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ORIGINAL RESEARCH PAPER

Transcript and metabolite alterations increase ganoderic acid content in *Ganoderma lucidum* using acetic acid as an inducer

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Abstract Acetic acid at 5–8 mM increased ganoderic acid (GA) accumulation in *Ganoderma lucidum*. After optimization by the response surface methodology, the GA content reached 5.5/100 mg dry weight, an increase of 105 % compared with the control. The intermediate metabolites of GA biosynthesis, lanosterol and squalene also increased to 47 and 15.8 μg/g dry weight, respectively, in response to acetic acid. Acetic acid significantly induced transcription levels of *sqs*, *lano*, *hmgs* and *cyp51* in the GA biosynthesis pathway. An acetic acid-unregulated acetyl coenzyme A synthase (*acs*) gene was selected from ten candidate homologous *acs* genes. The results indicate that acetic acid alters the expression of genes related to acetic acid assimilation and increases GA biosynthesis and the metabolic levels of lanosterol, squalene and GA-a, thereby resulting in GA accumulation.

Keywords Acetic acid · Ganoderic acid · Ganoderma lucidum · Lanosterol · Response surface methodology · Squalene

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Introduction

Ganoderma lucidum is a higher basidiomycete with health benefits that is widely used in China. Components of *G. lucidum* have several functions including the ability to inhibit tumor cell growth and to modulate both the immune and nervous systems (Joseph et al. 2011). More than 160 different chemical compounds have been isolated from *G. lucidum,* including ganoderic acids (GAs). GA is responsible for many important pharmacological activities, including protection of the liver, elimination of inflammation, inhibition of tumor cell growth and various other androgenic activities (Hajjaj et al. 2005; Jedinak et al. 2011; Li and Wang 2006). Therefore, GA content is often taken as a measure of the overall quality of the mushroom because many of the beneficial properties of *G. lucidum* can be directly attributed to GA.

Recently, the use of inducers to enhance active components has drawn interest (Ren et al. 2010; Sugimoto and Shoji 2012). To increase GA production, methyl jasmonate, salicylic acid (aspirin), phenobarbital and Cu^{2+} have been added to culture media (Ren et al. 2010; Liang et al. 2010; You et al. 2013; Tang and Zhu 2010). The transcription levels of the GA biosynthetic

genes were up-regulated by those inducers (Ren et al. 2010; Liang et al. 2010). As food and health products, however, the economy and security of the fermentation products of *G. lucidum* must be considered. As an inducer, compared with methyl jasmonate, aspirin, phenobarbital and copper, acetic acid has lower cost and is also safe for consumers. Acetic acid is the main component of vinegar and is safer than other inducers. In addition, it has also been used to reduce serum cholesterol and the expression of fatty acid synthase gene, increase protein *N*-α-acetylation, and stimulates growth in animals, plant and fungi (Fushimi and Sato 2005; Fushimi et al. 2006; Yi et al. 2011; Dang et al. 2009). However, whether acetic acid can induce GA biosynthesis in *G. lucidum* is still undetermined.

In the present paper, acetic acid was used as an inducer to improve ganoderic acid (GA) accumulation. Induction was optimized using central composite design (CCD) based on response surface methodology. GA and intermediates were subsequently investigated, and genes related to GA biosynthesis and acetic acid assimilation were also investigated at the transcriptional level.

Materials and methods

Organism and culture conditions

The HG strain of *Ganoderma lucidum* was obtained from the Edible Fungi Institute, Shanghai Academy of Agricultural Science, China. Details concerning the culture medium and growth conditions for *G. lucidum* are described nu Ren et al. (2010). Seed cultures were grown in 500 ml flasks containing 200 ml potato/dextrose/broth (PDB) and shaken at 150 rpm at 28 °C for 6 days. The fermentation experiments were performed in 250 ml flasks containing 100 ml CYM (1 % w/v maltose, 2 % w/v glucose, 0.2 % yeast extract, 0.2 % tryptone, 0.05 % $MgSO_4 \cdot 7H_2O$, 0.46 % KH_2PO_4, initial pH of 5.5) at 150 rpm at 28 °C for 7 days after inoculation with 5 % (v/v) of the seed culture. Before addition to the medium, the acetic acid was sterilized by filtration through a 0.2 μm membrane.

