J. Gen. Appl. Microbiol., **64**, 240–247 (2018)
doi 10.2323/jgam.2018.01.001

jGAM

Full Paper

5-Methylmellein is a novel inhibitor of fungal sirtuin and modulates fungal secondary metabolite production

(Received November 13, 2017; Accepted January 14, 2018; J-STAGE Advance publication date: May 23, 2018)

Ryosuke Shigemoto, Takara Matsumoto, Shunsuke Masuo, and Naoki Takaya*

Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Sirtuin is an NAD^+-dependent histone deacetylase that is highly conserved among prokaryotes and eukaryotes. Sirtuin deacetylates histones and non-histone proteins, and it is involved in fungal growth and secondary metabolite production. Here, we screened 579 fungal culture extracts that inhibited the histone deacetylase activity of Sirtuin A (SirA), produced by the fungus *Aspergillus nidulans*. Eight fungal strains containing three Ascomycota, two Basidiomycota and three Deuteromycetes produced SirA inhibitors. We purified the SirA inhibitor from the culture broth of *Didymobotryum rigidum* JCM 8837, and identified it as 5-methylmellein—a known polyketide. This polyketide and its structurally-related compound, mellein, inhibited SirA activity with IC_{50} of 120 and 160 μM, respectively. Adding 5-methylmellein to *A. nidulans* cultures increased secondary metabolite production in the medium. The metabolite profiles were different from those obtained by adding other sirtuin inhibitors nicotinamide and sirtinol to the culture. These results indicated that 5-methylmellein modulates fungal secondary metabolism, and is a potential tool for screening novel compounds derived from fungi.

Key Words: *Didymobotryum rigidum* JCM 8837; drug discovery; epigenetics; fungal sirtuin; histone modification

Introduction

Histone deacetylases (HDAC) are highly conserved enzymes found in organisms throughout all biological kingdoms. They deacetylate distinct lysine residues on the amino-terminal tails of histones and regulate their acetylation (Imai et al., 2000). The hypoacetylation of histones leads to the formation of heterochromatin, which is a tightly packed form of chromatin, and suppresses gene expression (Grunstein, 1997). Some HDAC deacetylate non-histone proteins, including transcription factors and mRNA-processing proteins, and control their activity (Kim et al., 2015; Shahbazian and Grunstein, 2007). Sirtuins are class III HDAC that require NAD^+ for the removal of an acetyl moiety (Landry et al., 2000). Several sirtuins are associated with pathological processes such as cancer and Parkinson's disease in mammals including humans, (Fraga et al., 2005; Outeiro et al., 2007). Therefore, inhibitors of human sirtuins are considered as attractive drug candidates, and have been screened from various chemical libraries (Bodner et al., 2006; Grozinger et al., 2001; Hirao et al., 2003; Napper et al., 2007; Vedadi et al., 2006).

Fungal sirtuins have highly pleiotropic functions. *Neurospora crassa* sirtuins Nst-1, Nst-3 and Nst-5 are required for telomeric silencing in a coordinated manner with histone methyltransferase (Smith et al., 2008). The sirtuin MpSir2 of the common rice pathogen *Magnaporthe oryzae* deacetylates the putative histone demethylase, MoJmjC, which regulates the expression of *MoSod1* that is required for neutralizing plant-derived reactive oxygen species during early infection (Fernandez et al., 2014). Pathogenic *Candida albicans* Hst3 deacetylates the acetylated 56th lysine residue on histone H3, and protects the fungus against genotoxic antifungal agents (Wurtele et al., 2010). Treatment with nicotinamide (NAM), which inhibits sirtuin, increases susceptibility to antifungal agents, suggesting that sirtuin inhibitors might function as new antifungal therapies. The sirtuin HstD of *Aspergillus oryzae* down-regulates biosynthetic gene expression and the production of kojic acid, which prevents the melanogenesis of skin cells (Kawauchi et al., 2013). We previously showed that *A. nidulans* sirtuin A (SirA) is a homolog of the *Saccharomyces cerevisiae* Sir2p, and it deacetylates

*Corresponding author: Naoki Takaya, Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.
Tel: +81-29-853-4937 Fax: +81-29-853-7191 E-mail: takaya.naoki.ge@u.tsukuba.ac.jp

None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

the acetylated 16th lysine residue on histone H4 and represses the expression of genes that produce secondary metabolites such as austinol, sterigmatocystin and penicillin (Itoh et al., 2017a,b; Shimizu et al., 2012). These findings indicated that fungal sirtuin inhibitors could increase production of fungal secondary metabolites and serve as useful tools for drug discovery. However, a systematic screen has not yet identified any inhibitor of fungal sirtuin.

