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Rational design for heterologous production of aurovertin-type compounds in *Aspergillus nidulans*

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Abstract Aurovertins are the structurally diverse polyketides that distribute widely in different fungal species. They feature a 2,6-dioxabicyclo[3.2.1]-octane ring in structure and exhibit the potential antitumor activity against breast cancer as F1-ATPase β subunit inhibitor. In this study, we constructed the biosynthetic pathway of aurovertin in an *Aspergillus nidulans* host and obtained seven aurovertin-type compounds. Surprisingly, three new aurovertin geometric isomers were characterized. By introducing an inducible promoter *xylP(p)* in the pathway gene acyltransferase *aurG*, we can control the product ratios among different aurovertin compounds by adding glucose and/or inducer xylose. The yields of aurovertins could be increased up to about 20 times by adding a constitutive promoter *gpdA(p)* to transcription factor AurF, which indicates AurF's positive role in the biosynthesis of aurovertin. Taken together, our results provided not only an efficient way to generate bioactive fungal natural products but also realized the rational controlling their yields with designed promoters.

Zihui Ma and Wei Li contributed equally to this work.

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Introduction

The aurovertins are highly reduced polyketide products with the basic structure of 2,6-dioxabicyclo[3.2.1]-octane ring system (DBO). Totally, near 30 aurovertin-type compounds including aurovertins A-S (Baldwin et al. 1964; Wang et al. 2005; Azumi et al. 2008; Zhou et al. 2009; Niu et al. 2010; Guo et al. 2013), asteltoxins (Adachi et al. 2015), avertoxins (Wang et al. 2015b), and citreoviridin (Franck and Gehrken 1980; Lin et al. 2016) were isolated from multiple fungal genus such as *Calcarisporium*, *Metarhizium*, *Aspergillus*, *Penicillium*, *Pochonia*, and mushroom *Albatrellus*. Due to the unusual polyketide-derived structure, the members of aurovertin-type compounds exhibit good bioactivities. Aurovertin B from *Calcarisporium arbuscular* (Baldwin et al. 1964) inhibits mitochondrial oxidative phosphorylation by binding to the β subunit of F1-ATPase (van Raaij et al. 1996), therefore uncompetitively inhibits ATP hydrolysis and has been implicated as potential therapeutics against cancer (Huang et al. 2008). With the similar mechanism, aurovertin D from *Pochonia chlamydosporia* showed strong toxicity toward the root-knot nematode *Meloidogyne incognita* and was found to mediate interactions between the fungus and its host (Wang et al. 2015a).

Due to the potent biological activities, the total synthesis and biosynthesis of aurovertins have been investigated since 1980s. Using D-glucose as starting material, Nishiyama et al. (1986) synthesized aurovertin B and determined its absolute configuration. The biosynthetic origin of aurovertin B was investigated by the labeling experiment to derive from a polyketide pathway (Steyn et al. 1981). Recently, the biosynthetic

gene cluster of aurovertin was characterized by the genome mining of *C. arbuscular* and genetic disruption as well as in vitro biochemical experiments (Mao et al. 2015a, b). The cluster includes seven genes (Fig. 1a). The encoded enzymes contains a high reducing polyketide synthase (HR-PKS) AurA, a methyltransferase (MT) AurB, a flavin-dependent mono-oxygenase (FMO) AurC, a hydrolase AurD, a cyclase AurE, a transcription factor (TF) AurF, and an acyltransferase AurG. Four enzymes were reported to be required to form aurovertin E with DBO system. FMO AurC and hydrolase AurD are identified to generate the complex natural product scaffolds by iterative oxidizing and the regioselective hydrolyzing reactions (Mao et al. 2015). More recently, Wang and co-workers reported the heterologous expression in *Aspergillus nidulans* for the biosynthetic gene cluster of citreoviridin from *A. terreus* var. *aureus* (Lin et al. 2016). Here, our effort is aimed to establish an efficient way to produce the aurovertins, the bioactive fungal natural products, in the heterologous host *A. nidulans*. Then, we can determine the production of acetylated compound, e.g., aurovertin B by the controlling *aurG* expression using an inducible promoter.