Measurement of ganoderic acids, individual GA-a, intermediates and acetic acid

Ganoderic acids were extracted from 100 mg dried fungal mycelium by 50 % (v/v) ethanol and measured as previously described (Ren et al. 2010). For the measurement of individual ganoderic acids (GA-a), 100 mg dried mycelium was extracted with methanol, and the GAs in the supernatant were monitored at 254 nm by UHPLC using an Agilent 1290 Infinity LC equipped with an Agilent Zorbax SB-C18 column (250 × 4.6 mm, 5 μm). The calibration curve for the measurement of GA-a in the fungal mycelium was constructed using (7,15,25R)-7,15-dihydroxy-3,11,23- trioxolanost-8-en-26-oic acid (ganoderic acid A). Lanosterol and squalene were extracted from cells and fractionated according to previously reported methods, with some modifications (Liang et al. 2010). Dried mycelium, 30 mg, were saponified with 1.2 ml 10 % (w/v) KOH/75 % (v/v) ethanol at 50 °C for 2 h. The supernatant was extracted three times with equal volumes of hexane. The hexane layers were combined and the solvent removed under N_2. The product was dissolved in 0.5 ml methanol for subsequent UHPLC analysis at wavelength of 210 nm. For the measurement of acetyl-COA, the fresh mycelium was extracted with PBS (pH 7.4) at 4 °C then centrifuged at 3,000×*g* for 10 min. The supernatant was tested for acetyl-CoA. The acetyl-CoA content was quantified according to an acetyl-CoA ELISA Kit (Jiancheng, Nanjing). Acetic acid was measured according to previously reported methods with some modifications (Dong et al. 2013). *G. lucidum* culture supernatant, 20 μl, was sterilized through a 0.22 μm filter and analyzed at 210 nm by HPLC using a Shimadzu C18 column (150 × 4.6 mm, 5 μm). Aqueous 0.01 % (v/v) H_2SO_4-/methanol (95:5 v/v) was used as the mobile phase at 0.8 ml/min. A calibration curve was constructed using acetic acid and was used for the measurement of acetic acid concentrations in the culture supernatant.

Optimization of ganoderic acid accumulation using RSM

Response surface methodology (RSM), a statistical method, is used for the optimization of fermentation conditions to maximize component production (Bhak et al. 2005). Exposure dosage and time of stimulation are the two main factors that affect the final product yield. In the present study, experimental combinations of the dosage and time of acetic acid stimulation were employed (Supplementary Table 1). The experimental data obtained by the procedure described above

were analyzed using a second order polynomial regression, as described in Eq. (1).

$$Y = \beta_{k0} + \sum_{i=1}^{5} \beta_{ki} X_i + \sum_{i=1}^{5} \beta_{kii} X_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta_{kij} X_i X_j \quad (1)$$

where y is the GA value (mg/100 mg); X_i and X_j are the coded independent variables; and β_{k0}, β_{ki}, β_{kii} and β_{kij} are constant coefficients corresponding to the intercept, linear, quadratic and interaction coefficients, respectively.

With the aim of maximizing the GA yield, the optimum condition (within the experimental range) was obtained by using the CCD in Design Expert 7.0.

Transcriptional analysis

Total RNA was extracted using RNAiso Plus (TaKaRa), and then treated with RNase-free DNaseI (TaKaRa). The cDNA was synthesized using a cDNA mixture (Takara) according to the manufacturer's protocol. The transcript levels of *18s*, hydroxymethylglutaryl-CoA synthase (*hmgs*), hydroxymethylglutaryl-CoA reductase (*hmgr*), mevalonate pyrophosphate decarboxylase (*mvd*), farnesyl pyrophosphate synthase (*fps*), squalene synthase (*sqs*), oxidosqualene cyclase (*osc*), acetyl-CoA acetyltransferase (*acat*), sterol 14α-demethylase, (*cyp51*), squalene epoxidase (*se*), isopentenyl diphosphate isomerase (*idi*) and acetyl coenzyme A synthase (*acs*) (see Supplementary Fig. 1) were determined by quantitative real-time PCR (qPCR) based on relative method using a Realplex2 System (Eppendorf) with SYBR Green I according to previously reported procedures (Ren et al. 2013a). The primer sets were used as previously described (Ren et al. 2010; Mu et al. 2012) and are listed in Supplementary Table 2. The design of exon–exon boundaries primer were designed according to the genomic DNA fragment and cDNA fragment of *hmgs* (see Supplementary Fig. 2). Sequences of *G. lucidum* homologues acetyl coenzyme A synthase genes were obtained by aligning *C. cinerea acs* sequence with the sequence of the *G. lucidum* genome (http://www.herbalgenomics.org/galu/). Genome sequence alignment was used to identify ten *acs* homolog genes (Accession numbers in *G. lucidum* gene models: GL19651-R1, GL20510-R1, GL20899-R1, GL21040-R1, GL23180-R1, GL23589-R1, GL23735-R1, GL24109-R1, GL28494-R1 and GL30345-R1) ($E < -5$) in the *G. lucidum* genome (Chen et al. 2012).

