JMB
Journal of Microbiology and Biotechnology

Online ISSN : 1738-8872
Pimt ISSN : 1017-7825

JMB Papers in Press. First Published online Feb 8, 2019
DOI: 10.4014/jmb.1812.12038

Manuscript Number: JMB18-12038

Title: Ammonium acetate supplement strategy for enhancement of chaetominine production in liquid culture of marine-derived Aspergillus fumigatus CY018

Article Type: Research article

Keywords: Chaetominine, Marine-derived fungus, Ammonium acetate, feeding strategy, Aspergillus fumigatus

Ammonium acetate supplement strategy for enhancement of chaetominine production in liquid culture of marine-derived *Aspergillus fumigatus* CY018

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Abstract

Chaetominine (CHA), a marine-derived quinazolinone alkaloid with significant cytotoxic activity, is restricted by low yields on pharmacological research, which needs to be settled urgently. In this work, the selection of additional nitrogen sources, optimization of the addition concentration and addition time of ammonium acetate, were investigated. CHA production was optimized to 62.1 mg/L with the addition of 50 mM ammonium acetate at 120 h of the fermentation in the shaker flask. This feeding strategy significantly increased 3-deoxy-arabino-heptulosonate-7-phosphate synthase activity and transcript levels of critical genes (*laeA*, *dahp* and *trpC*) in the shikimate pathway compared with the non-treatment group. In addition, the selection of feeding rate (0.01 and 0.03 g/L/h) was investigated in a 5-L bioreactor. And, CHA production was increased 57.9 mg/L with a 0.01 g/L/h ammonium acetate feeding rate. This work showed the strategy of ammonium acetate supplement had an effective role in improving CHA production by *Aspergillus fumigatus* CY018. This strategy could provide a precious experience for the large-scale fermentation of marine fungus in submerged culture.

Key words: Chaetominine; Marine-derived fungus; Ammonium acetate; feeding strategy; *Aspergillus fumigatus*

1. Introduction

Marine resources have received the attention of many scholars owing to the abundance of bioactive compounds with unique chemical structures by marine organisms [1-2]. Marine-derived microorganism, a renewable, prolific and efficient source, produces various secondary metabolites with remarkable bioactivities, such as antitumor, antibacterial, and anti-inflammatory effects [3-4]. However, the research and development of secondary metabolites within a distinct framework in the pharmacodynamics field are seriously limited by the low yield [5-6]. Therefore, effective biotechnological strategies imminently need to be exploited for enhancing the production of target compounds by the fermentation of marine microbes.

Many of secondary metabolites from marine symbiotic microbes possess excellent bioactivity and some have been developed into drugs for the treatment of various diseases [7-8]. *Aspergillus fumigatus* CY018, a symbiotic fungus of sea-crab, produces many valuable metabolites with antifungal, anticancer and cytotoxic activities [9-10]. Chaetominine (CHA), isolated and identified from *A. fumigatus* CY018, is a quinazolinone alkaloid (Fig. 1) with significant anticancer activity against the human leukemia K562 and colon cancer SW1116 cell lines. Yao and co-workers found the mechanism that CHA treatment induced apoptosis in human leukemia K562 cells via the mitochondrial pathway [11-12]. However, an effective regulation strategy for improving the production of CHA is not available thus far, which limits further research about the pharmacology and pharmacodynamic mechanism of the anticancer activity of CHA.

The supplement of medium components as a significantly effective strategy was widely

applied in the fermentation process for enhancing production of target metabolites. The nitrogen source as an important nutritional component affects the growth of microorganisms and synthesis of secondary metabolites [13]. On one hand, the effect of limiting nitrogen source can improve the production of secondary metabolites. As Wang et al. found the limitation of ammonium sulfate in culture medium could improve the production of pullulan by *Aureobasidium pullulans* [14]. On the other hand, the supplement of nitrogen source can also effectively promote the biosynthesis of target compounds. For instance, the feeding strategy of nitrogen source was beneficial for cell growth and curdlan production during the early fermentation stage of *Rhizobium radiobacter* [15].

CHA is speculated to be biosynthesized via the shikimate pathway in the previous work. Although the detailed polymerization of alanine, anthranilic acid and tryptophan is unclear, the early steps of CHA biosynthetic pathway were verified as involving the shikimate pathway in our previous work [16]. There are some key sites for control in the shikimate pathway, such as 3-deoxy-arabino-heptulosonate-7-phosphate synthase (DAHP, *dahp*), chorismate synthase (*cs*) and anthranilate synthase (*trpC*) [17-19]. In addition, *laeA*, a global regulator, plays an important regulation role in the biosynthesis of secondary metabolites, such as the effect of deletion/overexpression of *laeA* in *Aspergillus* app. [20]. Thus, the transcriptional expression of these key genes, *laeA*, *dahp*, *cs* and *trpC*, might have a promoting role in the biosynthesis of CHA by feeding nitrogen source strategy. And, the transcript levels of critical genes are verified to enhance the production of target compounds [21-22]. For example, limiting glutamine (a nitrogen source) improves the transcriptional expression of important related genes and the yield of ganoderic acids in *Ganoderma lucidum*

[23].

Feeding nutrient in the culture broth is a usual strategy for enhancement of the target compounds during fermentation, especially nitrogen source. Moreover, Zhang et al. found the optimization of nitrogen sources significantly influences CHA biosynthesis. Although the concentration of nitrogen source was optimized in the previous work [24], the CHA production was still low and this problem urgently requires solving.

In the present work, the supplement effect of different nitrogen sources in the broth was first investigated. Then, the optimal concentration and time of feeding ammonium acetate were determined in the shake flask. Metabolic parameters (dry cell weight (DCW), residual sugar, CHA production and the activity of DAHP synthase) and transcript levels of critical genes (*laeA*, *dahp*, *cs,* and *trpC*) were detected with the optimal fermentation condition. Furthermore, the different feeding rate of ammonium acetate was studied in a 5-L bioreactor. The results of this work are valuable for enhancing the production of a target compound by feeding nutrient in submerged fermentation.