Materials and methods

Strain and culture conditions The fungal strains used in this study are indicated in Table S1. *Calcarisporium arbuscula* NRRL 3705 was grown on potato dextrose agar medium (PDA) at 25 °C. *A. nidulans* strains were grown on glucose minimum medium (GMM) with or without appropriate supplementary (0.56 g uracil/L, 1.26 g uridine/L, and 0.5 μM pyridoxine HCl) at 37 °C for 72 h for sporulation. For aurovertin production, *A. nidulans* mutants were cultured in liquid GMM (supplemented with 5 g yeast extract/L, LMM) for 4 days at 25 °C, or were cultured in rice medium (rice 30 g, water 45 mL and supplemented with appropriate supplementary in 500-mL Erlenmeyer flasks) for 10 days at 25 °C for large fermentation. *Escherichia coli* DH5α strain was used routinely for DNA transformation and plasmids amplification, and LB media were used for *E. coli* culture. *Saccharomyces cerevisiae* strain BJ5464-NpgA (*MATαura3-52 his3-Δ200 leu2-Δ1 trp1 pep4::HIS3 prb1Δ1.6R can1 GAL*) was used for biosynthesis pathway reconstitution of plasmid construction with Yeast assembly method. For yeast culture, SD-drop out media were used for selection of plasmids transformed into *S. cerevisiae* at 30 °C.

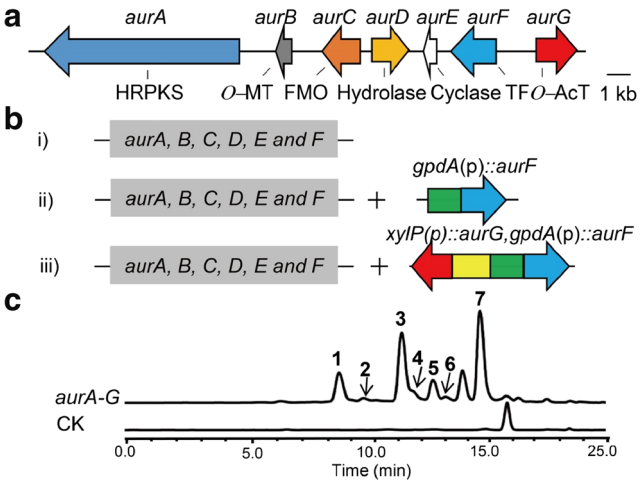

Fig. 1 Heterologous expression of aurovertin (*aur*) biosynthetic gene cluster in *Aspergillus nidulans*. **a** Aurovertin biosynthetic gene cluster from *C. arbuscular*. **b** Design for plasmid construction of different cluster gene combinations. (i) Five cluster genes *aurA*−*F*; (ii) *aurA*−*F* with overexpression transcription factor *gpdA(p)::aurF*; (iii) acyltransferase gene *aurG* with the inducible promoter *xylP(p)* and *gpdA(p)::aurF* in *aurA*−*F* (C) HPLC analysis of full *aur* cluster gene expression strains: TYZM14.2 (*aurA*−*G*) and *A. nidulans* RJMP1.59 (CK), seven new peaks were detected in the mutant at 363 nm

Gene cloning and plasmids construction The plasmids utilized in this work are listed in Table S1. The oligonucleotide sequences for PCR primers are given in Table S2. PCR reactions were performed with Phusion high-fidelity DNA polymerase (NEW ENGLAND Biolabs, NEB). PCR screening for transformants were carried out with 2 × TSINGKE® Master Mix (TSINGKE). The basic strategy for assembling of large PCR fragments is splicing by overlapping extension (SOE)-PCR and yeast homologous recombination (Wang et al. 2005). To clone aurovertin (*aur*) biosynthetic gene cluster, *aur* biosynthetic genes from *aurA* to *aurF* were divided into five fragments. Overlapping regions between adjacent flanking segments ranged from 230 to 250 bp and overlapping regions between the segments and the vector pYH-wA-pyrG were 40 bp. Each fragment (around 4 kb) was amplified with *C. arbuscula* genomic DNA by using designated primers (Table S2) respectively and then was assembled by homologous recombination to generate pWYaur in yeast (Wang et al. 2005). Positive yeast colonies were selected by screening PCR. Yeast plasmids were isolated by using Zymoprep™ (D2001) Kit (ZYMO RESEARCH) and transformed into *E. coli* DH5α. Bacterial plasmids were isolated by using a plasmid kit (OMEGA). Using Quick-change mutagenesis method, transcription factor gene *aurF* was inserted behind *gpdA(p)* in pYW25.16 to construct pYZM2.1 (Guo et al. 2013). Then, the inducible promoter *xylP(p)* and *aurG* gene were introduced to pYZM2.1 to get pYZM4.1 via an intermediate vector pYZM3.1. All plasmids were confirmed by restriction enzyme digestion (NEW ENGLAND Biolabs, NEB) and sequencing.