Screening of SirA inhibitors were performed in our study. We screened SirA inhibitors *in vitro* using high-throughput fluorometric measurements of SirA deacetylation activity followed by high-performance liquid chromatography (HPLC) to quantify the deacetylation product, NAM. We tested 579 crude fungal extracts obtained from 193 strains cultured in potato dextrose (PD), yeast-malt sucrose (YMS) and Czapek yeast (CY) media. We purified a SirA inhibitor produced by *Didymobotryum rigidum* JCM 8837, and identified it as 5-methylmellein. Adding 5-methylmellein to *A. nidulans* cultures increased the amount of secondary metabolites produced by the fungus, indicating that 5-methylmellein modulates fungal secondary metabolite production.

Materials and Methods

Fungal strains and cultures. We obtained 193 fungal strains from the RIKEN BRC-JCM library (Supplementary Table 1) and propagated them on potato dextrose (PD) (BD Biosciences, Franklin Lakes, NJ, USA) medium supplemented with 1.5% agar at 28°C for 7–14 days. *Aspergillus nidulans* A26 (*biA1*) obtained from the Fungal Genetic Stock Center (University of Missouri, Columbia, MO, USA) was maintained on glucose minimal (GMM) medium (10 g glucose, 6 g $NaNO_3$, 0.52 g KCl, 0.52 g $MgSO_4 \cdot 7H_2O$, 1.52 g KH_2PO_4, 0.2 % trace element solution (Hutner et al., 1950)) plates supplemented with 0.25 mg/mL biotin.

Preparation of crude fungal extracts. Fungi on plates containing 5 mm^3 of PD agar were inoculated into 50-mL test tubes containing 10 mL of liquid PD, YMS (10 g sucrose, 5 g peptone, 3 g yeast extract and 3 g malt extract in 1 L of distilled water) and CY (30 g sucrose, 5 g yeast extract, 1 g KH_2PO_4, 3 g $NaNO_3$, 0.05 g KCl, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.001 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$ and 0.0005 g $CuSO_4 \cdot 5H_2O$ in 1 L of distilled water) medium, and incubated at 28°C on a reciprocal shaker at 270 rpm for 7–14 days. Culture broths were alkalified with 2 M NaOH, extracted with 10 mL ethylacetate, and dried *in vacuo*. The residue was dissolved in 500 μL methanol.

Screening SirA inhibitors. Recombinant SirA was produced in *Escherichia coli* and purified by affinity chromatography (Shimizu et al., 2012). The primary screen used SIRT1/Sir2 deacetylase fluorometric assay kit (Cyclex Co. Ltd., Nagano, Japan) according to the manufacturer's protocol. Reaction mixtures containing 1 μL of crude fungal extracts were dispensed into 96-well plates. Reactions were initiated by adding 1 μg of recombinant SirA and monitored for 10 min at 30-s intervals using a Synergy HTX microplate reader (Biotek, Winooski, VT, USA) with excitation at 360 nm and emission at 460 nm. A secondary screen was based on NAM quantitation by HPLC. Reaction mixtures (50 μL containing 50 mM Tris-HCl (pH 8.8), 0.5 mM DTT, 98 μM [Lys(Ac)16]-histone H4 peptide (Eurogentec, Seraing, Belgium), 200 μM NAD^+, 1 μM trichostatin A, 1 μL fungal culture extract and 1 μg recombinant SirA) were incubated at 25°C for 4 min, then the reactions were terminated by adding 50 μL of 5% (v/v) trichloroacetic acid. Note that the reaction included trichostatin A to prevent possible deacetylation of the acetylhistone peptide by zinc-dependent HDAC contaminated in the fungal culture extract. The mixtures were analyzed using HPLC (1260 Infinity; Agilent Technologies, Palo Alto, CA, USA) equipped with a 250 × 4.6-mm Purospher® Star RP-18 end-capped column with a particle size of 5 μm (Millipore, Billerica, MA, USA). The mobile phase comprised 0.1% (v/v) ammonium bicarbonate:acetonitrile (90:10 v/v). The flow rate was 0.8 mL/min and the column was operated at 40°C. NAM was monitored at 230 nm of absorbance and appeared as a peak with a retention time of 5.3 min.