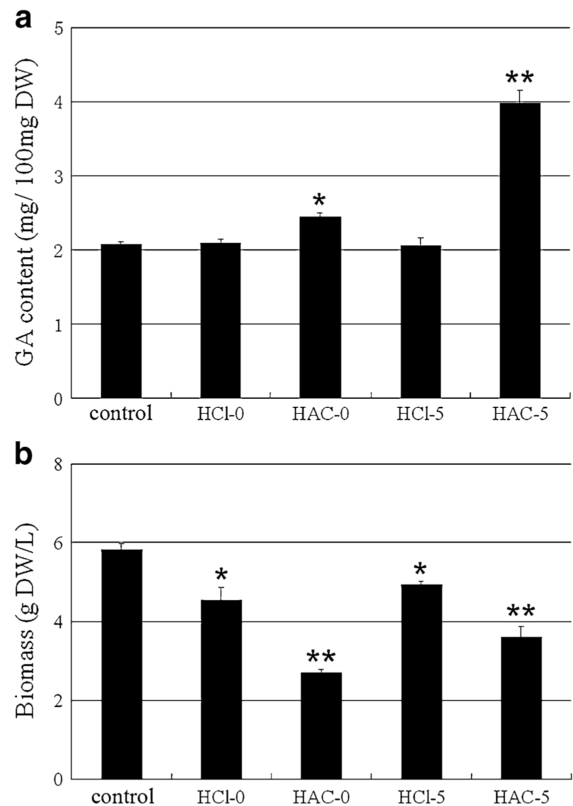

Fig. 1 The effect of acetic acid on the ganoderic acid biosynthesis and growth of *G. lucidum*. **a** acetic acid significantly induced total GA biosynthesis by *G. lucidum* at different times; **b** the time courses of cell growth at different times of acetic acid induction. The control indicates wild-type strain cultured on CYM for 7 days. HCl-0 and HAC-0 indicate the addition of acid to the medium on day 0. HCl-5 and HAC-5 indicate the addition of acid to the medium on day 5. All the samples are cultured for 7 days. Data are reported as the mean and standard error of the mean of at least three independent repetitions of each assay. $*p < 0.05$; $**p < 0.01$

Results and discussion

Acetic acid improves accumulation of ganoderic acid

The GA content increased from 2.1/100 mg DW to 3.98/100 mg DW in response to treatment with 5 mM acetic acid compared with the control group, representing a GA increase of 92.4 % (Fig. 1a). The *G. lucidum* growth results in response to 5 mM acetic

acid are shown in Fig. 1b. The results demonstrate that acetic acid significantly increased GA biosynthesis in *G. lucidum*. Among the effects of acetic acid in animals, the best known is that acetic acid can alter various metabolic processes, including glucose utilization, the acetyl-CoA level, lipid oxidation and lipid synthesis (Fushimi and Sato 2005; Fushimi et al. 2006; Yi et al. 2011; Li et al. 2013). As in animals, acetic acid can also influence metabolic pathways in plants, such as the glyoxylate cycle, lipid body synthesis and organic acid release (Blaby et al. 2013; Goodson et al. 2011; Zuo et al. 2012). The effects of acetic acid on metabolism in yeasts and fungi have also been described. In *Zygosaccharomyces bailii*, the production of fermentative metabolites increased when the media contained 1.25 % (v/v) acetic acid (Dang et al. 2009). In the present study, our results showed that acetic acid can increase GA biosynthesis in *G. lucidum*.