Transformation manipulation *A. nidulans* RJMP1.59 was used as the heterologous host (Table S1). The protocols for protoplasts preparation and transformation were described previously (Zhou et al. 2009). pWYaur was transformed into *A. nidulans* RJMP1.59 to generate TYZM1. Then pYZM2.1

or pYZM4.1 was transformed into TYZM1.5 to obtain TYZM13 and TYZM14, respectively. The transformants were verified by using diagnostic PCR with appropriate primers (Table S2).

RNA preparation and semi-quantitative RT-PCR For the analysis of function of *aurF*, 4×10^6 spores from TYZM1.5 and TYZM13.7 were inoculated into 20 mL LMM and cultivated at 25 °C for 4 days under dark conditions. To control acetylated aurovertin production with the expression of *aurG*, TYZM14.2 was inoculated into LMM with different carbon sources glucose or xylose. Three replicates were performed for each experiment. Then, mycelia were harvested and total RNA was extracted using TRIzol® Reagent (Life Technologies). Five micrograms of total RNA were reverse transcribed into first-strand cDNA using FastQuant RT-Kit (with gDNase) (TIANGEN Biotech, China). For transcription assessment, the coding regions of targeted genes were amplified using designated primer pairs, and the expression of ANactin was used as the internal control (Table S2).

Chemical analysis *A. nidulans* strains were cultivated in LMM (supplemented with 5 g yeast extract/L) at 25 °C for 4 days in the dark. 2.0×10^6 spores were added to 10 mL medium per 60 mm plate. The cultures were extracted with methanol-ethyl acetate (10:90, *v*/*v*), and then evaporated under reduced pressure. The residue was dissolved in 1 mL methanol, and 20 μL of the solution was directly injected for HPLC analysis. Analytical HPLC was conducted with a Waters HPLC system (Waters e2695, Waters 2998, Photodiode Array Detector) using an ODS column (C18, 250 × 4.61 mm, COSMOSIL, 5 μm) with a flow rate of 1 mL/min. Crude extract was analyzed on a gradient of 60 to 80% methanol (0.1% formic acid) in 20 min followed by 100% methanol for 5 min. The fold differences of these compounds between control and mutants were calculated according to the following formula: [Area (Sample)-Area (Blank)]/[Area (WT)-Area (Blank)]. All MS and LC-MS analyses were performed using a Kromasil 100–5 C18 column (4.6 × 250 mm; 25 °C) on an Agilent 6520 Accurate-Mass QTOF LC/MS system (Agilent Technologies) equipped with an electrospray ionization (ESI) source. NMR spectra were recorded at room temperature with a Bruker Avance-500 spectrometer.

Purification of compounds 1–7 TYZM1.5 was cultured at 25 °C in 500-mL Erlenmeyer flasks containing rice media (rice 30 g, water 45 mL, and 90 μL 0.1% pyridoxine HCl). Totally, 1.2 kg rice was fermented. After 10 days of cultivation, the fermentation from 40 flasks was extracted three times with methanol: ethyl acetate (10:90). To discard the residual starch, the residual was extracted with EtOAc/H_2O (1:1) for three times. The organic phase was evaporated to dryness under reduced pressure to afford the residue (5.44 g). The extracts were separated by a Sephadex LH-20 column with methanol. Further purification was carried out by a reverse-phase ODS column (25:75 → 100:0 MeOH/H_2O) to give eight fractions. Fractions 4 and 5 were applied to another Sephadex LH-20 column with methanol. The compound **1** (7.0 mg) and **3** (17.6 mg) were obtained by semi-preparative HPLC (30:70 CH_3CN/H_2O, 2 mL/min) with a reverse-phase column (YMC-Pack ODS-A 250 × 10 mml S-5 μm 12 nm).

TYZM13.7 was cultivated as described above for the purification of compounds 2 and 4. 1.5 kg rice in 50 flasks was fermented in total. The crude extracts (10.9 g) were subjected to a ODS column, eluting with CH_3CN-H_2O (10:90–100:0 gradient system) to afford nine fractions. Fraction 3 was further fractionated into Frs. 3.1–3.8. Frs. 3.1 and 3.2 were combined and separated over a Sephadex LH-20 column using methanol as the mobile phase, and compound 2 (2.2 mg) was then isolated by a semi-HPLC eluting with methanol-water (55:45, *v*/*v*) with a flow rate of 2.5 mL/min. Compound 4 (3.5 mg) was purified from a combination of Frs. 3.3–3.6 by applying the same separation procedure.

