable of transforming mogrosides belong to the genera of *Penicillium* and *Aspergillus* fungi, with a proportion reaching 50%. In addition to the hydrolytic activity towards mogrosides described in this paper, previous studies have revealed that *Penicillium* or *Aspergillus* fungi exhibited transformative capabilities for various natural glycoside compounds, such as glycyrrhizic acid and saponins (Zou et al., 2013; Liu et al., 2013; Ju et al., 2021). This may be attributed to the rich glycoside hydrolase harboring in *Penicillium* and *Aspergillus* fungi, indicating their potential for transforming a variety of glycoside compounds.

3.4 Production of rare mogrosides from the LHG extracts

According to our above results, diverse derivatives of mogroside were produced from the bioconversion of mogroside Ⅴ by plant endophytic fungi. Comparing to other fungi, *Aspergillus* sp. S125 could fast transform mogroside Ⅴ into two end-products (mogroside Ⅴ A and mogroside saponin, Fig. 1B**)**. Among various mogrosides, saimenoside I not only exhibits the highest level of sweetness but also has the most optimal taste, so it is widely recognized as the most of promising natural sweeteners. Therefore, it is of great significance to find and develop biocatalysts that can effectively convert mogroside V into saimenoside I. Fortunately, in the early stage of fermentation, *Muyocopron* sp. A5 was mainly able to efficiently convert mogroside Ⅴ to siamenoside I (Fig. 1B). To further evaluate the feasibility for mogroside production, two strains, namely *Muyocopron* sp. A5 and *Aspergillus* sp. S.125 were selected to produce corresponding mogrosides. Previous studies have shown high level of β-glucosidase activities meaning excellent ability for bioconversion of mogrosides (Chen et al., 2022), as this glycosidase is usually responsible for hydrolyzing glycosidic bond of mogrosides. Thus, β-glucosidase activities were selected as a parameter to optimize cultural conditions. Different amount of substrates (from 1 to 30 g/L of LHG extract), and various nitrogen sources were employed to culture *Muyocopron* sp. A5 and *Aspergillus* sp. S125, respectively. Our results demonstrated significant effect of substrates and nitrogen sources on β-glucosidase activity which produced by two selected strains (Fig. 4). After 7 days of fermentation, the highest β-glucosidase activity (190.15 U/mL) was generated by *Muyocopron* sp. A5 cultivated in medium containing 15 g/L of LHG extract (Fig. 4A). In addition, various nitrogen sources, including $NaNO_3$, beef extract, peptone, urea, NH_4NO_3, yeast powder and NH_4Cl, were employed to culture *Muyocopron* sp. A5, and highest β-glucosidase activity (284.17 U/mL) was assayed at peptone (Fig. 4B). Then, different dosages of peptone (1 ~ 11 g/L) were further used to culture *Muyocopron* sp. A5, and our results shown 5 g/L of peptone contributing to highest β-glucosidase activity (306.03 U/mL) (Fig. 4C). Meanwhile, as shown in Fig. 4D **~ F**, 20 g/L of LHG extract, and 7 g/L of peptone caused highest β-glucosidase activity of *Aspergillus* sp. S125, respectively.

Subsequently, the fermentation of strains A5 and S125 were carried out for rare mogrosides production under optimal conditions, respectively (Fig. 5). The HPLC method was further employed to analyze the transformation process of the products. Consistent with the results of the initial screening mentioned earlier, *Aspergillus* sp. S125 demonstrated a strong conversion ability to mogroside V, which was completely transformed in only 3 days, producing 56.07% of mogroside II A and 43.93% of glycone (**S** Fig. 2 **and** Fig. 5A). It is worth noting that *Muyocopron* sp. A5 showed different performance in the conversion of mogroside V under optimized fermentation conditions compared to the initial screening. During the initial screening, *Muyocopron* sp. A5 converted mogroside V into various mogrosides, including siamenoside I, mogroside IV E, mogroside III E, and finally into mogroside II E (Fig. 1). Surprisingly, after optimization, we found that *Muyocopron* sp. A5 selectively converted mogroside V into siamenoside I during the early stage of fermentation (1–2 days), and after 3 days of fermentation, it was mainly converted into mogroside III E, and ultimately into mogroside II E (**S** Fig. 3). Therefore, in order to obtain siamenoside I, which is of great value as a high-intensity sweetener, the fermentation of strain A5