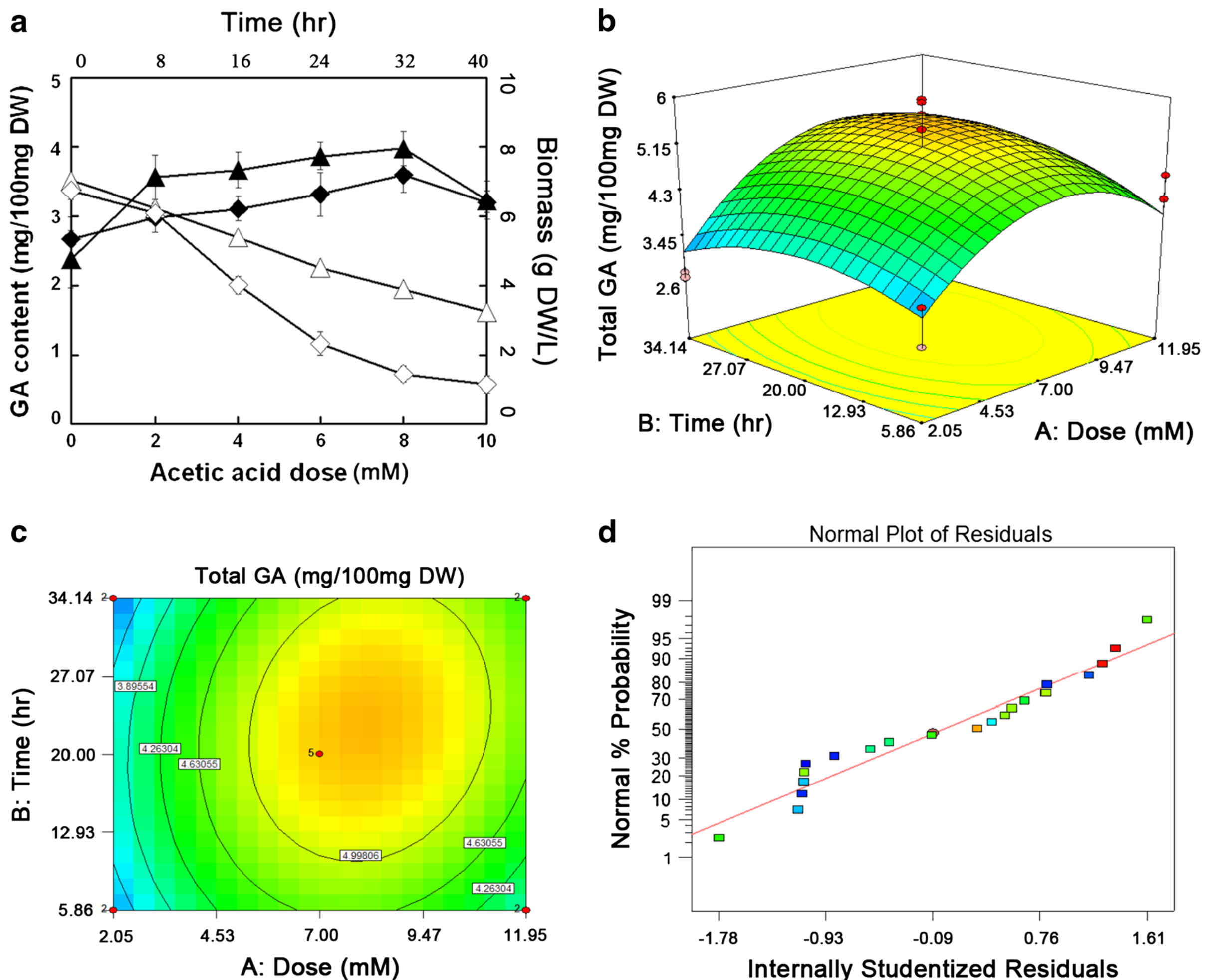

Fig. 2 Optimal conditions for acetic acid induction of ganoderic acid biosynthesis. **a** a single parameter selection study was performed initially to select the influencing parameters for GA content and biomass. *Filled diamonds* GA content with different dose of acetic acid, *filled triangles* GA content with the time courses of acetic acid induction, *empty diamonds* cell growth with different doses of acetic acid, *empty triangles* cell growth with the time courses of acetic acid induction. **b** a six level, two factor central composite design was employed for maximizing ganoderic acid accumulation. The independent variables were acetic acid dose (mM) and time (h). The ranges and levels of the independent variables are given in Supplementary Table 1. Levels of variables were determined according to single factor analysis. **c** the contour plot represents the combined effect of time and residence time on GA yield. **d** the normal probability plot of the standardized residuals

To investigate whether changes of pH after the addition of acetic acid were responsible for these effects, HCl was added to the fermented liquid of *G. lucidum* until the same pH as that obtained with acetic acid was reached. The GA content after HCl treatment was not significantly different from the control (Fig. 1a). In *Chlamydomonas reinhardtii*, the effect of acetic acid on programmed cell death depended on its concentration, not the pH, as no changes were observed when the same medium pH was obtained after adjusting with HCl (Zuo et al. 2012). In this study, we also found that the effect of acetic acid on the GA content is not attributed to a change of the pH of the liquid fermentation system of *G. lucidum* (Fig. 1a).

Optimal conditions for acetic acid induction

A single parameter selection study was conducted initially to identify the parameters that influence the production of GA. High concentrations of acetic acid inhibit the growth of microorganisms (Dang et al. 2009), therefore organisms require a suitable concentration and induction time of acetic acid to promote mycelial growth. To evaluate this effect, the concentration of acetic acid was varied from 0 to 40 mM. At 30 mM, no mycelial growth was observed. GA accumulation increased with increasing concentrations of acetic acid until a limiting maximum concentration (14 mM) was reached, after which the growth rate decreased rapidly (data not show). The *G. lucidum* growth results at different concentrations and induction times of acetic acid are shown in Fig. 2a. With different concentrations of acetic acid, the highest concentration of GA (3.59/100 mg DW) was with 8 mM acetic acid (Fig. 2a). In response to varying acetic acid induction times (0–40 h), the highest concentration (3.98/100 mg DW) was at 32 h (Fig. 2a).