TYZM14.2 was cultivated as described above for the purification of compounds 5, 6, and 7. Seven liters LMM media were used in total. The crude extracts (3.5 g) were subjected to a ODS column, eluting with CH_3CN-H_2O (10:90–100:0 gradient system) to afford six fractions. Fraction 4 was further fractionated into Frs. 4.1–4.8. Frs. 4.2 and 4.4 (24.5 mg) were combined and isolated by a semi-HPLC eluting with methanol-water (55:45, *v*/*v*) with a flow rate of 2.5 mL/min. And then compounds 5 (5.4 mg) and 6 (1.3 mg) were purified. Compound 7 (3.7 mg) was purified from Fr. 4.6 by applying the same separation procedure.

Analytical methods and equipment overview HPLC analyses were performed on Waters 2695 Separations Module 241 with a RP C18 column (38020-41 COSMOSIL 5C18-MS-II Packed 242 Column; 4.6 × 250 mm) at a flow rate of 1 mL/min. Water and methanol were used as solvents. For analysis of the extracts, a linear gradient of methanol in water (60–80%, *v*/*v*) in 20 min was used. The column was then washed with 100% (*v*/*v*) methanol for 5 min and equilibrated with 30% (*v*/*v*) methanol for 5 min. Detection was carried out with a photodiode array detector and illustrated at 363 nm in this paper. For isolation of compound 1, 2, and 3, Shimadzu LC-6AD with a YMC-Pack ODS-A column (250 × 10 mm, 5 μm) was used. Water and methanol/acetonitrile were used as solvents. For isolation of 2 and 3, 40% acetonitrile was used with a flow rate at 3.0 mL/min. The column was then washed with 100% (*v*/*v*) acetonitrile for 10 min and equilibrated with 40% (*v*/*v*) acetonitrile for 10 min. All LC-MS analyses were performed on an Agilent 6520 Accurate-Mass QTOF LC/MS system (Agilent Technologies) equipped with a RP C18 column (Kromasil 100-5 C18 column; 4.6 × 250 mm; 25 °C) and an electrospray ionization (ESI) source. NMR

spectra were recorded at room temperature with a Bruker Avance-500 spectrometer.

Statistical analysis For statistical analyses, data were analyzed using the GraphPad Instate software package, version 5.01 (GraphPad software Inc) according to the Tukey-Kramer multiple comparison test at $P \leq 0.05$. Mean values with asterisks are significant.

Results

Construction and transformantion of aurovertin biosynthetic gene cluster

The genomic DNA containing the biosynthetic cluster genes of aurovertins (Fig. 1a) described by Mao et al. (Mao et al. 2015a) was extracted from *C. arbuscular* NRRL 3705. We amplified the cluster gene fragment in three combinations to reach different expectations with the oligonucleotide primers (Fig. 1b): (1) clone *aurA* to *aurF* to get the simplest compound 3 (aurovertin E); (2) the use of the constitutive promoter *A. nidulans gpdA(p)* is to identify the function of TF AurF in the basis of (1) and control the production of aurovertins; (3) introduction of an inducible promoter *xylP(p)* derived from the *P. chrysogenum* endoxylanase (xylP) gene to the acyltransferase aurG is to control the yields of acetylated aurovertin compounds by using inducer xylose or xylan (Haas et al. 1993; Zadra et al. 2000). Then, three plasmids were constructed by using yeast recombination strategy (Colot et al. 2006) (Fig. 1b). To obtain the transformants of aurovertin cluster gene, *A. nidulans* RJMP1.59 protoplasts were transformed with plasmids pWYaur, which contained *aurA* to *aurF* in aurovertin cluster with auxotroph marker *pyrG*. Transformants were selected by auxotroph 0.5 μM pyridoxine HCl, then gDNA of the transformants were isolated and verified by PCR with the screening primers (Table S2). The PCR product showed an amplicon of 527 bp in the positive transformants named TYZM1. Then, TYZM1 protoplasts were transformed with plasmids pYZM2.1 which contained *pyroA*::*gpdA(p)*::*aurF* cassette and pYZM4.1 which contained *pyroA*::*gpdA(p)*::*aurF* and *xylP(p)*::*aurG* cassette, respectively. The gDNA of the transformants were isolated and verified by PCR with the screening primers (Table S2). The PCR product showed an amplicon of 1210 or 989 bp in the transformants, which named TYZM13 and TYZM14, respectively (Fig. S1 and Table S1).