was performed under the optimized conditions for only 48 hours. Our results showed that after 36 hours of fermentation, the content of siamenoside I accounted for 88.74% of the total mogrosides, and the concentration was 4.88 g/L (Fig. 5B). Wang et al. (2019) screened 5 yeast strains for the conversion of mogroside V and found that siamenoside I accounted for approximately 30–54% of the total glycosides after 7 days of fermentation, along with considerable amounts of Mogroside III E, ranging from 7–59%. Compared to other catalytic strains that can convert mogroside V into S I, plant endophytes *Muyocopron* sp. A5 not only exhibited better selectivity, but also achieved significantly higher efficiency, with 88.74% of siamenoside I obtained in just 36 hours. Indeed, plant endophytes *Muyocopron* sp. A5 and *Aspergillus* sp. S125 exhibit higher substrate tolerance and product yield compared to other microbe-mediated mogroside V bioconversions. Our results further suggest the great potential and practical value of endophytic fungi in the biotransformation of mogrosides.

4. Conclusions

Selective hydrolysis of the different glucose moieties of mogroside V, the main bioactive compounds of the LHG, is important and practical strategy to the preparation of other various glycosides. However, the complicated spatial structures of mogroside V pose a challenge for selective hydrolysis of specific glucose moieties. Biocatalytic strategies show significant advantages in preparing rare mogrosides due to their excellent region-selectivity. Therefore, it is critical to screen microorganisms and enzymes that can efficiently and selectively transform. In this study, we randomly selected 20 strains from our plant endophytic fungi library and investigated their ability to transform mogroside V using LHG extract (containing 50% of mogroside V) as a substrate. Six strains were found to be capable of transforming mogrosides and producing multiple different products, including siamenoside I, mogroside IV E, mogroside III, mogroside III E, mogroside II E, mogroside II A, Mogroside I E, mogroside I A and aglycone, indicating the diversity of transformation pathways. Among 6 strains, strain S125 showed efficient transformation ability by completely converting mogroside V in 1–2 days of fermentation, while strain A5 was able to selectively convert mogroside V into a high-intensity natural sweetener siamenoside I. By optimizing the fermentation condition such as substrate addition and nitrogen source, strain S125 was able to completely transform mogroside V in 3 days of fermentation, generating 4.5 g/L of mogroside II A and 3.6 g/L of aglycone. Strain A5 selectively transformed mogroside V into siamenoside I during the early stage of fermentation and was able to convert 93.2% of mogroside V in only 36 hours, producing 4.88 g/L of siamenoside I. In addition, morphological and molecular identification of the six plant endophytic fungi strains revealed that multiple strains are potential novel species. This study not only provides diverse candidates for bioconversion of mogroside which significantly facilitate the development of mogroside industry, but also demonstrates that endophytic fungi are a great potential biocatalytic resource for natural products.

Authors' contributions

WXL, BLG and QJ conducted the experiments. BLG, YWX, YW and YMD provided resources. DZ and BLG supervised the project, designed the experiments, and wrote the manuscript. All authors read and

approved the final manuscript.

Declarations

Authors' contributions

WXL, BLG and QJ conducted the experiments. BLG, YWX, YW and YMD provided resources. DZ and BLG supervised the project, designed the experiments, and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read this article and have approved its submission to *Bioresources and Bioprocessing*.