2. Materials and methods

2.1. Microorganism

The CHA producing by *A. fumigatus* CY018 utilized in this work was kindly provided by Nanjing University (Nanjing, China). Mycelia were first cultured on potato dextrose agar (PDA) medium for seven days at 28°C in the constant temperature incubator then sub-cultured monthly [25].

2.2. Fermentation conditions

A 1.0 cm^2 agar tablet was cut from cultivated PDA and transferred to a 500 mL shake

flask containing 200 mL potato dextrose broth (PDB). After inoculation, the flask was cultured as seeds at 180 rpm, 28°C, for 72 h in the oscillating constant temperature shaker. Then, a 250 mL shake flask with 50 mL fermentation medium was inoculated with 7 mL of seeds for culturing at 180 rpm, 28°C, for 336 h in the oscillating constant temperature shaker. The fermentation medium composed of (g/L): sucrose, 100; ammonium acetate, 5; sodium tartrate, 2; sodium glutamate, 2.4; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 0.84; and $FeSO_4 \cdot 7H_2O$, 0.016.

2.3. Optimization of the nitrogen source

The effects of different nitrogen sources (ammonium sulfate, ammonium chloride, and ammonium acetate) with 5 and 10 mM concentration at 120 h of fermentation on CHA production and DCW were investigated. The effects on CHA production and DCW with ammonium acetate were further examined with different concentrations (5, 10, 20, 50, and 100 mM) at 120 h during the fermentation. Then, ammonium acetate (screened concentration) was supplied at 72, 120, 168, 216, 264 and 312 h in fermentation process, respectively. The non-treatment groups were performed in cultivation medium without addition of ammonium acetate.

2.4. Sampling and analyse

At the end of the fermentation process, DCW was determined to analyze the biomass of *A. fumigatus*. The anthrone-sulfuric acid method was utilized to measure the residual sugar of fermentation broth at different detection time. High performance liquid chromatography was used to assay the content of CHA in the broth as described by Liu et al. [26]. One milliliter of broth was centrifuged and the underlying mycelium was analyzed for the activity of a key enzyme DAHP synthase in the shikimate pathway. The analysis method of DAHP synthase

was quoted by the published articles [16]. After the reaction was end, the final solution was determined by measuring the decrease of PEP with spectrophotometer at 549 nm.

2.5. Measurement of the expression of key enzyme genes by the quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The TRIzol solution was employed to extract total RNA of taken samples for all experiments. The RNA concentrations in the taken samples were determined using a spectrophotometer at $A_{260/280}$. The method of Li et al. was utilized to detect mRNA expression levels of *laeA*, *dahp*, *cs*, *trpC* and actin [27]. Total RNA served as the initial template and the PremixScript™ Reagent Kit (Takara Bio, Kusatsu, Japan) was used for reverse transcription. Premix Ex Taq™ II (Takara Bio) was utilized to determine mRNA levels of key genes according to the manufacturer's procedure. Primer pair sequences for qRT-PCR amplification of *laeA*, *dahp*, *cs*, *trpC*, and actin are displayed in Table 1. The qRT-PCR was performed as displayed by the published paper [16].

2.6. Batch-fed fermentation in a 5-L bioreactor

The research of feeding flow rate with ammonium acetate was worthy in the bioreactor. The feeding flow rates of ammonium acetate (0.01 g/L/h, 0.03 g/L/h and non-treatment) on batch-fed fermentation were investigated in a 5-L stirred bioreactor. A 1 L shake flask containing 400 mL PDB was cultured on shaker at 180 rpm, 28°C, for 72 h in the oscillating constant temperature shaker. Then, this was inoculated into the bioreactor containing 2.6 L medium and fermented for 384 h at 28 ± 0.2°C with a stirring speed of 300 rpm. Air flow was controlled at 1.5 ± 0.1 vessel volume/min during the fermentation process. Antifoam (a mixture of organic polyether dispersions) was added at 0.3% (v/v) before autoclaving.

147 Fermentation broth (20 mL) was taken at intervals of 48 h for detecting the metabolic
148 parameters.

149 *2.7. Statistical analyse*

150 All experiments in the shake flasks were performed in triplicate. Error bars in the figures
151 indicate the corresponding standard deviations. A value of $P < 0.05$ was considered
152 statistically significant. Origin 8 software (OriginLab, Northampton, MA, USA) was utilized
153 to create the figures and CFX Manager (Bio-Rad, Hercules, CA, USA) was used to analyze
154 the qRT-PCR data.

155

156 **3. Results and discussion**

157 *3.1. Effects of adding different nitrogen sources*

158 Nutrients in the culture broth, especially nitrogen sources, played important roles in the
159 biosynthesis of secondary metabolites. Moreover, Zhang et al. discovered that the effects of
160 different nitrogen sources and concentrations on CHA production were obviously significant
161 [24]. However, the production of CHA was also at a low level. Therefore, we fed the
162 supernumerary nitrogen source for enhancement of CHA production. The effects of feeding 5
163 and 10 mM three different nitrogen sources (ammonium sulfate, ammonium chloride, and
164 ammonium acetate) were investigated. As shown in Table 2, the production of CHA was 31.6
165 ± 3.1 and 25.4 ± 1.6 mg/L with 5 and 10 mM ammonium sulfate, respectively. This indicated
166 that CHA production was decreased with an increasing content of ammonium sulfate, which
167 implied that effect of ammonium sulfate addition was not significant on CHA production.