Analysis and isolation of aurovertins in the heterologous host *A. nidulans*

The strains TYZM1.5 (*aurA*−*aurF*) and TYZM14.2 (*aurA*−*aurG*) were cultivated in LMM media, respectively. And the metabolites were extracted and analyzed by HPLC and LC-MS (Figs. 1c and S4). Compared to the isogenic control, four or seven new peaks were observed with UV absorptions identical to those of aurovertin-type compounds, respectively. The expression of *aurG* gene led to the production of the peaks 5, 6, and 7. Surprisingly, some peaks appeared in pairs, e.g., peaks 1, 2; 3, 4, and 5, 6 which suggested the production of aurovertin isomers (Fig. 1c).

To identify these new peaks, large-scale fermentation of TYZM1.5 and TYZM14.2 strains was conducted. After the extraction and isolation, peaks 1, 3, 5, and 7 were characterized as known compounds: aurovertins I (1), E (3), C (5), and B (7), respectively (Fig. 2), according to their MS and NMR data in comparison to the known compounds (Zhou et al. 2009; Niu et al. 2010; Mao et al. 2015b) (Tables S3, S5, S7, S9 and Supplementary Figures of NMR spectra). Following the above isolation, the compounds 2, 4, and 6 were purified carefully due to their instabilities and then immediately analyzed by MS and NMR. Mass analysis showed that they have the identical molecular weights at m/z = 404, 419 and 447 $[M + H]^+$ with the related compounds 1, 3, and 5, respectively (Fig. S4), which suggest that they are paired isomers.

Stability assessment and structural determination of compounds 2, 4, and 6

From the previous purifications for compound 2, 4, and 6, we found that they are not stable and quickly converted into their stable forms. To assess their stabilities, we took 2 as an example for the analysis. HPLC analysis showed that compound 2 was a major peak when injection immediately after purification, then compound 2 was transformed to compound 1 near 1:1 ratio after 4 days. After 30 days, over 90% of compound 2 was transformed to compound 1 which showed compound 2's instability (Fig. 3a, b). By comparisons of the ^{1}H NMR spectra, almost all proton signals between compound 1 and compound 2 are consistent except for the H-13 (δ_H 7.09, J = 11.3, 15.0 Hz, for 1; δ_H 7.42, J = 12.0, 11.3 Hz, for 2) and H-14 (δ_H 6.60, J = 15.0 Hz, for 1; δ_H 6.16, J = 12.0 Hz, for 2) indicating that compound 2 was the specific 13-*Z*-isomer of compound 1 (Table S4 and Supplementary NMR spectra). Additionally, the *Z*-isomer (2) was found to isomerize readily to its *E*-isomeric form (1) under laboratory conditions, which could be explained by the light-driven *cis* to *trans* isomerization (Zaki et al. 2013). Compounds 4 and 6 showed a similar ^{1}H NMR pattern at C13/C14 double bond as compound 2, which demonstrated the *Z*-configuration of compound 4 and compound 6 (Tables S6 and S8). Hereafter, compounds 2, 4, and 6 are named as *iso*-aurovertin I, *iso*-aurovertin E, and *iso*-aurovertin C, respectively.