In the present study, a six-level, two-factor CCD was employed to maximize GA production (Fig. 2b). The *p* value of the model was <0.001, which indicates that the model is significant. The high value of the determination coefficient ($R^2 = 0.7919$) and the adjusted determination coefficient (adjusted $R^2 = 0.7226$) also indicate that the model is significant. Figure 2c shows that the acetic acid dose was not a limiting parameter once it reached ~8 mM. The normal probability plot of the standardized residuals demonstrated that there was no abnormality in this study (Fig. 2d). The final equation was as follows:

$$Y = 3.35 + 0.50 \times \text{dose} + 0.090 \times \text{time} - 0.023 \times \text{dose} \times \text{time} - 0.30 \times \text{dose}^2 - 0.35 \times \text{time}^2 \quad (2)$$

With acetic acid at 8.21 mM and an induction time of 22.68 h, the maximum predicted GA accumulation was 5.36/100 mg DW. To confirm the predicted response, independent experiments were conducted in triplicate. Experimentally, the maximum conversion was 5.52/100 mg DW (data not shown), which was close to the predicted value.

In previous reports, methyl jasmonate-induced GA biosynthesis resulted in GA reaching 4.52/100 mg DW (Ren et al. 2010). Crude GA reached 4.21/100 mg DW over 9 d under phenobarbital induction (Liang et al. 2010). GA up to 3/100 mg DW was obtained with supplementation of Cu^{2+} (Tang and Zhu 2010). GA accumulation demonstrated in the present study represents a definite improvement over those reported in previous studies. Thus, the present findings confirm that direct production of GA in response to acetic acid is possible.

The alterations of intermediate metabolites and GA-a

GA is synthesized by the mevalonate pathway in which lanosterol and squalene are intermediates (Mu et al. 2014). In the process of increasing GA with 8.21 mM acetic acid and 22.68 h of induction, the

Table 1 Quantitation of metabolites of *G. lucidum*

Target compound	Control (μg/g DW)	HCl (μg/g DW)	HAC (μg/g DW)
GA-a	0.64 ± 0.01	1.04 ± 0.21*	3.86 ± 0.11**
Lanosterol	18.59 ± 1.98	23.10 ± 2.94	47.13 ± 0.70**
Squalene	8.88 ± 0.61	9.45 ± 0.16	15.80 ± 0.19**

The quantitation of metabolites in methanolic extracts of *G. lucidum* mycelia grown in flasks. HCl = mycelia treated with 1.16 mM HCl, HAC = mycelia treated with 5 mM acetic acid. The values are the mean ± standard error (*n* = 3 biological replicates). Lanosterol and squalene were separated using 100 % methanol as the mobile phase at a constant flow rate of 0.5 ml/min. The specific peaks of lanosterol and squalene were identified based on their retention times and UV spectra at 210 nm compared to those obtained with squalene and lanosterol standards (Sigma)

* $p < 0.05$; ** $p < 0.01$

content of lanosterol and squalene was also observed to increase (Table 1). Lanosterol increased from 18.6 μg/g DW to 47.1 μg/g DW. The squalene content increased from 8.9 to 15.8 μg/g DW. GA-a, also increased from 1.28 μg/g DW to 7.72 μg/g DW. These results are consistent with the observation of total GA accumulation. Recent research has focused on the effect of acetic acid on primary metabolism (Fushimi et al. 2006; Rodrigues et al. 2012), while its effect on secondary metabolism has rarely been reported. This work demonstrates that acetic acid influences the mevalonate pathway in *G. lucidum,* leading to an increased accumulation of GA.

Transcript alterations

Figure 3 shows that in the acetic-acid-induced cultures at 6 h, the transcription levels of *hmgs*, *sqs*, *lano*, and *cyp51* were significantly increased compared with the control group by factors of 5.7, 8.6, 11.5 and 6.1,