Isolation and structure elucidation of 5-methylmellein. *Didymobotryum rigidum* JCM 8837 was propagated in a 500-mL Erlenmeyer flask containing 100 mL PD medium on a rotary shaker at 120 rpm and 28°C for one week, and then transferred into a 5-L Erlenmeyer flask containing 1.5 L PD medium and incubated under the same conditions. The culture was filtered through Miracloth, acidified with 2N HCl and extracted three times with the same volume of ethyl acetate. The organic layer was concentrated *in vacuo* to obtain a brown paste (546 mg), which was applied to an open column packed with Wakogel 100C18 (Wako Pure Chemical Industries Ltd., Osaka, Japan) and separated into 13 fractions by stepwise elution with water:acetonitrile from 100:0 to 0:100. The 40:60 fraction (18.5 mg dry weight) was separated by preparative HPLC equipped with a Purospher® Star RP-18 end-capped, 250 × 10-mm column with a particle size of 5 μm (Millipore) using a mobile phase of 50% aqueous acetonitrile at a flow rate of 3.0 mL/min. The peak at a retention time of 19 min was collected and evaporated to obtain a pure compound 1 (5.1 mg).

The mass spectrum of compound 1 was acquired using a LCMS-8030 (Shimazu, Kyoto, Japan) under the following conditions: mobile phase, 0.05% (v/v) ammonium formate (pH 7.0):acetonitrile (1:1 v/v), flow rate, 0.8 mL/min; capillary voltage, 4.5 kV; detection range, *m/z* 50–600; desolvation line, 250°C; heat block, 400°C; nebulizer nitrogen gas 3 L/min; drying gas, 15 L/min. Ion source polarity was set in the negative mode. The NMR spectra were recorded in DMSO-d_6 (Cambridge Isotope Laboratories Inc., Andover, MA, USA) on an AVANCE III-600 Spectrometer (Bruker, Billerica, MA, USA), and referenced to the peaks of tetramethylsilane (δ_H 0) for ^1H and of DMSO-d_6 (δ_C 39.5) for ^{13}C.

Quantitation of intracellular 5-methylmellein. *Didymobotryum rigidum* JCM 8837 was propagated in 500-mL Erlenmeyer flasks containing 100 mL PD medium on a rotary shaker at 120 rpm and 28°C for one week. Resulting mycelia were collected by filtration and ex-

Table 1. Fungal crude extracts that inhibited SirA.

Strain	Medium	SirA inhibition (%)	
		First screen	Second screen
Chaetomella raphigera JCM9995	YMS	55	90
Trichoderma reesei JCM22676	PD	39	86
Didymobotryum rigidum JCM8837	PD	39	61
Podosporium beccarianum JCM8095	CY	39	21
Rhytidhysteron rufulum JCM14423	YMS	55	20
Didymostilbe aurantiospora JCM5089	CY	37	18
Rhytidhysteron rufulum JCM14423	CY	42	16
Cerinosterus luteoalbus JCM2923	PD	39	13
Sphacelotheca pamparum JCM2007	PD	32	8

Table 2. ^{1}H and ^{13}C NMR data for compound 1.

Position	Compound 1[a]		(−)-5-Methylmellein[b]	
	δ_C	δ_H, multi. (*J* in Hz)	δ_C	δ_H, multi. (*J* in Hz)
1	169.9		170.2	
3	75.5	4.75, *m*	75.4	4.68, *m*
4α	31.0	3.05, *dd* (3.4, 16.8)	31.9	2.86, *dq* (4.7, 10.6, 16.8), 2H
4β		2.73, *dd* (11.6, 16.8)		
5	125.2		124.9	
6	137.8	7.38, *d* (8.6)	137.0	7.28, *d* (8.5)
7	114.9	6.79, *d* (8.6)	115.6	6.78, *d* (8.5)
8	159.4		160.5	
9	108.1		108.1	
10	138.2		137.8	
11	20.4	1.45, *d* (6.3), 3H	20.9	1.54, *d* (6.1), 3H
12	17.6	2.17, *s*, 3H	18.0	2.18, *s*, 3H
8-OH		10.91, *s*		10.98, *s*

Data recorded in [a]DMSO-d_6 (^{1}H: 600 MHz, ^{13}C: 150 MHz) and [b]$CDCl_3$ by Okuno T. et al. (1986, ^{1}H: 60 MHz, ^{13}C: 15 MHz).

tracted with 5 mL of ethyl acetate. The organic layer was dried *in vacuo*, resuspended in 200 μL of methanol, and analyzed by HPLC as described (Itoh et al., 2017a). Cells dried at 60°C for 6 h were weighed.

Quantitation of IC_{50}. Commercial 5-methylmellein (AdipoGen Life Sciences Inc., San Diego, CA, USA) and mellein (Cayman Chemical, Ann Arbor, MI, USA) and NAM (Wako) were dissolved in DMSO:methanol (1:1 v/v). Half maximal inhibitory concentrations (IC_{50}) were measured using the fluorometric assays described above, and data were fitted to single exponential decay using OriginPro 7.5J software (OriginLab, Northampton, MA, USA).