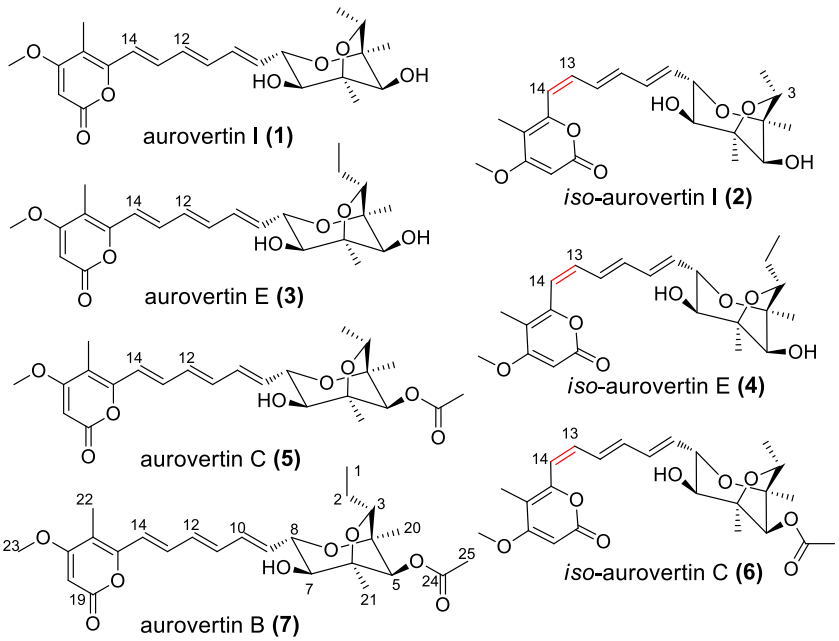

Fig. 2 Structures of aurovertins identified in the heterologous host *A. nidulans*. Seven compounds are characterized as aurovertin I (**1**), *iso*-aurovertin I (**2**), aurovertin E (**3**), *iso*-aurovertin E (**4**), aurovertin C (**5**), *iso*-aurovertin C (**6**), and aurovertin B (**7**)

AurF is a positive transcription factor in the biosynthesis of aurovertin

We examined the transcription factor aurF's function by the quantitative analysis of auroverin's yields in the *aurF* overexpression (OE) strain. Both of *aurA−F* (TYZM1.5) and *gpdA(p)*::*aurF*, *aurA−F* (TYZM13.7) strains were cultivated in LMM media for 4 days at 25 °C. HPLC analysis of crude extracts showed that the yields of compound 1 and compound 3 in OE::*aurF* strain are much higher than the isogenic control (Fig. 4a, b). Quantitative analysis indicated that 20 times of compound 1 and compound 3 were produced in the OE::*aurF* strain in comparison to the control (Fig. 4c). To assess the role of *aurF* in the regulation of the *aur* cluster genes, RT-PCR was carried out and showed the transcription levels of *aurB-F* were greatly increased (Fig. 4d).

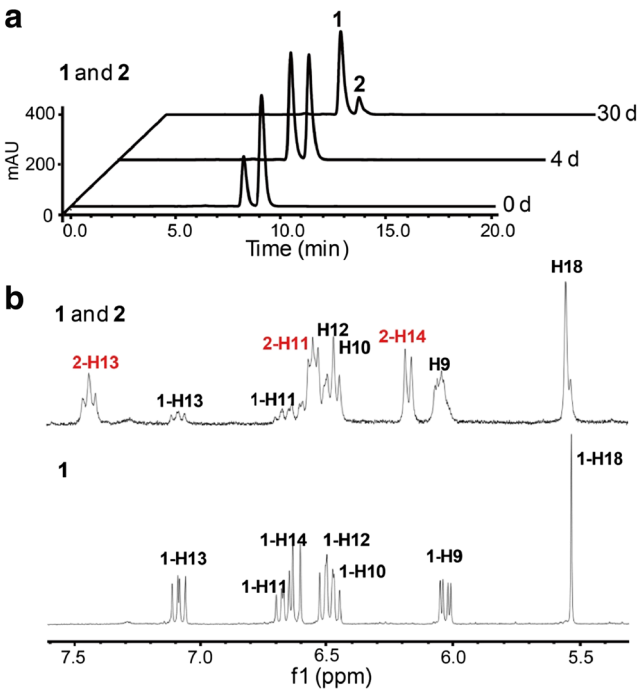

Fig. 3 Compound **2** is not stable and converted into **1** automatically. **a** **2**'s stability assessment by HPLC analysis in different days after isolation. **b** ^{1}H NMR spectra of the **1** and **2**. **2** is mixed with **1** (i). Proton signals of **2**-H13/H14 are clearly different with **1** (ii)

Promoter controlling aurovertin-type compound production

To control the production of different compounds, we constructed *gpdA(p)::aurF* with *xylP(p)::aurG* in the same vector and transformed it into the *aurA−F* strain. Thus, the yields of aurovertin-type compounds can be controlled by mediating of the expression of *aurG* with different carbon sources such as glucose and inducer xylose. By the analysis of transformants, seven peaks were found in the mutant as expected (Fig. 1c). To meidiate the production of different aurovertin-type compounds, we added glucose and xylose to the culture media at different ratios. When using glucose as sole carbon source, four major compounds 1, 3, 5, and 7 were produced whether 2 or 1% glucose in the culture media. While adding 1% xylose with 1% glucose to the media, the proportions and of 5 and 7 were greatly increased in comparison to the media only with glucose (Fig. 5a–c). When adding xylose as sole carbon source to the media, 5 and 7 are the major compounds and their yield are much higher in 2% xylose media than 1% xylose media suggesting the higher expression of *aurG*.