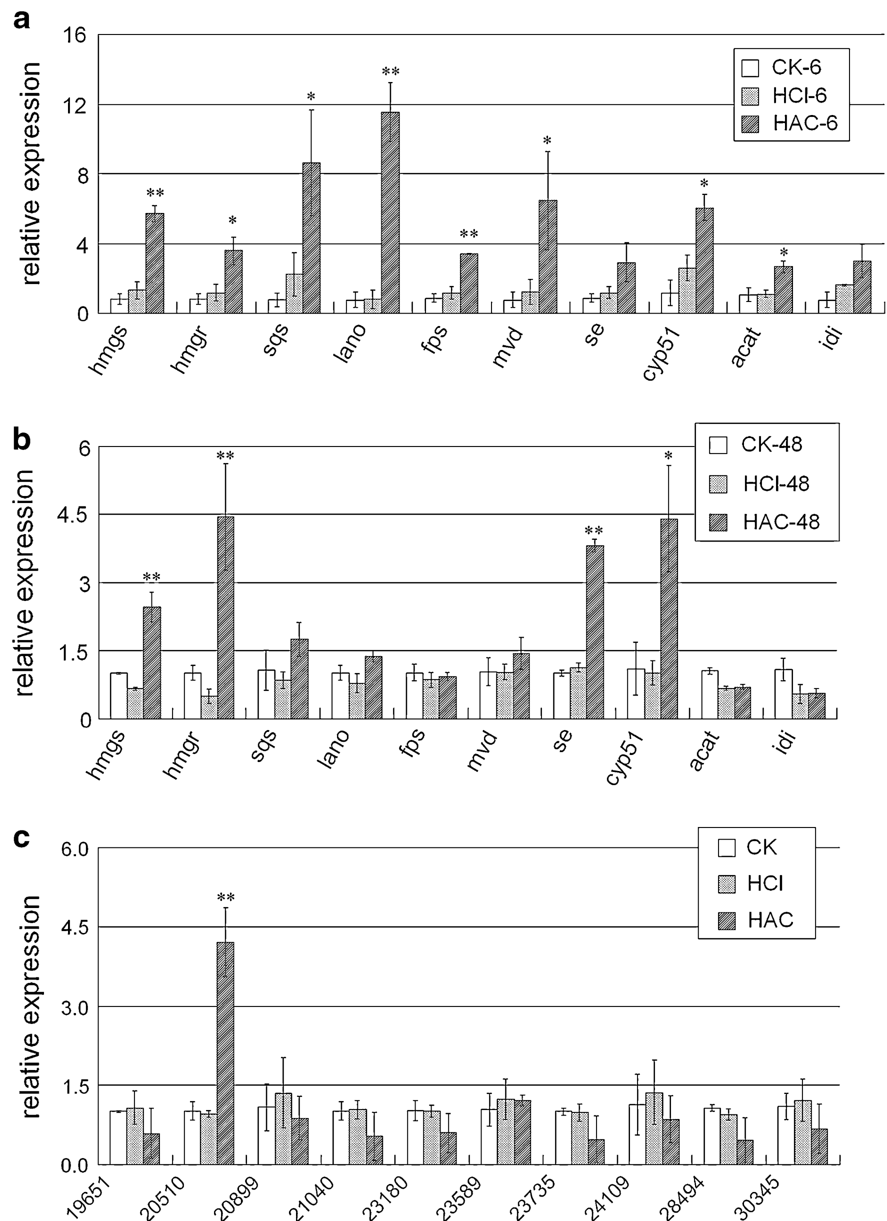

Fig. 3 Transcription analysis of genes encoding enzymes of GA biosynthesis and acetate metabolism in response to 5 mM acetic acid at different times. **a** HCl-6 and HAC-6 indicate the genes response to acid at 6 h. CK-6 is a control at 6 h. **b** HCl-48 and HAC-48 indicate the genes response to acid at 48 h. CK-48 is a control at 48 h. **c** HCl and HAC indicate the acetate metabolism genes response to acid at 48 h. CK is a control at 48 h. All 20 GA biosynthesis and acetic acid assimilation genes were detected using quantitative real-time PCR. The relative expression is shown as a mean value ± standard deviation ($n = 3$ biological replicates). The genes related to GA biosynthesis were *hmgs*, *hmgr*, *mvd*, *fps*, *sqs*, *osc*, *acat*, *cyp51*, *se* and *idi*. The homologous acetyl-coenzyme A synthetase genes (*acs*) were Gl19651, Gl20510, Gl20899, Gl21040, Gl23180, Gl23589, Gl23735, Gl24109, Gl28494 and Gl30345

respectively. For cultures induced with acetic acid at 48 h, the transcription levels of *hmgs*, *hmgr*, *se* and *cyp51* genes were the most significantly increased, at 2.5, 4.5, 3.8 and 4.4 times control levels. These results are consistent with the up-regulation expression of *hmgs*, which codes for acetyl coenzyme A, the first enzyme in the GA biosynthetic pathway (Ren et al. 2013b). Consistent with our results, other studies also suggest that acetic acid can act as a signaling molecule that modulates the expression of *aox* and *CYP7A1* genes in lipid metabolism in hepatocytes (Fushimi et al. 2006). Transcriptions of four genes encoding *hmgs*, *sqs*, *lano*, and *cyp51* in the GA biosynthetic pathway were up-regulated under acetic acid induction (Fig. 3).