Analysis of A. nidulans secondary metabolites. *Aspergillus nidulans* A26 conidia (0.5×10^7) were transferred to 20-mL test tubes containing 5 mL of GMM supplemented with 0.25 mg/mL biotin, yeast-malt glucose (YMG; 10 g glucose, 5 g peptone, 3 g yeast extract and 3 g malt extract in 1 L of distilled water) and PD media, and incubated at 28°C on a reciprocating shaker at 270 rpm. After 48 h, 5-methylmellein, NAM, and sirtinol (100 μM each) were added, and the cultures were further incubated for 120 h. Culture broth was acidified with 2N HCl and extracted with 5 mL of ethyl acetate. The organic layer was evaporated, dissolved in 200 μL methanol, and analyzed by HPLC as described (Itoh et al., 2017a).

Results and Discussion

Screening SirA inhibitors

Fungal strains were cultured in PD, YMS and CY media at 28°C for one week, and SirA inhibition was tested in ethyl acetate extracts of culture broth. The primary screen comprised fluorometric assays that measured the amounts of fluorophore covalently bound to the histone H4-peptide that were released by a fluorescence quencher. Among 579 extracts prepared from 193 strains cultured in any of PD, YMS and CY media, 19 extracts from the primary screen with >30% SirA inhibition were evaluated in a secondary screen comprising HPLC quantitation of NAM. Nine extracts prepared from eight fungal strains inhibited SirA in the secondary screen (Table 1). We did not find a taxonomic bias in strains that produced sirtuin inhibitors. However, *Chaetomella raphigera*, *Rhytidhysteron rufulum* and *Sphacelotheca pamparum* are plant pathogens (Murillo et al., 2009; Wang et al., 2015; Zhang et al., 2014). *Trichoderma reesei* and *Cerinosterus luteoalbus* are fungi that decay wood (Held and Blanchette, 2017; Ooshima et al., 1990). These results suggest that these producers of SirA inhibitors are associated with plants.

Identification of 5-methylmellein as a novel sirtuin A inhibitor

We isolated and identified the SirA inhibitor from

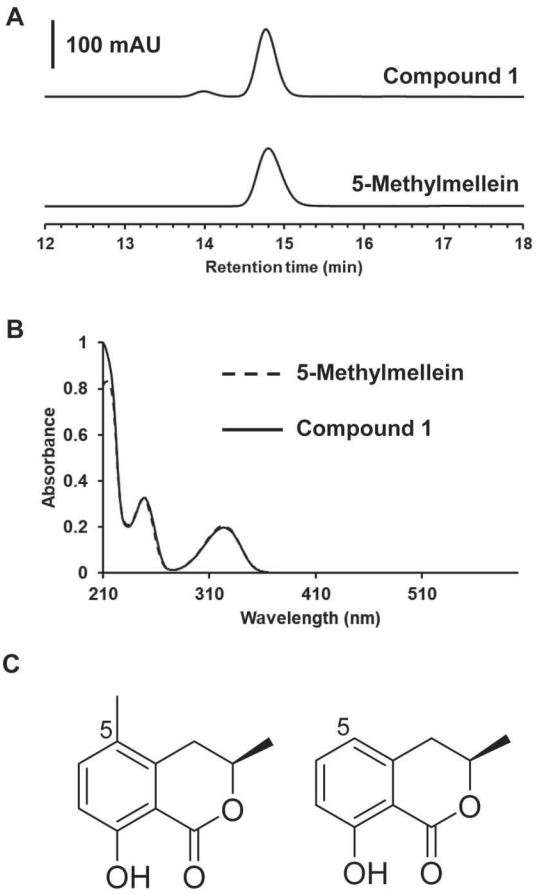

Fig. 1. Identification of 5-methylmellein.

A, HPLC profiles of compound 1 and commercial 5-methylmellein. B, UV/visible spectra of compound 1 and commercial 5-methylmellein. C, Structures of 5-methylmellein (left) and mellein (right).

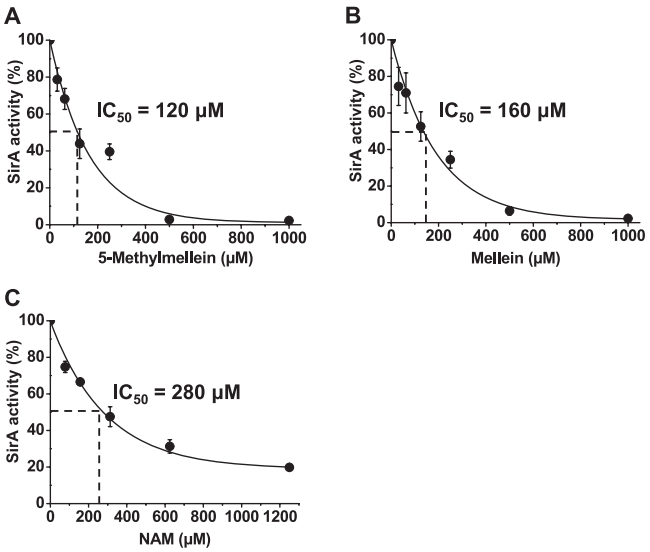

Fig. 2. Inhibition of SirA activity.