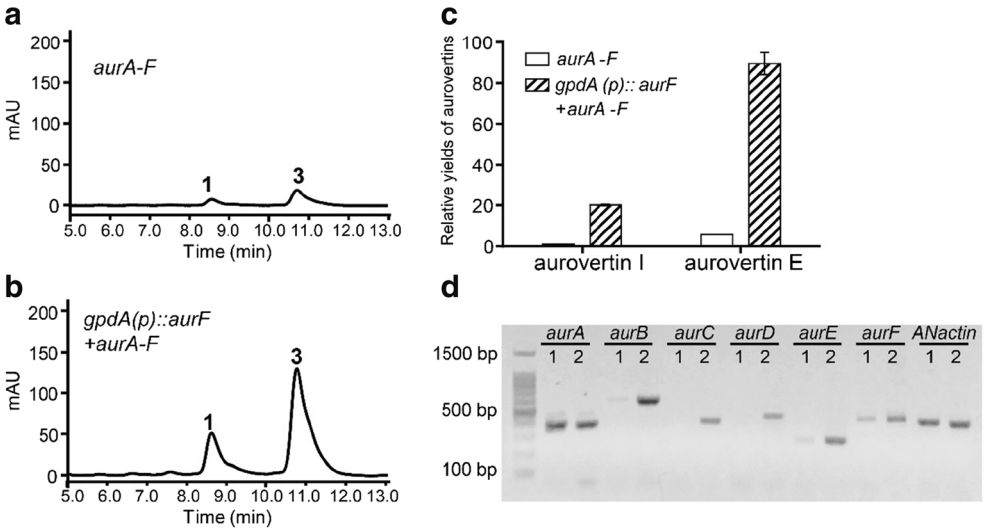

Fig. 4 AurF is a positive transcription factor in the biosynthesis of aurovertin. **a** HPLC analysis of crude extracts from *aurA−F* strain and **b** *aurF* overexpressed strain; **c** Quantitative analyses of aurovertin I and E production in both strains. **d** RT-PCR analysis of *aur* cluster gene expression. 1: cDNAs from *aurA−F* strain; 2: cDNAs from *aurF* overexpressed strain; *Anactin* was used as internal standard

Indeed, RT-PCR analysis of *aurG* in TYZM14.2 showed the higher expression with the induction of xylose (Fig. S3).

Discussion

Calcarisporium arbuscular was reported to produce the aurovertin-type compounds including aurovertins A, B, C (Mulheirn et al. 1974; Silber et al. 2013), D (Baldwin et al. 1964), E (Wang et al. 2005), J, M, T, and U (Zhao et al. 2016). However, the natural host *C. arbuscular* grows very slowly. The average growth rate is 1.75 mm per day in the defined media (Watson Pauline 1965). In this study, we aim to reconstruct the biosynthetic pathway of aurovertin quickly and express the cluster in an *A. nidulans* host.

For fungal heterologous expression, we chose *A. nidulans* RJMP1.59 as host (Palmer et al. 2010; Yin et al. 2012) (Table S1) which has been used for the heterologous production and activations of silent gene clusters such as immunosuppressant neosartoricins from dermatophytes and cryptic fungal pigment melanin from an endophytic fungus *Pestalotiopsis fici* previously (Yin et al. 2013; Zhang et al. 2017; Liu et al. 2017). The resultant plasmids containing the designated genes were transformed into *A. nidulans*. Transformants were obtained by PCR verification of the integration of *aur* cluster genes.