168 The production of CHA (32.5 ± 1.6 and 34.5 ± 3.2 mg/L) was slightly increased with 5

and 10 mM ammonium chloride. The highest production of CHA was 42.5 mg/L with the addition of 10 mM ammonium acetate in the selection experiments, which production was 45.3% higher than the non-treatment group. Therefore, the addition of ammonium acetate played a significantly promoting role in CHA production, suggesting that feeding ammonium acetate might be an effective strategy for enhancement of CHA production.

3.2. Concentration-and time- effects of ammonium acetate addition on CHA production

Because feeding ammonium acetate was beneficial for increasing CHA production by *A. fumigatus* CY018, the optimal concentration and time of ammonium acetate supplement were investigated. As shown in Fig. 2A, the effects of different concentrations of ammonium acetate (5, 10, 20, 50 and 100 mM) at 120 h were promoted CHA biosynthesis at different levels. The trend of CHA production was improved when concentration of ammonium acetate was increased from 5 to 50 mM. Specifically, the optimal concentration of ammonium acetate was 50 mM, where CHA production reached the maximum (64.7 mg/L) which was 2.2 folds compared that in non-treatment group. 100 mM concentration of ammonium acetate resulted in the CHA production decreasing to 41.9 mg/L, indicated that a high content of ammonium acetate had a negative effect on CHA biosynthesis. Therefore, the optimized concentration of ammonium acetate supplement for enhancing CHA production was 50 mM.

The feeding time of ammonium acetate also played an important role in CHA biosynthesis. The determinate ammonium acetate (50 mM) was added at different time of the fermentation (72, 120, 168, 216, 264 and 312 h) in the shake flasks (Fig. 2B). The production of CHA was first increased then decreased with feeding time, which reached a maximum (62.0 mg/L) at 120 h. The promoting effect of ammonium acetate supplement on CHA

191 production at early times might be related to the beneficial effects of nutrient addition on
192 fungal growth and product synthesis [28]. Overall, the optimal condition of ammonium
193 acetate supplement for CHA biosynthesis was feeding 50 mM ammonium acetate at 120 h
194 after inoculation. Hence, the supplement of ammonium acetate was an effective strategy for
195 improving CHA production (Fig. 3).

196 *3.3. Effects of the feeding strategy on metabolic parameters*

197 The strategy of feeding ammonium acetate (50 mM at 120 h) was confirmed to have a
198 significant positive role in CHA biosynthesis. Thus, metabolic parameters (DCW, residual
199 sugar, CHA production, and DAHP synthase activity) were further investigated during the
200 fermentation process in shake flask.

201 As shown in Fig. 4A, the trends of DCW in the feeding ammonium acetate and
202 non-treatment groups were similar before 120 h culture time. Thereafter, the growth of cells in
203 the group that was fed with ammonium acetate was higher than the non-treatment group. The
204 maximal DCW in the test and non-treatment groups were 19.4 and 16.7 g/L at 288 h,
205 respectively.

206 The consumption tendency of residual sugar continued to decline in both groups, while
207 residual sugar was consumed quickly after feeding ammonium acetate (Fig. 4B). As shown in
208 Fig 4C, the trend of CHA production was first increased and then decreased. Moreover, CHA
209 production with the addition of ammonium acetate was 1.9 folds higher than that in
210 non-treatment (33.7 mg/L). Moreover, the yield of CHA with ammonium acetate addition was
211 higher than non-treatment group in supplemental data 1, which meant the increasing CHA
212 production appeared to be in the growth-dependent manner.

In previous research, we speculated and verified that the biosynthesis of CHA was through the shikimate pathway, and that DAHP synthase was a key enzyme in the process. Thus, the activity of DAHP synthase was investigated with ammonium acetate supplement at different taken points of the fermentation process (Fig. 4D). The activity of DAHP synthase increased and then decreased in both the test and non-treatment groups. Importantly, the activity of DAHP synthase increased to a maximum, which was 52.6% above the non-treatment group at 240 h. These data indicated that the strategy of ammonium acetate supplement was beneficial for improving the biosynthesis of CHA.

Yang et al. also found that various fermentation parameters, the activities of key enzymes and the content of metabolites were enhanced when either urea or ammonium chloride was used as the sole source of nitrogen [29]. It can clearly be seen the strategy of feeding ammonium acetate could significantly promote the activity of key enzyme to optimize fermentation parameters for enhancing CHA production.

3.4. Gene expression in response to ammonium acetate addition

The feeding strategy we used was benefited for cell growth and DAHP synthase activity to enhance CHA production. We next researched the mRNA transcript levels of *laeA*, *dahp*, *cs* and *trpC* using qRT-PCR to explore the mechanism of this effective strategy. The transcriptional levels of these key genes with supplement of ammonium acetate were taken samples at 48, 144, 240 and 336 h during the fermentation.

As shown in Fig. 5A, feeding ammonium acetate significantly increased the expression of *laeA* 4.2 fold and 2.4 fold at 144 and 336 h, respectively, compared to the non-treatment group. The mRNA transcript levels of *dahp* were also improved remarkably (43.4 fold and

5.5 fold at 144 and 240 h, respectively) with supplement of ammonium acetate (Fig. 5B). The trend of *trpC* expression was similar to the formers (Fig. 5D). In contrast, the influence of ammonium acetate addition was not significant on mRNA transcript level of *cs* (1.5- and 1.3- folds increases at 144 and 336 h, respectively) (Fig. 5C). These results demonstrated the supplement of ammonium acetate could promote the mRNA transcript expression of key genes in the CHA synthesis pathway.

In general, an effective strategy for enhancing production of target compound could affect mRNA or protein levels of regulated factors in the biosynthesis pathway of target compound. For example, Lu et al. found the biosynthesis of dalesconols A and B was enhanced via calcium/calmodulin signaling with addition of Ca^{2+} [30]. In addition, Long et al. demonstrated that production of cellulase and hemicellulase was improved via the increased transcription factor, *creA*, in *Trichoderma orientalis* EU7-22 [31]. Moreover, Pandey and co-workers found that vindoline production was promoted by up-regulation of the Catharanthus AP2-domain protein and down-regulation of Cys2/His2-type zinc finger protein family transcriptional repressors [32].