Acetic acid is a substrate for acetyl-coenzyme A synthetase (acs) and acetyl-CoA, is used in many metabolic pathways (Yi et al. 2011). Acetic acid can change the level of intracellular acetyl CoA through acetyl coenzyme A synthetase (*acs*) and then regulate intracellular metabolism and gene expression (Yi et al. 2011). In *C. reinhardtii*, acetyl-CoA synthetase, which catalyzes acetate assimilation, was up-regulated when it was grown mixotrophically on acetate (Blaby et al. 2013). Using quantitative real time PCR, one acetic acid-upregulated homologous gene was selected. Under acetic acid-induced conditions, the transcription levels of homologous *acs* (GL20510-R1) were 2.3- and 4.2-times that of the control at 6 h and 48 h, respectively (Fig. 3). To monitor the metabolic process of ACS, cellular acetyl-CoA was extracted and analyzed by ELISA. Acetyl-CoA increased from 174 ng/μg protein to 337 ng/μg protein under acetic acid treatment (Supplementary Fig. 3). We also quantified acetic acid in the culture supernatant of *G. lucidum*. The results demonstrate that acetic acid is decreased to 1.24 mM after 2 days in culture (Supplementary Fig. 4). Up-regulation of the gene encoding the acs enzyme, together with the increase in acetyl-CoA and decrease of acetic acid suggests that intracellular acetyl-CoA synthetase may be the enzyme that catalyzes the formation of acetyl coenzyme A, which then enters the GA biosynthetic pathway in *G. lucidum*.

Conclusion

Acetic acid enhances GA biosynthesis in *G. lucidum*. Optimum conditions for stimulating GA yield was acetic acid at 8.21 mM with an induction time at 22.68 h. GA-a, lanosterol and squalene were also increased. The transcription levels of genes in the GA biosynthesis pathway were up-regulated. One acetic-acid-upregulated *acs* homologous gene was detected by qPCR from 10 candidate genes. This work provides a convenient, economical and safe approach to improving GA accumulation through the alteration of transcripts and metabolites by acetic acid in *G. lucidum*.

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Supporting information Supplementary Table 1—Design matrix of CCD and response

Supplementary Table 2—Primer sets used for real time PCR

Supplementary Figure 1—The ganoderic acid biosynthesis pathway of *G. lucidum*

Supplementary Figure 2—The genomic DNA sequence of hmgs from *G. lucidum*

Supplementary Figure 3—The acetyl-CoA content under acetic acid treatment

Supplementary Figure 4—The acetic acid content in the culture supernatant

References

Bhak G, Song M, Lee S, Hwang S (2005) Response surface analysis of solid state growth of *Pleurotus ostreatus* mycelia utilizing whey permeate. Biotechnol Lett 27: 1537–1541

Blaby IK, Glaesener AG, Mettler T, Fitz-Gibbon ST, Gallaher SD, Liu B, Boyle NR, Kropat J, Stitt M, Johnson S, Benning C, Pellegrini M, Casero D, Merchant SS (2013) Systems-level analysis of nitrogen starvation-induced modifications of carbon metabolism in a *Chlamydomonas reinhardtii* starchless mutant. Plant Cell 25:4305–4323

Chen S, Xu J, Liu C, Zhu Y, Nelson DR, Zhou S, Li C, Wang L, Guo X, Sun Y, Luo H, Li Y, Song J, Henrissat B, Levasseur A, Qian J, Li J, Luo X, Shi L, He L, Xiang L, Xu X, Niu Y, Li Q, Han MV, Yan H, Zhang J, Chen H, Lv A, Wang Z, Liu M, Schwartz DC, Sun C (2012) Genome sequence of the model medicinal mushroom *Ganoderma lucidum*. Nat Commun 3:913

Dang TD, Vermeulen A, Ragaert P, Devlieghere F (2009) A peculiar stimulatory effect of acetic and lactic acid on growth and fermentative metabolism of *Zygosaccharomyces bailii*. Food Microbiol 26:320–327

Dong BY, Chen YF, Zhao CC, Zhang SJ, Guo XW, Xiao DG (2013) Simultaneous determination of furfural, acetic acid,

and 5-hydroxymethylfurfural in corncob hydrolysates using liquid chromatography with ultraviolet detection. J AOAC Int 96:1239–1244

Fushimi T, Sato Y (2005) Effect of acetic acid feeding on the circadian changes in glycogen and metabolites of glucose and lipid in liver and skeletal muscle of rats. Br J Nutr 94:714–719

Fushimi T, Suruga K, Oshima Y, Fukiharu M, Tsukamoto Y, Goda T (2006) Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. Br J Nutr 95:916–924

Goodson C, Roth R, Wang ZT, Goodenough U (2011) Structural correlates of cytoplasmic and chloroplast lipid body synthesis in *Chlamydomonas reinhardtii* and stimulation of lipid body production with acetate boost. Eukaryot Cell 10:1592–1606