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Figures

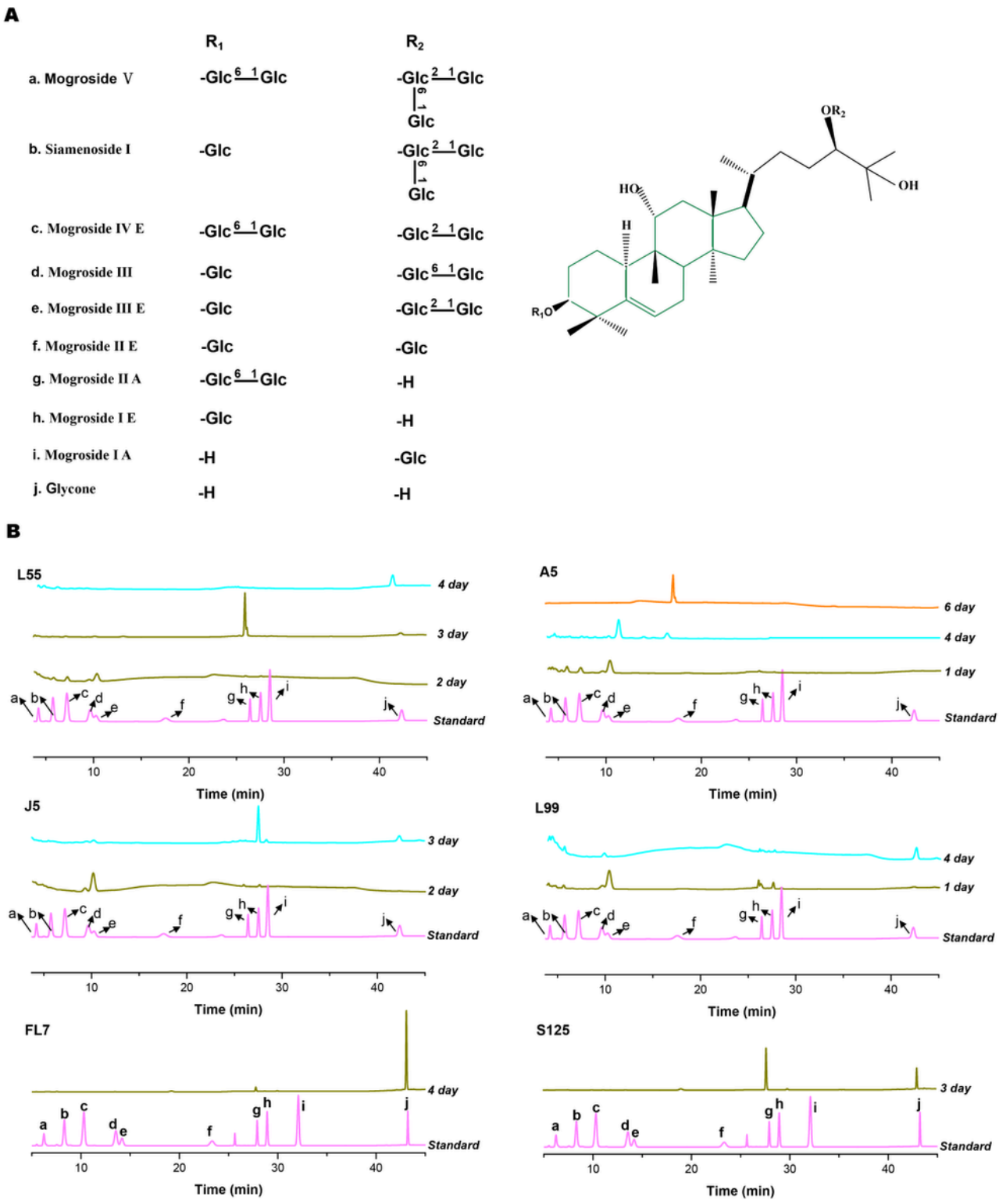


Figure 1

HPLC analysis of products produced by bioconversion mogroside V of 6 selected fungal endophytes. A: the chemical structure of different mogrosides; B: Strains L55, A5, J5, L99, FL7, and S125 were cultured and fermented in a medium containing 0.5% (w/v) Luo Han Guo extract (LHG extract). The fermentation supernatant was collected per day, and the mogrosides in the fermentation broth were analyzed using HPLC method.