We researched the mRNA transcript levels of important genes/enzymes for revealing the effect of our strategy on CHA production. In the current work, feeding ammonium acetate increased the transcription levels of *laeA*, *dahp* and *trpC*, which were key genes involved in DAHP synthase activity and CHA production. The activity of DAHP synthase was also improved by the feeding strategy. This meant supplement of ammonium acetate positively regulated gene expression to improve enzyme activity. Therefore, the obtained results showed and verified that the strategy of ammonium acetate supplement up-regulated the transcription

levels of key genes in the biosynthetic pathway to promote the biosynthesis of CHA by *A. fumigatus* CY018.

3.5. Ammonium acetate feeding strategy in a 5-L bioreactor

The feeding rate of nutrient is a helpful strategy in scale-up fermentation. Zhang et al. found that feeding glucose was beneficial for Antrodin C accumulation with 40 g/L glucose in a 15-L bioreactor [33]. Therefore, for scaling up the fermentation with feeding ammonium acetate, the feeding rate (0.01 and 0.03 g/L/h) was investigated in a 5-L stirred bioreactor compared to non-treatment (Fig. 6C). The time profiles of dissolved oxygen (DO), residual sugar, CHA production, DCW, and pH were first investigated with the feeding rate of 0.03 g/L/h (Fig. 6A).

The trend of DO was similar to non-treatment but decline slowly and maintained 0 from 216-312 h. Cell growth and CHA biosynthesis resulted in DO declining and cell death resulted in the DO rebound. DCW was also similar to non-treatment and the value of DCW with addition was higher than control. Residual sugar in the 5-L bioreactor was consumed rapidly compared to non-treatment and finally reached 11.3 g/L. CHA production in the 5-L bioreactor was increased to the maximum value of 46.99 mg/L at 312 h and then decreased. CHA concentration decreased towards the end of the fermentation which indicated metabolic instability for the target secondary metabolite. One known example of this was clavulanic acid [34-35]. The phenomenon demonstrated a novel routes in fermentations would be open to lead to the product of CHA decrease. The pH increased early during the fermentation process, then decreased and remained at approximately 4.0 earlier than non-treatment. This pH was beneficial to cell growth and target compound synthesis.

CHA production in the 5-L bioreactor with a 0.03 g/L/h feeding rate was lower than the results in the shake flasks (62.10 mg/L). This indicated that the rate of feeding ammonium acetate needed to be adjusted. A 0.01 g/L/h rate of feeding ammonium acetate was applied in the 5-L bioreactor (Fig. 6B). DO, residual sugar, and pH were all increased for a short time (96-144 h) after feeding, which were similar to non-treatment. The trend of DCW was greater the 0.03 g/L/h rate. Moreover, CHA production reached 57.9 mg/L, which was more close to the 62.10 mg/L in the shake flask than 0.03 g/L/h rate and non-treatment (46.11 mg/L). This information meant that a 0.01 g/L/h rate of feeding ammonium acetate was more beneficial for CHA biosynthesis than a 0.03 g/L/h rate and non-treatment in the 5-L bioreactor. Moreover, the detail comparison of each parameter (DO, residual sugar, chaetominine production, DCW, and pH) was shown in the supplemental data 2.

Hence, the fermentation of *A. fumigatus* CY018 for producing CHA was successfully scaling-up with feeding 0.01 g/L/h rate ammonium acetate in 5-L bioreactor. The results of the current work indicated that the supplement strategy of ammonium acetate was beneficial for CHA biosynthesis by *A. fumigatus* CY018. Meanwhile, this provided a valuable experience for enhancing target compound production in submerged fermentation.

Finally, in this paper, the supplement of selected ammonium acetate was beneficial for CHA biosynthesis. The optimization of addition concentration and time of ammonium acetate was first performed in the shake flask. Maximal CHA production (62.1 mg/L) was achieved with addition of 50 mM ammonium acetate at 120 h of fermentation. The activity of key enzyme and transcription levels of key genes (*laeA*, *dahp* and *trpC*) were promoted with supplement strategy of ammonium acetate, which enhanced CHA production ultimately.

Moreover, the feeding rate of ammonium acetate was researched and determined to be 0.01 g/L/h in 5-L bioreactor. These results demonstrated that supplement of ammonium acetate was a significant strategy to improve the production of CHA. Furthermore, this work provided a precious experience for promoting target compound from marine fungus by supplement of effective nutrients in submerged fermentation.

Acknowledgements

This work is sponsored by the National Natural Science Foundation of China (81741156), the Shanghai Sailing Program (17YF1403700), and the Fundamental Research Funds for the Central Universities (222201714023, 22221818014).

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure Captions

Fig. 1 Chemical structure of chaetominine.

Fig. 2 Effects of addition concentration (A) and addition time (B) of ammonium acetate on chaetominine (CHA) production and dry cell weight (DCW) at 336 h (180 rpm, 28 °C) by *Aspergillus fumigatus*. Non-treatment group: without addition of ammonium acetate. Experimental of addition concentration: 5, 10, 20, 50 and 100 mmol/L. Experimental of addition time: 72, 120, 168, 216, 264 and 312 h. * Indicates statistical significance ($P < 0.05$) compared to the control group.

Fig. 3 HPLC chromatograms of CHA at 227 nm in the non-treatment group and ammonium acetate supplement group (50 mmol/L, 120 h) at 336 h (180 rpm, 28 °C) by *Aspergillus fumigatus*. Condition of HPLC: acetonitrile: water = 65: 35, flow = 1 mL/min, temperature = 25 °C, injection volume = 20 μL.