5-Methylmellein (A), mellein (B), and NAM (C) dose-dependent inhibition of SirA *in vitro*. Data are means ± standard error of three experiments.

Didymobotryum rigidum JCM 8837 culture extracts. Although the screening used alkaline ethyl acetate extracts of the fungal cultures, we purified the inhibitor from acidic ethyl acetate extract since it more strongly inhibited HDAC activity of SirA than the alkaline one. The fungus was cultured in 1.5 L of PD medium, then culture supernatants were acidified and extracted with ethyl acetate. The organic layer was fractionated by reverse-phase column chromatography with resins, and active fractions were further purified by preparative HPLC. This process yielded 5.1 mg of pure compound 1, which was subsequently analyzed by spectrometry.

Compound 1 appeared as a brown syrup. A molecular formula of $C_{11}H_{12}O_3$ was deduced based on the deprotonated molecular ion at m/z = 191.2 by LC/ESI-MS analysis. The NMR spectra were analyzed in DMSO-d_6. Table 2 summarizes the chemical shifts in ^{13}C- and ^{1}H-. The ^{13}C-NMR spectrum comprised one carbonyl (δ_C 169.9), four aromatic quaternary carbons (δ_C 108.1, 125.2, 138.2 and 159.4), two aromatic methine carbons (δ_C 114.9, 137.8), one methine carbon (δ_C 75.5), one methylene carbon (δ_C 31.0) and two methyl carbons (δ_C 17.6, 20.4). Resonance for one D_2O-exchangeable proton (δ_H 10.91), two aromatic protons (δ_H 6.79, 7.38), one methine proton (δ_H 4.75), one methylene proton (δ_H 2.73/3.05) and two methyl protons (δ_H 1.45, 2.17) was evident in the ^{1}H-NMR spectrum. These chemical shift values matched those of a 3,4-dihydroisocoumarin skeleton. Consecutive 2D-NMR analyses (COSY, HSQC and HMBC) revealed that the structure of compound 1 was that of 5-methylmellein, a known polyketide (Fig. 1C). Identical retention times on HPLC and identical UV/Vis spectra confirmed that compound 1 and commercial (–)-5-methylmellein were the same (Figs. 1A and B). 5-Methylmellein has been isolated from endophytic and plant-pathogenic fungi such as *Xylaria psidii* (Arora et al., 2016), *Mollisia nigrescens* (Ibrahim et al., 2017) and *Biscogniauxia mediterranea* (Evidente et al., 2005). However, this is the first documented production of 5-methylmellein by *D. rigidum*. We quantified the extracellular and intracellular amounts of 5-methylmellein produced by *D. rigidum* and they were 61 ± 11 and 0.21 ± 0.049 μg/100 mL, respectively. Therefore, the amount of 5-methylmellein was 290-fold higher outside, than inside the cells. These findings indicated that large amounts of 5-methylmellein are secreted into the natural environment, and that 5-methylmellein potentially functions in the regulation of sirtuin activity across species. For example, *D. rigidum* might compete in an environmental niche by inhibiting the sirtuin activity of other fungi.

SirA-inhibitory activity of 5-methylmellein

Reports indicate that 5-methylmellein has antifungal (Carvalho et al., 2016; Silva-Hughes et al., 2015), antibacterial (Zheng et al., 2017), and anticancer activities (Arora et al., 2016), whereas its ability to inhibit sirtuin activity has not been described. We analyzed the abilities of 5-methylmellein and structurally analogous mellein to inhibit SirA activity and found that both dose-dependently inhibited SirA activity at micromolar levels (Fig. 2). The IC_{50} of 5-methylmellein and mellein against SirA activity were 120 ± 20 and 160 ± 30 μM, respectively. The structural similarity of these compounds suggests that the methyl group at C-5 is insignificant for inhibitory activity