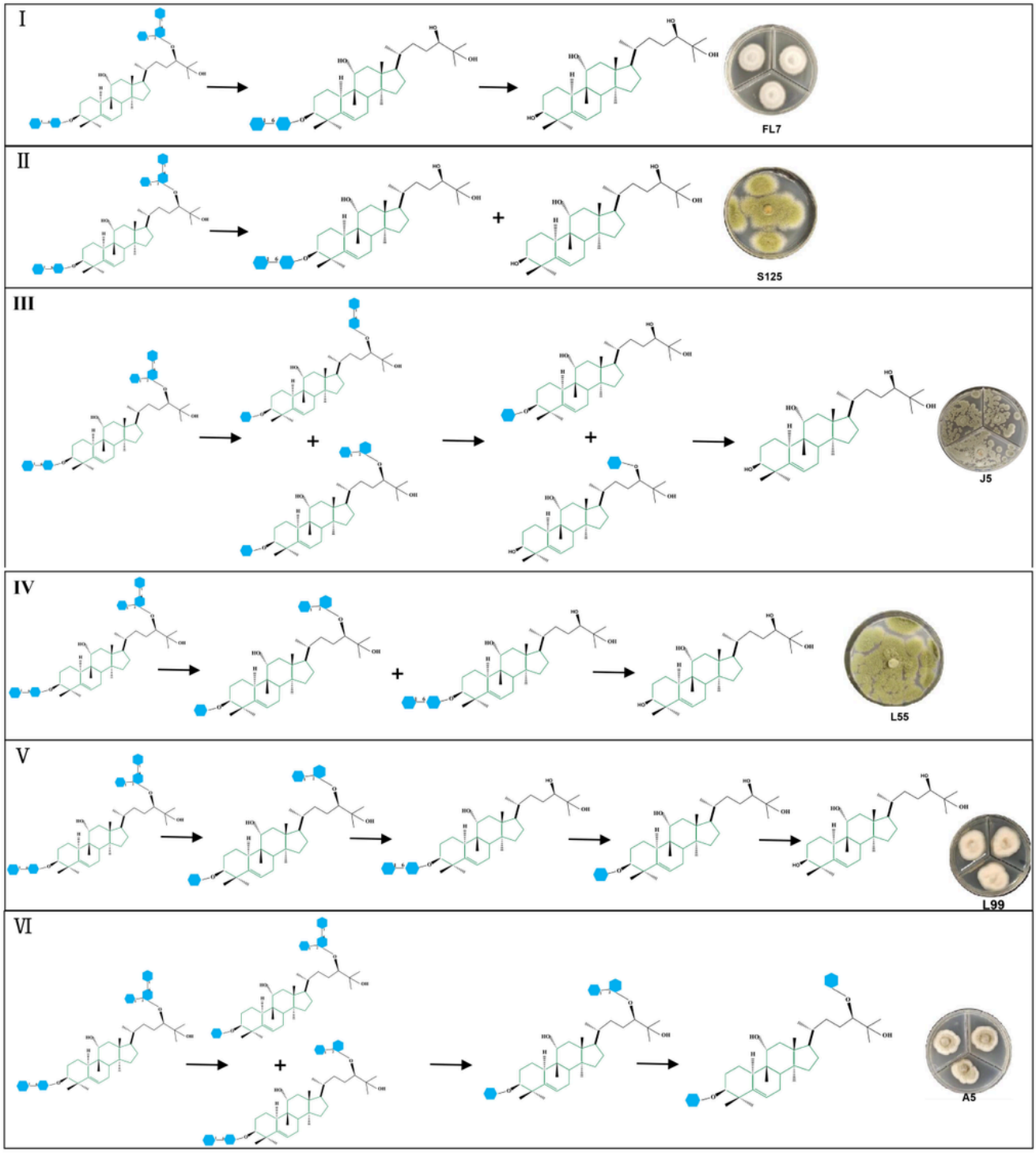


Figure 2

The bioconversion pathways of mogroside V during the conversing processes by endophytic fungi L55, A5, J5, L99, FL7 and S125.