Fig. 4 Time courses of DCW (A), residual sugar (B), chaetominine production (C) and DAHP synthase activity (D) in shake flask with non-treatment and ammonium acetate feeding strategy. *Aspergillus fumigatus* CY018 was cultured 360 h at 180 rpm and 28 °C. * Indicates statistical significance ($P < 0.05$) compared to the non-treatment group.

Fig. 5 Response of transcript level of genes related to chaetominine biosynthesis when feeding strategy and non-treatment were shown. *laeA* as a global regulator (A), *dahp* as 3-Deoxy-arabino-heptulosonate-7-phosphate synthase (B), *cs* as chorismate synthase (C), and *trpC* as anthranilate synthase (D). *Aspergillus fumigatus* CY018 was cultured 360 h at 180 rpm and 28 °C. * Indicates statistical significance ($P < 0.05$) compared to the non-treatment group.

Fig. 6 Time courses of DO, chaetominine production, residual sugar, pH, and DCW in *Aspergillus fumigatus* cultured in 5-L bioreactor with feeding rate of 0.03 g/L/h (A), 0.01 g/L/h (B) and non-treatment (C) The culture condition of *A. fumigatus* CY018 was 300 rpm, 28 °C and 1.5 ± 0.1 vessel volume/min of air flow. The 11.55 g of ammonium acetate (50 mM, 3 L broth) was supplied at 120 h.

448 **Table Captions**

449 **Table 1.** Sequences of primer pairs for quantitative real-time RT-PCR (qRT-PCR) assay.

Target gene	Primer name	Primer sequence (5'-3')
laeA	*laeA*-forword	TTCTTTCGAGCTGCCGTCAA
	laeA-reverse	TCCATGGTATGTTCGTCCGT
dahp	*dahp*-forword	GACAAGGGCCCATCTTCACA
	dahp-reverse	TGGCAGACGTGTTATAGCGG
cs	*cs*-forword	CCCCCGAACAAATGAATCGC
	cs-reverse	GGTACTTCTCCGCAATGGCT
trpC	*trpC*-forword	AGGTCGACGTTTTCGGTGAA
	trpC-reverse	AGTCACTTCAACCGCAGGAG
actin	*actin*-forword	TTCGAGACCTTCAACGCTCC
	actin-reverse	ATGGGGACAACGTGAGTGAC

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451 **Table 2.** Effect of different nitrogen sources addition on CHA biosynthesis. * Indicates

452 statistical significance ($P < 0.05$) compared to the non-treatment group.

Nitrogen source	Dry cell weight (g/L)	Production of CHA (mg/L)
Non-treatment group	21.98 ± 1.60	29.27 ± 3.04
5 mM ammonium sulfate	19.43 ± 2.04	31.62 ± 3.05
10 mM ammonium sulfate	19.46 ± 1.99	25.35 ± 1.55
5 mM ammonium chloride	20.53 ± 1.27	32.52 ± 2.11
10 mM ammonium chloride	20.61 ± 1.28	34.54 ± 3.17
5 mM ammonium acetate	20.47 ± 1.13	32.73 ± 1.25
10 mM ammonium acetate	21.83 ± 2.11	42.52 ± 2.26*

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Ammonium acetate supplement strategy for enhancement of chaetominine production in liquid culture of marine-derived *Aspergillus fumigatus* CY018

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Figures

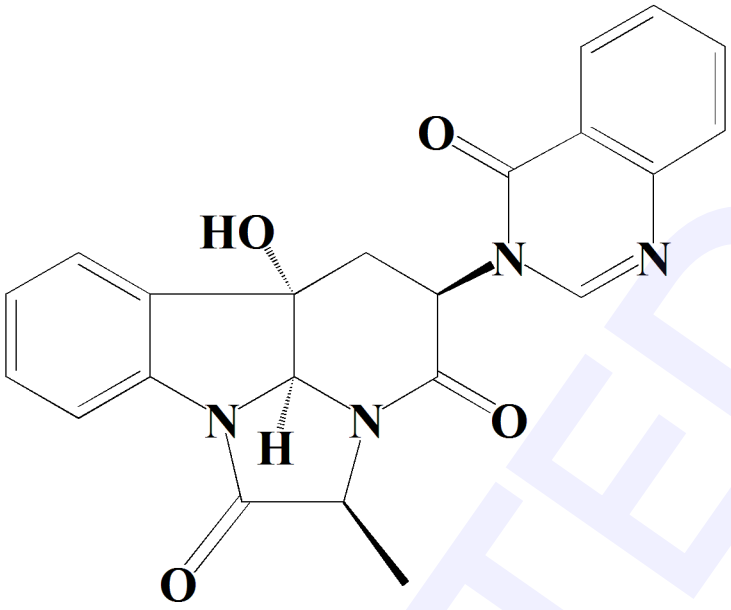
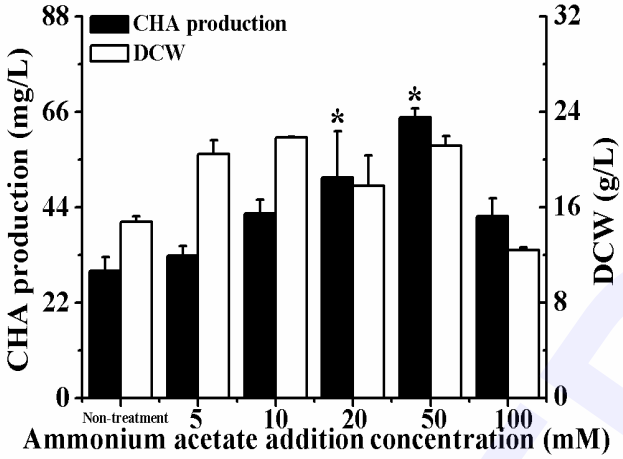