Hajjaj H, Mace C, Roberts M, Niederberger P, Fay LB (2005) Effect of 26-oxygenosterols from *Ganoderma lucidum* and their activity as cholesterol synthesis inhibitors. Appl Environ Microbiol 71:3653–3658

Jedinak A, Thyagarajan-Sahu A, Jiang J, Sliva D (2011) Ganodermanontriol, a lanostanoid triterpene from *Ganoderma lucidum*, suppresses growth of colon cancer cells through ss-catenin signaling. Int J Oncol 38:761–767

Joseph S, Sabulal B, George V, Antony KR, Janardhanan KK (2011) Antitumor and anti-inflammatory activities of polysaccharides isolated from *Ganoderma lucidum*. Acta Pharm 61:335–342

Li YQ, Wang SF (2006) Anti-hepatitis B activities of ganoderic acid from *Ganoderma lucidum*. Biotechnol Lett 28:837–841

Li X, Chen H, Guan Y, Lei L, Liu J, Yin L, Liu G, Wang Z (2013) Acetic acid activates the AMP-activated protein kinase signaling pathway to regulate lipid metabolism in bovine hepatocytes. PLoS ONE 8:e67880

Liang CX, Li YB, Xu JW, Wang JL, Miao XL, Tang YJ, Gu T, Zhong JJ (2010) Enhanced biosynthetic gene expressions and production of ganoderic acids in static liquid culture of *Ganoderma lucidum* under phenobarbital induction. Appl Microbiol Biotechnol 86:1367–1374

Mu D, Shi L, Ren A, Li M, Wu F, Jiang A, Zhao M (2012) The development and application of a multiple gene co-silencing system using endogenous URA3 as a reporter gene in *Ganoderma lucidum*. PLoS ONE 7:e43737

Mu D, Li C, Zhang X, Li X, Shi L, Ren A, Zhao M (2014) Functions of the NADPH oxidase family in *Ganoderma lucidum*: an essential role in ganoderic acid biosynthesis regulation, hyphal branching, fruiting body development, and oxidative-stress resistance. Environ Microbiol 16:1709–1728

Ren A, Qin L, Shi L, Dong X, da Mu S, Li YX, Zhao MW (2010) Methyl jasmonate induces ganoderic acid biosynthesis in the basidiomycetous fungus *Ganoderma lucidum*. Bioresour Technol 101:6785–6790

Ren A, Li MJ, Shi L, Mu DS, Jiang AL, Han Q, Zhao MW (2013a) Profiling and quantifying differential gene transcription provide insights into ganoderic acid biosynthesis in *Ganoderma lucidum* in response to methyl jasmonate. PLoS ONE 8:e65027

Ren A, Ouyang X, Shi L, Jiang AL, Mu DS, Li MJ, Han Q, Zhao MW (2013b) Molecular characterization and expression analysis of GlHMGS, a gene encoding hydroxymethylglutaryl-CoA synthase from *Ganoderma lucidum* (Ling-zhi) in ganoderic acid biosynthesis pathway. World J Microbiol Biotechnol 29:523–531

Rodrigues F, Sousa MJ, Ludovico P, Santos H, Corte-Real M, Leao C (2012) The fate of acetic acid during glucose co-metabolism by the spoilage yeast *Zygosaccharomyces bailii*. PLoS ONE 7:e52402

Sugimoto T, Shoji H (2012) Indigestible dextrin is an excellent inducer for alpha-amylase, alpha-glucosidase and glucoamylase production in a submerged culture of *Aspergillus oryzae*. Biotechnol Lett 34:347–351

Tang YJ, Zhu LW (2010) Improvement of ganoderic acid and Ganoderma polysaccharide biosynthesis by *Ganoderma lucidum* fermentation under the inducement of Cu^{2+}. Biotechnol Prog 26:417–423

Yi CH, Pan H, Seebacher J, Jang IH, Hyberts SG, Heffron GJ, Vander Heiden MG, Yang R, Li F, Locasale JW, Sharfi H, Zhai B, Rodriguez-Mias R, Luithardt H, Cantley LC, Daley GQ, Asara JM, Gygi SP, Wagner G, Liu CF, Yuan J (2011) Metabolic regulation of protein *N*-alpha-acetylation by Bcl-xL promotes cell survival. Cell 146:607–620

You BJ, Lee MH, Tien N, Lee MS, Hsieh HC, Tseng LH, Chung YL, Lee HZ (2013) A novel approach to enhancing ganoderic acid production by *Ganoderma lucidum* using apoptosis induction. PLoS ONE 8:e53616

Zuo Z, Zhu Y, Bai Y, Wang Y (2012) Acetic acid-induced programmed cell death and release of volatile organic compounds in *Chlamydomonas reinhardtii*. Plant Physiol Biochem 51:175–184