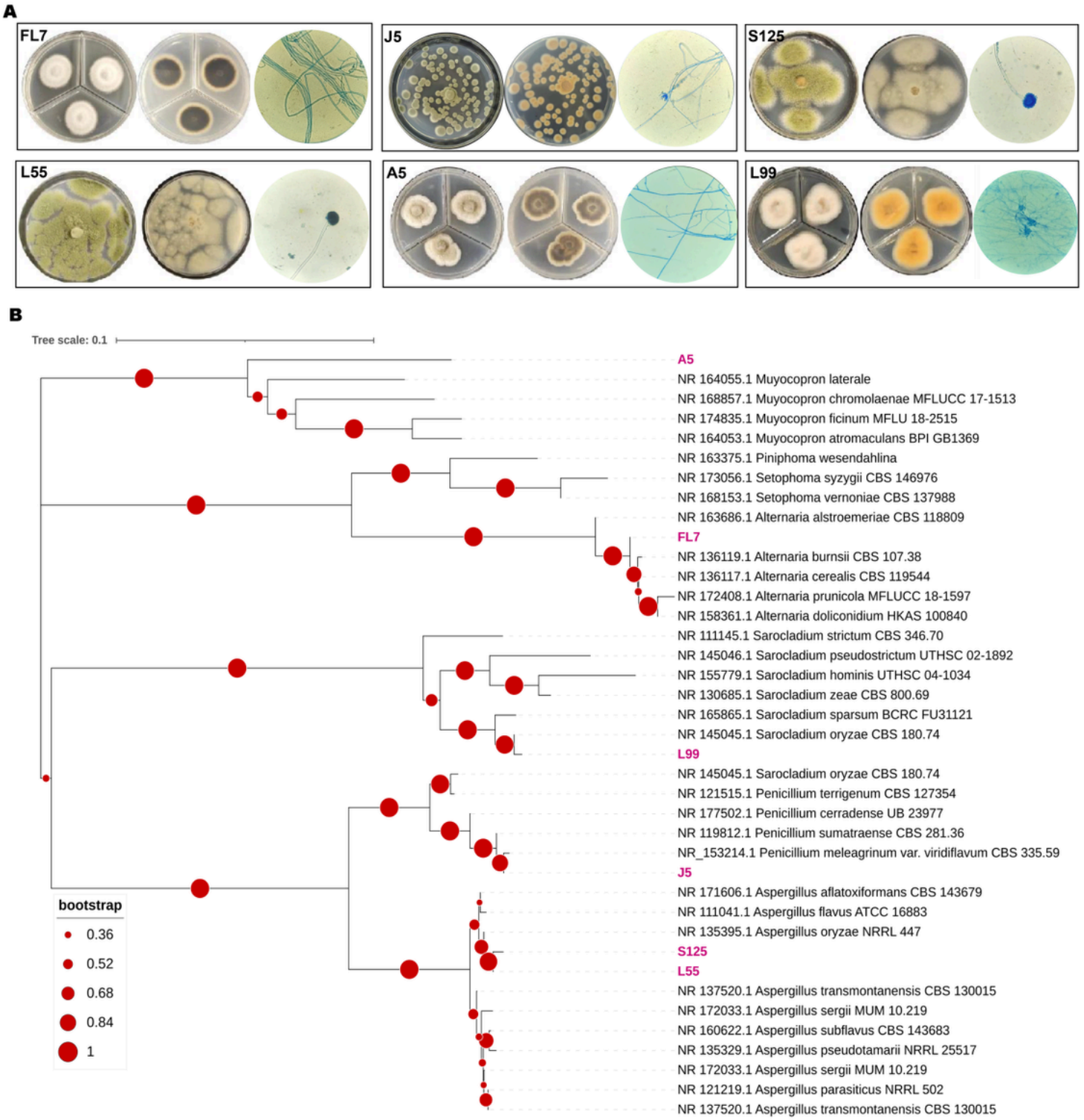


Figure 3

Morphological characteristics of endophytic fungi L55, A5, J5, L99, FL7 and S125 (A), and Maximum-likelihood (ML) trees based on ITS sequences using MEGA software (version 6.0.6) with the Kimura 2-parameter model for calculations of evolutionary distances. The bootstrap values (1000 replicate runs) greater than 50% are listed.