Fig. 1



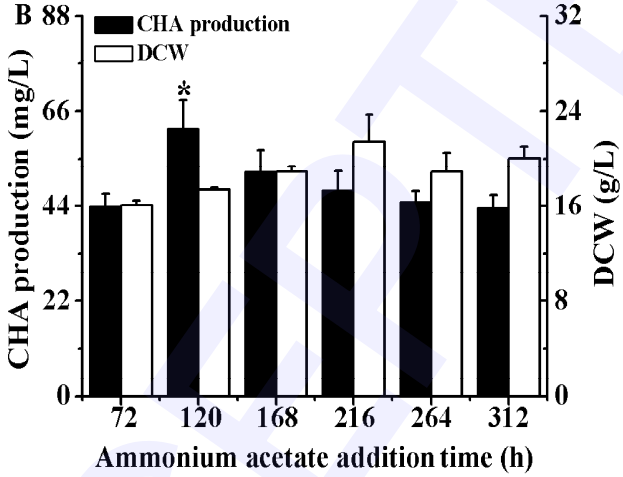
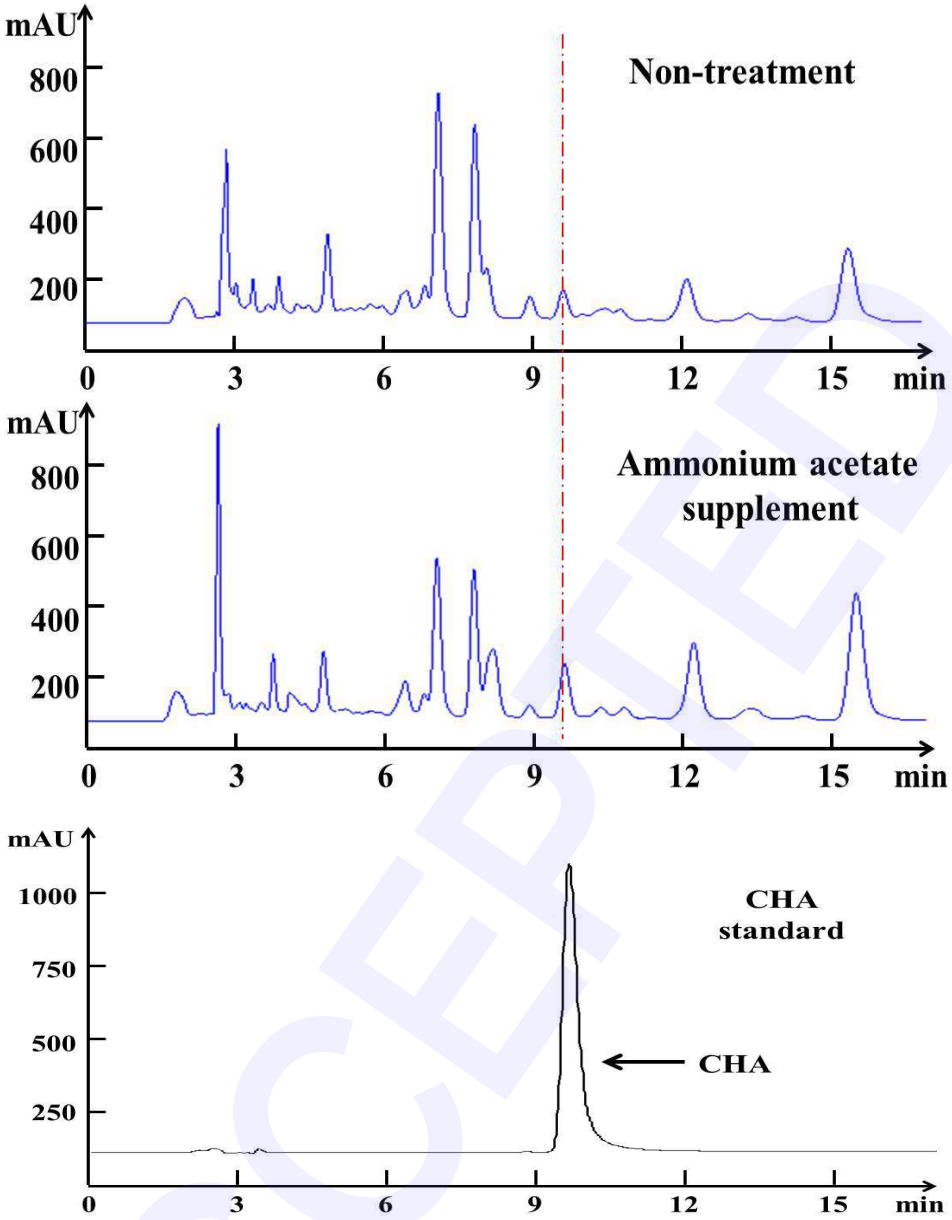