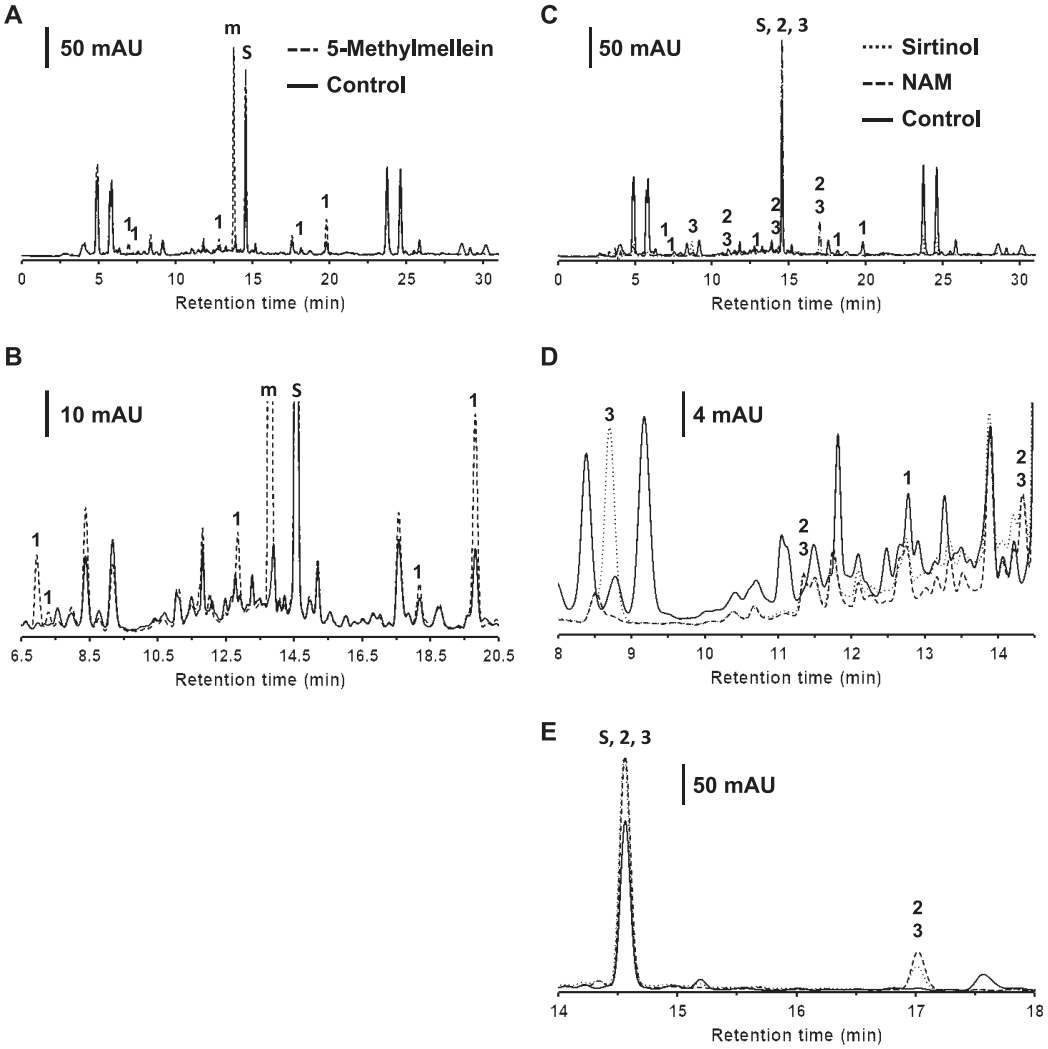

Fig. 3. Effect of 5-methylmellein and NAM on secondary metabolisms of *A. nidulans* cultured in GMM medium.

A, B, HPLC profiles of ethyl acetate extracts of *A. nidulans* cultured in GMM medium with or without 100 μM 5-methylmellein for 7 d. B is an enlarged view of panel A. C-E, HPLC profiles of ethyl acetate extracts of *A. nidulans* cultured in GMM medium with or without NAM and sirtinol (100 μM each) for 7 d. D, E, Enlarged views of the HPLC profiles. 5-Methylmellein (m), sterigmatocystin (s), and metabolites increased in the presence of 5-methylmellein (1), NAM (2), and sirtinol (3) are highlighted.

against SirA. Dihydrocoumarin and NAM inhibit human SIRT1 activity with IC_{50} of 208 and 175 μM, respectively, and de-repress fungal secondary metabolite production (Asai et al., 2012; Feldman et al., 2015; Hu et al., 2017; Olaharski et al., 2005). Here, we found that the measured IC_{50} of NAM for inhibiting SirA was 280 ± 40 μM. These findings indicated that the SirA-inhibitory activity of 5-methylmellein was 2-fold more potent than that of NAM, indicating that 5-methylmellein is a fungal sirtuin inhibitor.