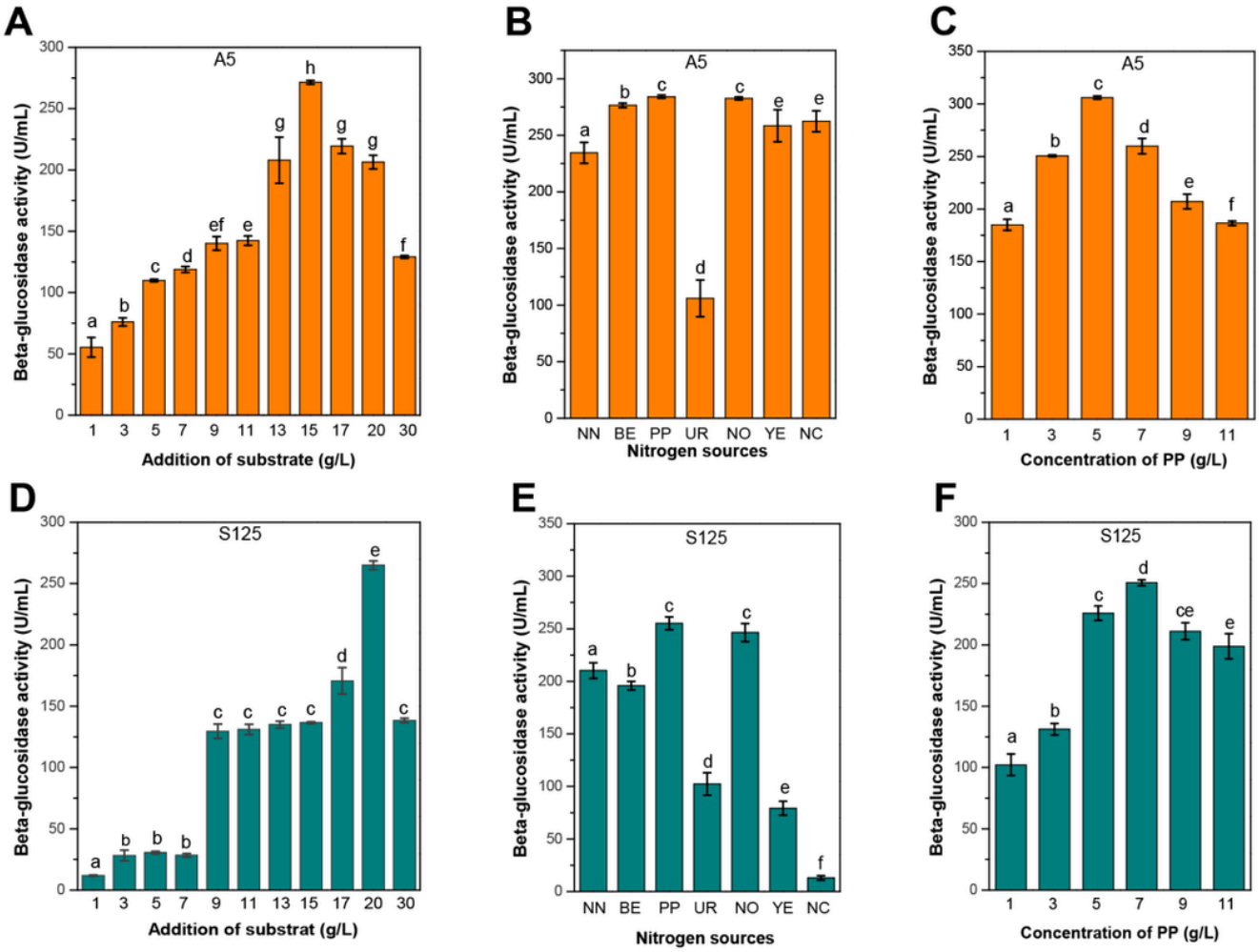


Figure 4

Effect of LHG extract dosages (A, D), different nitrogen sources (B, E) and concentration of peptone (C, F) on β-glucosidase activity of *Muyocopron* sp A5 and *Aspergillus* sp S125, respectively.