Fig. 2



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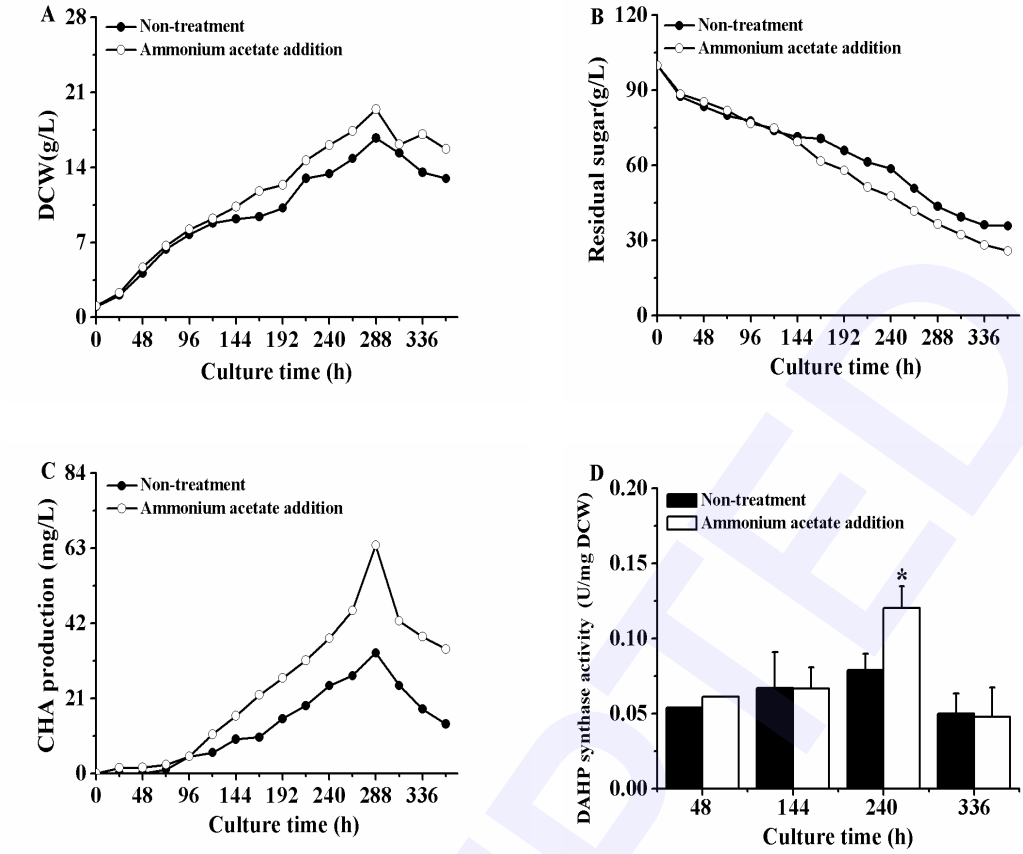

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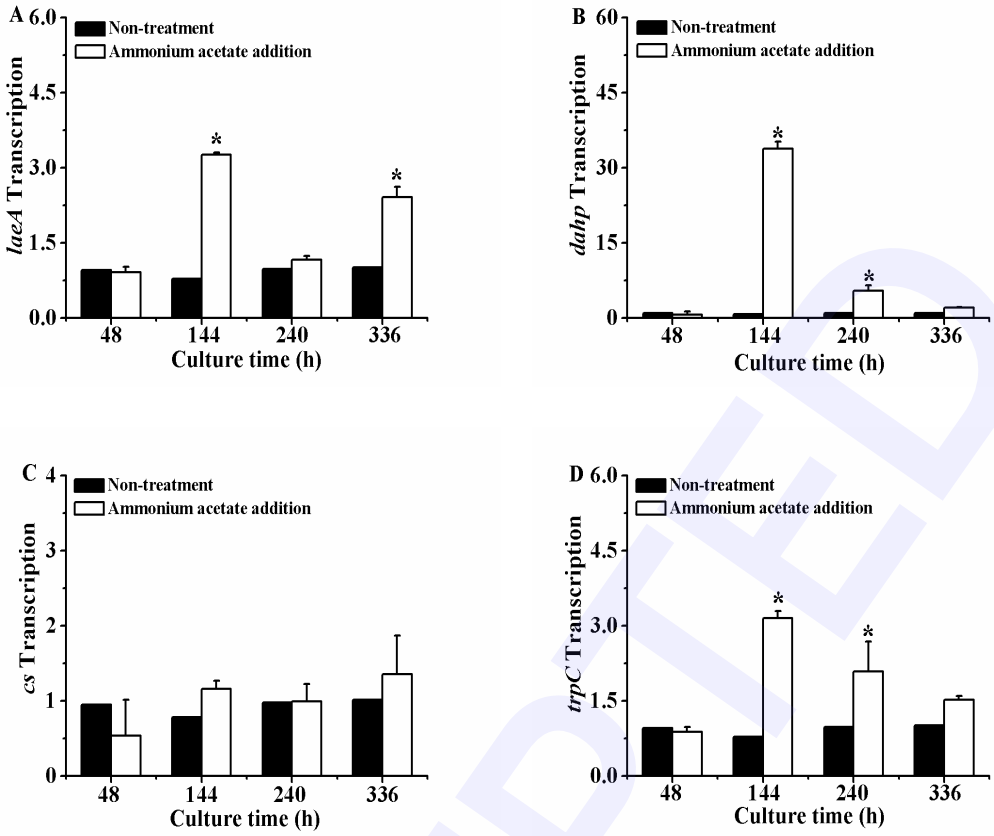

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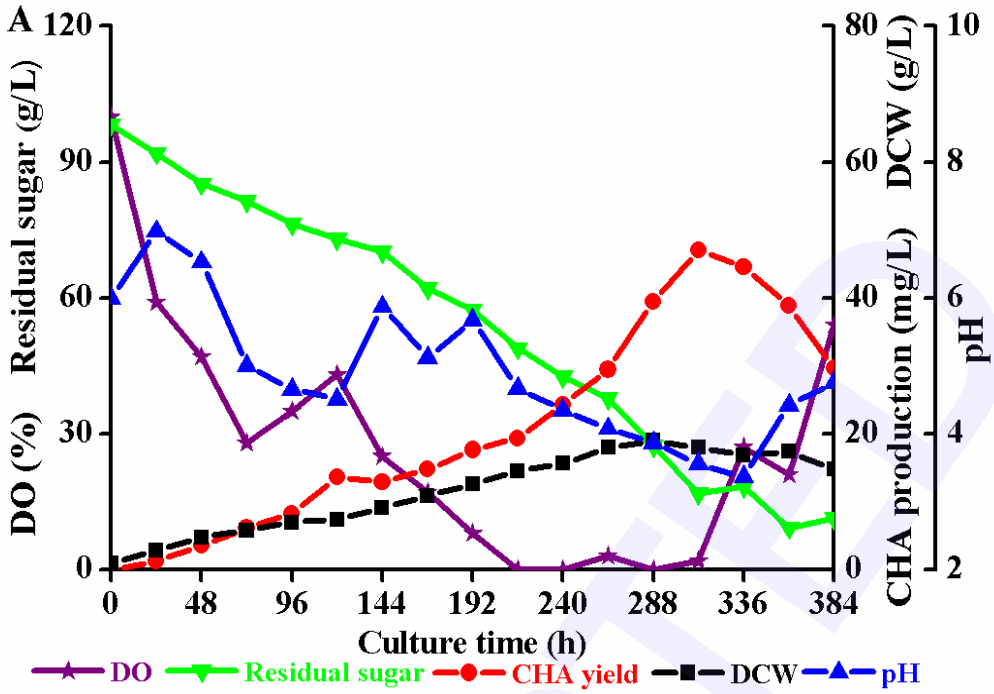