Effect of 5-methylmellein on fungal secondary metabolism

We recently reported that the gene disruption of SirA increases sterigmatocystin production by *A. nidulans* cultured in liquid GMM medium (Itoh et al., 2017b). To determine the potential of 5-methylmellein to alter fungal secondary metabolism, *A. nidulans* was cultured in GMM medium with or without 100 μM 5-methylmellein for one week, and then the culture broth was extracted with ethyl acetate and analyzed by HPLC. We detected 52 peaks on HPLC using a UV detector set at a wavelength of 230 nm (Fig. 3 and Supplementary Table 2). Added 5-methylmellein was observed at retention time of 13.7 min. Sterigmatocystin (retention time = 14.6 min) was the major secondary metabolite of *A. nidulans* under the above culture conditions. Fungal sterigmatocystin production did not significantly differ between cultures with or without 5-methylmellein (2.4 ± 0.1 vs. 2.0 ± 0.2 μg/mL culture, respectively). This result was unexpected since 5-methylmellein inhibits SirA *in vitro*, and suggests that 5-methylmellein targets another sirtuin isozyme(s) that regulates sterigmatocystin production. SirE of this fungus could be the most likely candidate since its gene disruption decreases sterigmatocystin production (Itoh et al., 2017b) and might cancel the possible positive effect of 5-methylmellein on sterigmatocystin production through SirA inhibition. The amounts of metabolites at retention times of 6.9, 12.8, 18.2 and 19.8 min were increased > 1.5-fold by 5-methylmellein ($P < 0.03$) ("1" in Figs. 3A

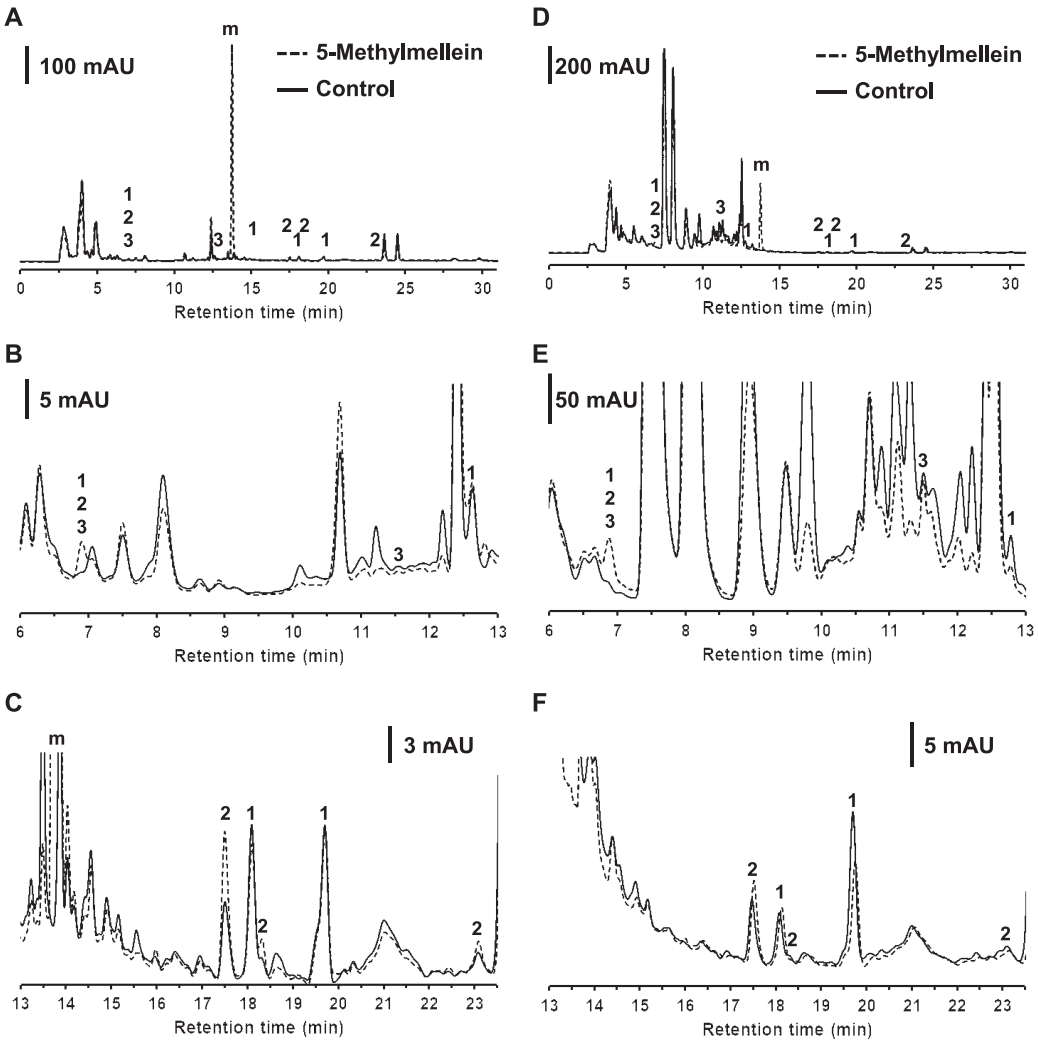

Fig. 4. Effect of 5-methylmellein and culture media on secondary metabolisms of *A. nidulans*.