As expected, the transformant strain TYZM1.5 (*aurA−aurF*) can produce compound 3, precursor to other aurovertin derivatives, and TYZM14.2 (*aurA−aurG*) can produce compound 3, 5, and 7. Interestingly, compound 1 (aurovertin I) is not reported

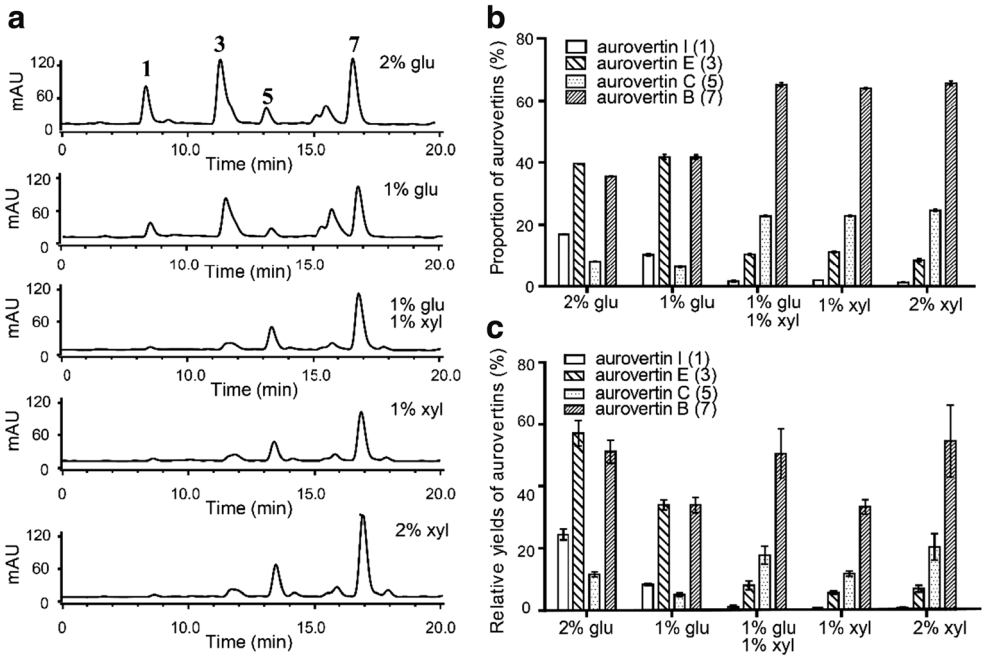

Fig. 5 Promoters controlling aurovertin-type compound production in *A. nidulans* hosts. The strains were cultivated in the minimal media with inducer xylose. Glu: glucose, xyl: xylose. **a** HPLC analysis of crude extracts from *aurA−G* strain with different concentrations of glucose or xylose. **b** Quantitative analysis of the proportions of different aurovertin-type compounds. **c** Quantitative analysis of the relative yields of different aurovertin-type compounds

in *C. arbuscular* but was only found in *P. chlamydosporia* (Niu et al. 2010) and *Albatrellus confluens* (Guo et al. 2013). Heterologous expression of *aur* cluster indicated that the genes from *C. arbuscular* can synthesize compound 1 too. It may because that compound 1 turns into compound 5 almost completely so that it could not be detected in *C. arbuscular*. Following the above isolation, the compounds 2, 4, and 6 were purified carefully due to their instabilities and then immediately analyzed by MS and NMR, which were suggested that they are paired isomers of compounds 1, 3, and 5, respectively.

AurF was speculated to be mostly the transcriptional activator of the *aur* cluster (Mao et al. 2015b). In this study, improving about 20 times of compound 1 and compound 3 produced in the *OE::aurF* strain confirmed the function of *aurF*, which is consistent with the production of aurovertin-type compounds which demonstrated the positive role in the biosynthesis of aurovertins. Compounds 1 and 3 can be produced without the acyltransferase gene *aurG*. The acetylated aurovertin compounds such as compounds 7 often exhibited good activities (van Raaij et al. 1996). So, to acquire more product of compounds 7, we designed to control acyltransferase gene *aurG* by introducing of inducible promoter *xylP(p)*. As shown in the results, we can not only improve the total products of aurovertins including compounds 3 only with glucose in the media, but also greatly increase the compound 7 which has potential therapeutics against breast cancer with adding xylose as sole carbon source to the media. Therefore, acetylated compounds of aurovertins could be controlled by the inducible expression of acyltransferase gene *aurG* in the heterologous *A. nidulans* host strain.

In summary, our studies demonstrate an efficient way to construct the biosynthetic pathway and produce the bioactive fungal natural products. Using this heterologous system, we expressed successfully seven aurovertin-type compounds, specifically; we characterized three new aurovertin isomers. Introduction of constitutive and inducible promoters to the expression system, we identified the TF AurF's positive role in the biosynthesis of aurovertins and realized the control of acetylated aurovertins compounds production.

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Compliance with ethical standards

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Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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