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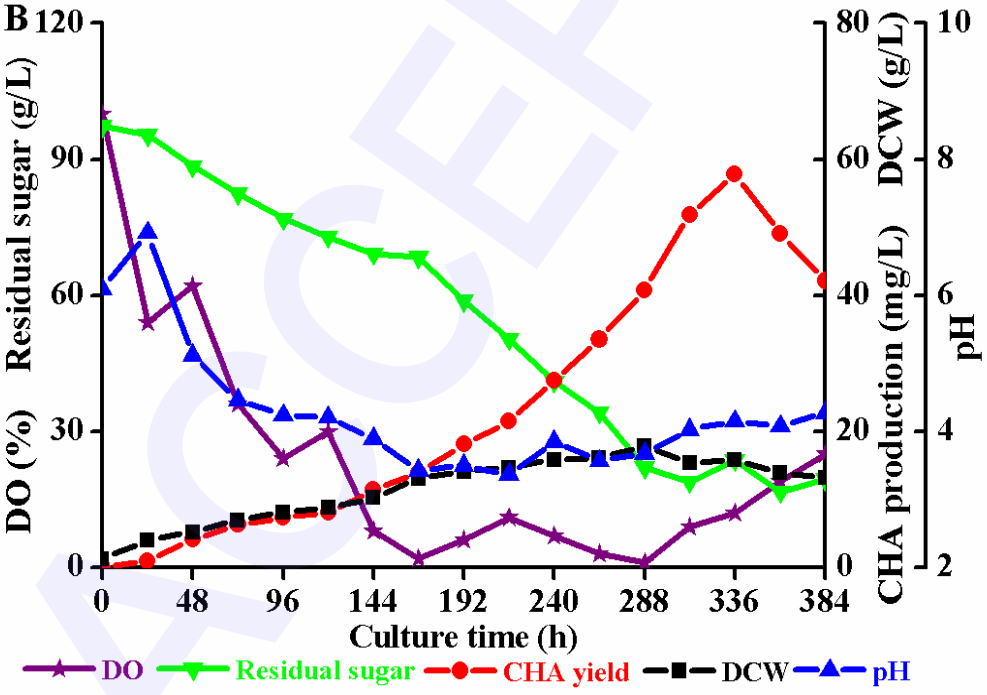

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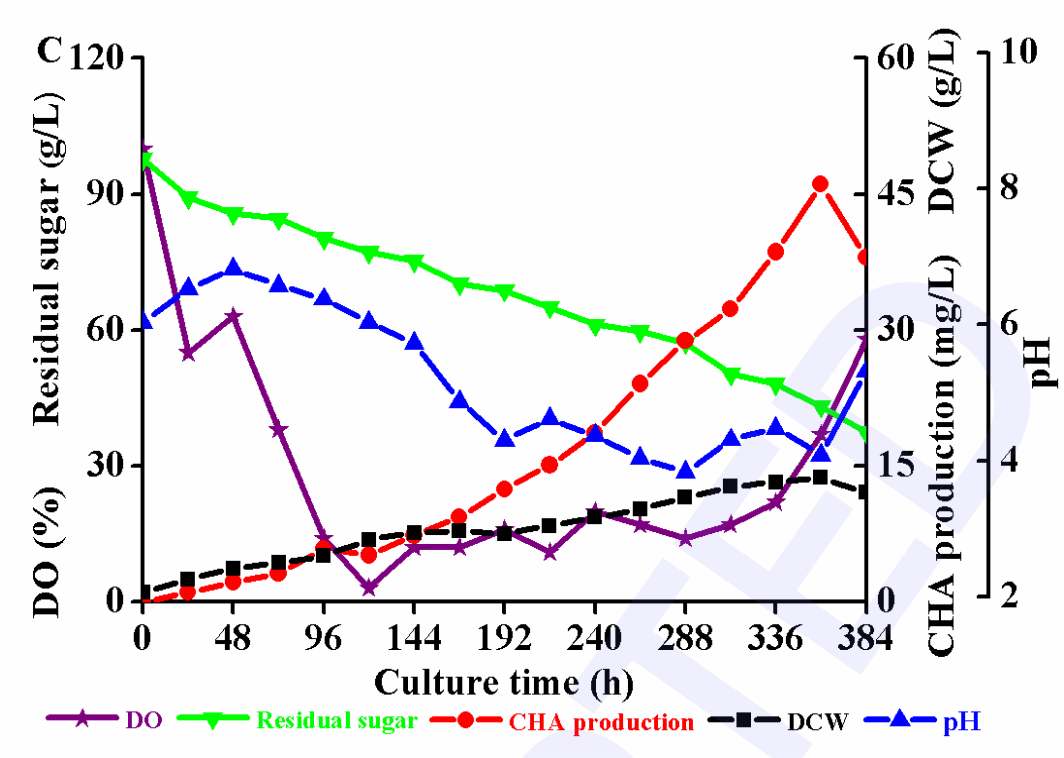

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42 **Fig. 6**

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