A–C, HPLC profiles of ethyl acetate extracts of *A. nidulans* cultured in YMG medium with or without 100 μM 5-methylmellein for 7 d. B and C are enlarged views of panel A. D–F, HPLC profiles of ethyl acetate extracts of *A. nidulans* cultured in PD medium with or without 100 μM 5-methylmellein for 7 d. E and F are enlarged views of panel D. 5-Methylmellein (m), and metabolites increased in the presence of 5-methylmellein in GMM (1) and YMG (2) and PD (3) media are highlighted.

and B). An additional compound in cells cultured with 5-methylmellein that eluted as a peak at 7.3 min in the chromatogram, was undetectable in the untreated fungus. These results suggested that 5-methylmellein affects fungal secondary metabolism.

Effects of other sirtuin inhibitors and culture media

We cultured *A. nidulans* in GMM medium in the presence or absence of 100 μM NAM, and compared secondary metabolites to those cultured with 5-methylmellein. Adding NAM to GMM medium increased the production of sterigmatocystin 1.5-fold (3.0 ± 0.1 μg/mL culture), and of a metabolite eluted as a peak at 17.0 min 15-fold ("2" in Figs. 3C–E and Supplementary Table 2). The amount of metabolites eluted as peaks at 11.3 and 14.5 min also increased. Another sirtuin inhibitor, sirtinol, also increased the production of sterigmatocystin and metabolites eluted at 11.3, 14.5 and 17.0 min ("3" in Figs. 3C–E), indicating that the effect of sirtinol is similar to NAM, while sirtinol induced the production of a compound eluted at 8.7 min that was not detected in the culture with NAM. The metabolites which increased by adding 5-methylmellein ("1" in Fig. 3) were not increased by adding NAM and sirtinol. These results indicate that the sirtuin inhibitors affect the production of different secondary metabolites.

We cultured *A. nidulans* in YMG and PD media, and compared the activation patterns of metabolites by 5-methylmellein. Adding 5-methylmellein to an *A. nidulans* culture in YMG medium increased the amount of metabolites eluted at 6.9, 17.6, 18.3 and 23.2 min by >1.5-fold ("2" in Fig. 4 and Supplementary Table 3). Adding 5-methylmellein to *A. nidulans* cultured in PD medium increased the metabolites eluted at 6.9 and 11.5 min ("3" in Fig. 4). The metabolite eluted at 6.9 min was increased by the addition of 5-methylmellein to all the tested media whereas the metabolites eluted at 7.3, 12.8, 18.2, and 19.8 min, which were increased in the culture with GMM medium ("1" in Figs. 3A, 3B, and 4), were not detected in the cultures with YMG and PD media (Fig. 4). These findings indicated that the culture conditions affected the

sirtuin inhibitors to regulate the fungal secondary metabolite productions.

Three reports indicate that disruption of the gene encoding sirtuin in *Fusarium graminearum*, *A. oryzae* and *A. nidulans* de-repress secondary metabolite production (Itoh et al., 2017a, b; Kawauchi et al., 2013; Li et al., 2011). Adding sirtuin inhibitors to fungal cultures also increases secondary metabolite production (Asai et al., 2012; Hu et al., 2017), which is advantageous for exploiting novel fungal secondary metabolites without the need for genetic manipulation. To date, human sirtuin inhibitors have been used for this purpose, whereas this study is the first to screen fungal SirA inhibitors. We showed that 5-methylmellein up-regulates secondary metabolite production by *A. nidulans*. Therefore, this inhibitor of fungal sirtuin might serve as a potential tool for drug discovery by altering fungal secondary metabolism.

Acknowledgments

The authors thank Drs. Gen Okada and Moriya Ohkuma (Japan Collection of Microorganisms, Bioresource Center, RIKEN, Japan) for providing fungal strains, and Norma Foster for critical reading of the manuscript.

Supplementary Materials

Supplementary tables are available in our J-STAGE site (http://www.jstage.jst.go.jp/browse/jgam).

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