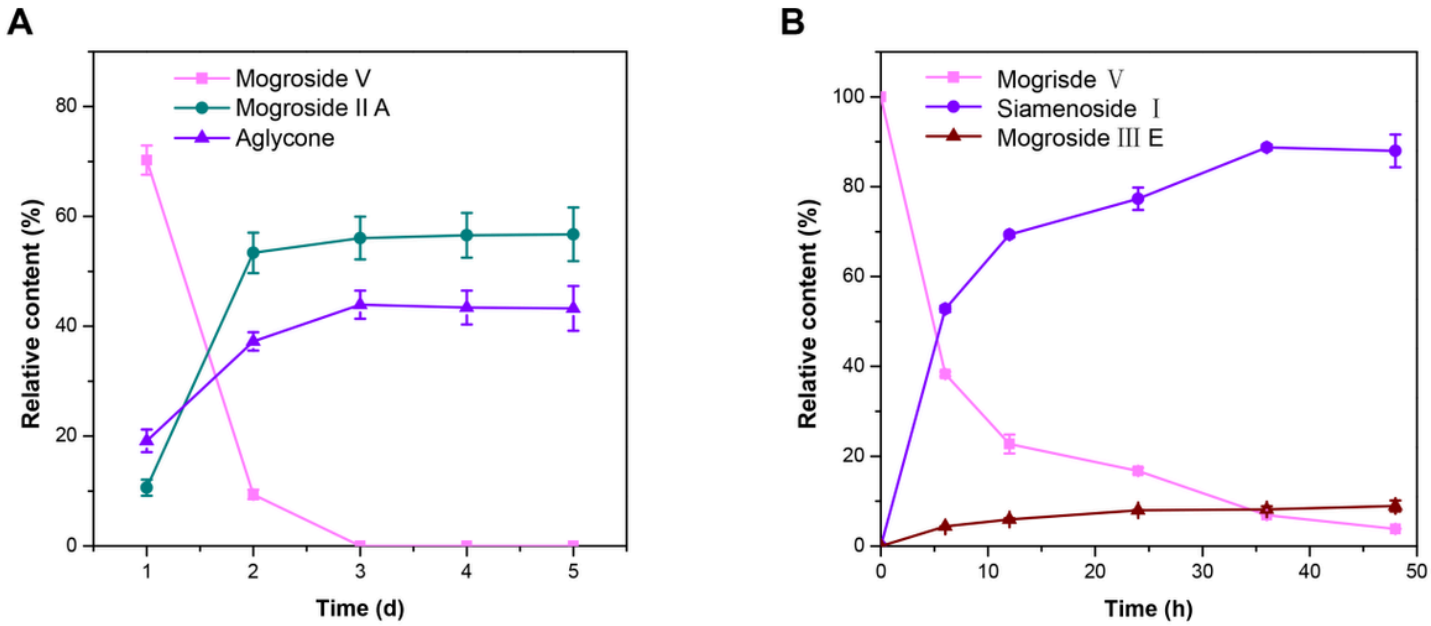


Figure 5

The relative content profiles of mogrosides during bioconversion of *Aspergillus* sp S125 (A) and *Muyocopron* sp A5 (B) under optimal fermentive conditions.

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